

## Exploring baseline behaviour in group-housed, pre-weaned dairy calves

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### ABSTRACT

With increasing public concern for farm animal welfare, understanding their current welfare status is paramount. Animal welfare can be inferred from their behaviour, as behaviour represents the combination of internal and external cues. The aims of this study were to quantify a behavioural baseline for group-housed, pre-weaned dairy calves that were reared under conventional management conditions, and to determine how different internal (i.e. age) and external (i.e. temperature) factors affected this behaviour. Female dairy calves (n=47) were allocated to 1 of 3 pens based on birth date and reared under conventional Irish management conditions; after 3–4 d in individual pens, calves were moved into group pens where they had *ad libitum* access to water, concentrates, and forage (first barley straw, then hay). Milk replacer (6 L/d) was fed through an automatic milk feeder; calves were gradually weaned from day 42–84. A 24 h period/week of video recording was used for behaviour scoring for 8 consecutive weeks (scan sampling at 10-min intervals). Behaviours included posture (lying or standing) and activity (17 behaviours). Calves were scored for clinical health twice weekly and only healthy calves were used in the analysis (n=39). Behaviour proportions were analysed using generalised linear mixed models. Proportion of time spent lying decreased as calves aged (week 1 vs. 9; percentage mean  $\pm$  standard deviation;  $79.8 \pm 4.04$  vs.  $72.1 \pm 6.52$  %;  $P=0.004$ ), while time spent ruminating ( $2.0 \pm 2.51$  vs.  $14.1 \pm 8.72$  %;  $P<0.001$ ), eating bedding ( $0.8 \pm 1.16$  vs.  $6.1 \pm 4.66$  %;  $P<0.001$ ), eating forage ( $0.9 \pm 1.20$  vs.  $1.8 \pm 1.81$  %;  $P=0.007$ ), and eating concentrates ( $0.5 \pm 1.15$  vs.  $2.2 \pm 1.72$  %;  $P=0.018$ ) increased with age. On days when the minimum shed temperature was  $<4^{\circ}\text{C}$  compared to  $>6^{\circ}\text{C}$ , calves spent more time lying ( $75.9 \pm 5.27$  vs.  $72.3 \pm 5.78$  %;  $P<0.001$ ) and less time eating concentrates ( $0.8 \pm 1.11$  vs.  $1.4 \pm 1.49$  %;  $P=0.035$ ), eating forage ( $0.8 \pm 0.91$  vs.  $1.5 \pm 1.57$  %;  $P=0.005$ ), eating bedding ( $2.7 \pm 2.87$  vs.  $4.0 \pm 4.78$  %;  $P=0.003$ ), and walking ( $1.5 \pm 1.20$  vs.  $2.0 \pm 1.40$  %;  $P=0.017$ ), independent of age. These findings provide a normal behavioural baseline for future calf behaviour studies and highlight potential areas of improvement in current, conventional calf rearing practices.

### 1. Introduction

Consumers and the general public are increasingly concerned about the welfare of production animals (Busch et al., 2017; Hotzel et al., 2017; Sweeney et al., 2022), making understanding current welfare status of farm animals paramount. Welfare is defined here as the balance between, and accumulation of, positive/pleasant and negative/unpleasant experiences over time (Webb et al., 2019; Reimert et al., 2023). Inferences about welfare can be made using animal behaviour, as it is key to understanding the motivational states of

animals (e.g. Dawkins, 2003; Wechsler, 2007; Mason and Bateson, 2009). Animal behaviour is governed by internal (i.e. calf breed or age) and external (i.e. ambient temperature or space allowance) stimuli, as well as their interaction (Darwin, 1873; Jensen and Toates, 1993; Fraser, 2009). It therefore is important to consider, measure, and account for various internal and external factors when quantifying behaviour.

Many studies use behaviour to infer welfare states, and recently there has been a rise in studies using sensors to monitor behaviour, as they allow for long-term, continuous assessment with minimal labour input (Rutten et al., 2013; Steensels et al., 2017; Riaboff et al., 2022). Often

*Abbreviations:* ADG, average daily gain; ADGw, weekly average daily gain; ADGb, average daily gain from birth; AvgT, average daily pen temperature; MinT, minimum daily pen temperature; EBI, Economic Breeding Index; THI, Temperature-humidity index; TNZ, Thermo-neutral zone.

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these sensor-based systems are based on the principle of detecting deviations from 'normal' to identify periods of illness (Belaid et al., 2020; Duthie et al., 2021; Sun et al., 2021) or other low welfare states, such as social distress (Bus et al., 2021). Consequently, calf health status is also important to consider when defining 'normal ranges' of behaviour.

During the pre-weaning period, the dairy calf undergoes a substantial amount of development, both in terms of growth and development, in particular rumen development. New-born calves are considered monogastric, as they are born with a non-functional rumen and rely solely on milk for nutrient intake at the beginning of their life (Khan et al., 2016). Consumption of solid feeds (concentrates, hay, or straw) triggers the start of rumen development and rumination. Calves typically start ruminating at approximately 2–3 weeks of age (Swanson and Harris, 1958; Noller et al., 1959; Wang et al., 2022), but individual calf variability is high (Wang et al., 2022). High milk allowances can delay rumen development, as they discourage consumption of solid feeds (de Passillé et al., 2011; Eckert et al., 2015; Steele et al., 2016). There are a lack of studies describing normal feeding behaviours during the pre-weaning period in dairy calves under normal management conditions.

Lying behaviour is commonly used as a welfare indicator in dairy cattle (see review by Tucker et al., 2021) and calves (Webster et al., 1985; Færevik et al., 2008; Webb et al., 2017). It is known that young, pre-weaned calves will spend the majority of their day lying (Dwyer, 1960; Calvo-Lorenzo et al., 2016), and the amount of time they spend lying will decrease as they age (Hutchison et al., 1962; Vitale et al., 1986; Kerr and Wood-Gush, 1987). However, the majority of studies defining baselines of calf lying behaviour are dated and on a mixture of breeds (i.e. dairy, beef, and zebu), social groups (i.e. calves alone vs. calves with dams), and environments (i.e. indoors vs. outdoors on pasture). Additional factors, such as temperature, may also affect lying and other behaviours (Hänninen et al., 2003; Tripon et al., 2014; Sawalbah et al., 2016).

The objective of this research was to observe calf behaviour during the pre-weaning period to establish a behaviour baseline for group-housed, pre-weaned dairy calves reared under conventional management conditions in Ireland. Specifically, we wanted to quantify the proportion of time per day (using scan sampling) calves spent performing specific behaviours related to feeding and lying, observe how the proportion of time spent performing these specific behaviours changed as calves aged, and determine whether the proportion of time calves spent performing specific behaviours was related to different internal (i.e. breed, colostrum amount and quality) and external (i.e. ambient temperature) factors. We hypothesised that as calves aged during the pre-weaning period, they would increase the proportion of time spent consuming solid feed, ruminating, and drinking water. Moreover, as they aged calves were expected to become more active.

## 2. Materials and methods

### 2.1. Ethics statement

This study was conducted from 13 January to 16 April 2022 at Teagasc Moorepark Dairy Research Farm, County Cork, Ireland. Ethical approval for this study was received from the Teagasc Animal Ethics Committee (TAEC2021–319). Experiments were performed in accordance with European Union Regulations 2021 (Protection of Animals Used for Scientific Purpose; S.I. No. 543 of 2012).

### 2.2. Animals and experimental design

Forty-seven female dairy calves were enrolled in the experiment on a rolling basis, as they were born during the spring calving season. This experiment consisted of three pens of calves, or replicates. Calves were allocated into the pens (replicates) by (mean  $\pm$  standard deviation) date of birth, birthweight (kg), and breed (Holstein-Friesian, Jersey, or

Holstein-Friesian x Jersey): replicate 1, 27 January 2022  $\pm$  10.0 d; 33  $\pm$  5.8 kg; 15 Holstein-Friesian and 2 Holstein-Friesian x Jersey; replicate 2, 30 January 2022  $\pm$  7.4 d; 33  $\pm$  5.2 kg; 12 Holstein-Friesian and 5 Holstein-Friesian x Jersey; and, replicate 3, 15 February 2022  $\pm$  3.2 d; 34  $\pm$  6 kg; 5 Holstein-Friesian, 3 Jersey, and 2 Holstein-Friesian x Jersey. Replicate 1 and 2 enrolled calves at the same time; replicate 3 enrolled calves after 1 and 2 had been filled to minimise the difference in ages between the youngest and oldest calf in each pen. Calves in replicate 3 were approximately 3 weeks younger than calves in replicates 1 and 2, thus replicates were running simultaneously for the majority of the experiment. In the third replicate, one calf died of diarrhoea around 3 weeks old, thus no behaviour observations were used from that calf. There were two additional calves housed in the third replicate pen that were not used in this experiment (to maintain pen stocking density).

### 2.3. Animal management and housing

#### 2.3.1. Birth and colostrum management

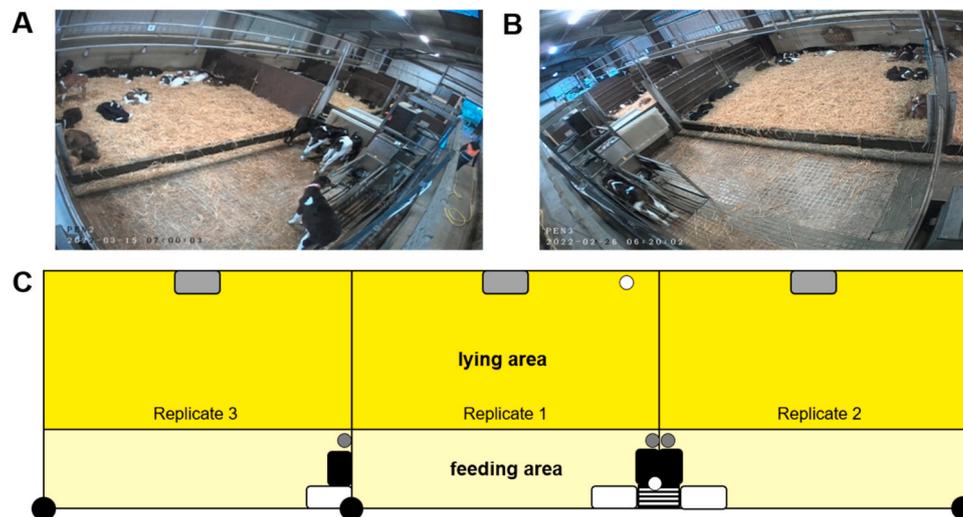
All calves were managed as per the conventional calf rearing system in Ireland (i.e. Barry et al., 2020), and all final treatment and management decisions were made by the farm manager. Calves were separated from their dam within 1 h post-birth, were weighed, ear-tagged, and had an iodine solution sprayed on their navel, and then were placed in an individual calf pen. Calves were fed a standardised amount (3 L) of high quality colostrum (>22 % using a Brix refractometer; Bielmann et al., 2010) within 2 h post-birth. Following colostrum feeding, calves received five feeds of transition milk (2.5 L fed twice/d) by a bucket fitted with a teat (Conneely et al., 2014). After the feedings of transition milk, calves were fed two feeds per day of 2.5 L of milk replacer (125 g/L; Heiferlac, Volac, Hertfordshire, United Kingdom; 26 % crude protein) in the individual pens using a bucket with a teat attached until they were moved into the group pens, which occurred twice weekly. When in the individual pens calves were fed milk replacer twice daily at 08:00 h and 15:00 h.

#### 2.3.2. Group housing and nutrition

The shed containing the calf pens was a converted shed with natural ventilation, and also contained the dry cows close to calving and individual calving pens, meaning calves shared airspace with mature animals. Ventilation within the shed could be manually altered by opening doors on opposing ends of the shed. Due to internal walls, calves could only see other calves, but could hear mature cows. Due to the calving pens, the lights were on in the shed 24 h/d, but the amount of light calves received differed day to night, due to sunlight. The calf pens had canopies at the back of the lying area (visible in Fig. 1A and B) that could be manually lowered during cold temperatures, and also were each equipped with three heat lamps that automatically turned on when the ambient shed temperature near the sensor was  $<4^{\circ}\text{C}$ .

The calves were housed in group pens (Fig. 1C), consisting of a grooved concrete standing area (12.6 m<sup>2</sup>) and a straw-bedded lying area (23.6 m<sup>2</sup>) separated by a wooden divider, giving a total space of 36.1 m<sup>2</sup> per pen. Replicates 1 and 2 (Fig. 1A) housed 17 calves while replicate 3 housed 12 calves (10 on the experiment plus two extras), giving space allowances of 2.13 m<sup>2</sup>/calf and 3.01 m<sup>2</sup>/calf, respectively. Once in the group pens, calves were fed milk replacer by an automatic milk feeder (Förster-Technik) at a rate of 125 g/L. Milk replacer allowance depended on each calf's number of days in the pen and corresponded to age. The plan was as follows: from d 1–7, calves were increased from 5 L/d to 6 L/d; from d 7–42, calves remained at 6 L/d; from d 42–56, calves were reduced from 6 L/d to 4 L/d; from d 56–89 calves were reduced from 4 L/d to 1 L/d. Daily milk allowance was split over four equal periods throughout the day (i.e. a quarter of their total allowance/d was offered every 6 h). Calves were fully weaned at 84 d of age (12 weeks) by removal from their group pen.

Upon entering the group pen, calves were offered *ad libitum* access to concentrates (first four weeks: Prime Elite Krispi Kaf, DairyGold Agri



**Fig. 1.** Diagram showing the camera views for replicates 1 (A) and 2 (B) and the set-up of the three replicate's pens (C). In the diagram (A), the black circles represents the camera locations in each pen, the grey circles represent the water bowls, the white circles represent the two temperature and humidity logger positions (hanging 1.5 m off the ground, out of calf reach), the white rectangle represents the automatic milk feeder, the black rectangle represents the concentrate trough feeder, and the grey rectangle represents the forage (barley straw or hay) feeder.

Business Limited, Mitchelstown, Co. Cork, Ireland, 18 % protein; five weeks old until weaning: Prime Elite Kaf Gro, DairyGold Agri Business Limited, Mitchelstown, Co. Cork, Ireland, 16 % protein), water through a water bowl installed in each pen, and forage available from a rack installed on the wall over the lying area (Fig. 1C). The forage provided to calves differed: during the first four weeks of the study, barley straw was provided in the forage feeder and after this, hay was provided.

### 2.3.3. Health checks and treatments

All medical treatments (i.e. an injection of an antibiotics or an NSAID) were provided at the discretion of the farm manager and were recorded. All calves were checked twice a day by the farm manager. On five occasions (5 calves, 1 occasion per calf), calves were temporarily removed from the group pens and placed into an individual or group sick pen. Calves were returned to their original group pen once deemed recovered by the farm manager (i.e. able to drink independently from the automatic milk feeder and no longer requiring a higher level of attention). All instances of movements in and out of sick pens were noted by the researchers.

### 2.3.4. Dehorning and vaccinations

Following normal farm management procedure, calves were dehorned in three batches (midday on a Tuesday: 8 February 2022, 22 February 2022, and 29 March 2022), at an average age of  $17 \pm 10.7$  d (7–13 d = 16 calves; 14–20 d = 22 calves; 21–27 d = 4 calves; 35–41 d = 2 calves). All calves were provided with a local nerve block (2 mL lidocaine/side), applied at least 10 minutes before dehorning commenced. Calves were restrained in a dehorning crate for the procedure, during which they were also vaccinated for Clostridium (Blackleg, Braxy, Black Disease, and Tetanus; Tribovax, MSD, Ireland; subcutaneous injection) and coccidiosis (Bovicox; oral suspension). One of the purebred Jersey calves was polled, and thus was vaccinated but not dehorned. Calves were carefully monitored by the farm manager in the days following this procedure.

### 2.4. Shed measurements

Temperature and relative humidity were recorded every 10 minutes using data loggers (Tinytag TGP 4017 Temperature Data Logger; Gemini Data Loggers, West Sussex, United Kingdom). Only two loggers were available, thus were both placed in the middle of the three adjacent pens

(replicate 1; Fig. 1). Two loggers were placed in one calf pen (replicate 1 pen; Fig. 1) to get an average temperature across the pen. One logger was positioned under the canopy, overtop of the calves' lying area, but high enough so the calves could not reach them (1.5 m from bedding surface). The other logger was positioned in the feeding area of the pen, also out of calf reach (1.5 m from ground). The first logger's position under the canopy allowed it to capture the temperature under the canopy when the heating lamps were turned on.

### 2.5. Video recording and calf identification

Each pen of calves was continuously recorded by a video camera (8MP-4K Varifocal Dome CCTV Camera with 40 m night vision, Equicom Limited, Cobh, Co. Cork, Ireland) connected to a digital video recorder (PRIMA XR5 8MP 4 K, Equicom Limited, Cobh, Co. Cork, Ireland). The camera in each pen was positioned approximately 2.7 m above the ground, so that the majority of the pen was within view of the camera (Figs. 1A and B); only the entrance gate of the pen, directly under the camera, was not visible. For calf identification purposes, pictures were taken of all calves from several different angles (front, sides, back, and above). The majority of calves within each pen were wearing collars in three different colours (red, blue, or yellow) which also helped to identify the calves.

### 2.6. Calf health measurements

All calves were clinically health scored twice a week for the duration of the experiment, using a modified health scoring system by Barry et al. (2019). Ten aspects of calf health (demeanour, ocular discharge, ear position, nasal discharge, cough, dehydration, mobility, interest in surroundings, faecal hygiene, and naval score) were scored on a 4-point scale, from 0 to 3, except interest in surroundings (2-point scale). A score of 0 indicated there were no issues while a score of 3 indicated the calf is severely affected (i.e., for nasal discharge, a score of 0 = no discharge, eyes are bright and pronounced, while a score of 3 = dull and sunken eyes with excessive non-clear discharge present in both eyes). Health scoring was performed by three individuals throughout the pre-weaning period (inter-observer reliability; weighted kappa = 0.939). A composite clinical health score was calculated for each health-scoring event by summing all individual scores. Calf body weight (kg) was recorded weekly from birth using a weighing scale (TrueTest

XR 3000, Tru-test Limited, Auckland, New Zealand). Average daily gain (ADG) for each week was calculated by subtracting the previous week's weight from the current week's weight, then dividing by the number of days between the weight measurements.

## 2.7. Behaviour scoring

### 2.7.1. Ethogram

Calf behaviour was scored using scan sampling from the video recordings, using the ethogram in Table 1. Behaviours were separated into two categories that were scored at each time-point: posture and activity. Postural behaviours included standing and lying; the calf was always performing one or the other. Activity behaviours included anything else the calf might be doing; if the calf was idle or sleeping, nothing was noted for that time-point.

### 2.7.2. Training and validation

Five independent observers performed the behaviour scoring for this experiment. Of the five observers, only one had had previous behaviour scoring experience. Before commencing scoring, all observers were trained and then completed a two-part validation process. To train all the observers, they were first given the ethogram (Table 1), practice videos (not videos from the scoring days), and calf identification materials (see Section 2.5). Once they felt confident in their scoring ability, they completed the first round of validation (validation 1). The validation video was one hour long, and was scored using 5-minute scan samples (13 observations total). Each calf in the pen ( $n = 17$ ) was scored. This validation was completed within 1 day and independently; the observers were not allowed to ask questions or compare answers. The results for each observer were then compared for validation 1, and a percent of agreement was calculated for posture (98.55 % agreement) and activity (80.00 % agreement). After validation 1, any major scoring issues (i.e. clarifying of behaviours, incorrect labelling, or times when a calf was scored differently by all) were viewed, discussed, and a common behaviour classification was agreed upon. Sometimes, the issue was not the behaviour but misidentification of the calves. After the issues had been discussed, validation 2 was performed. Validation 2 was performed using the same method and video as validation 1. The final percent agreements were 99.19 % for posture and 81.99 % for activity.

### 2.7.3. Scan sampling behaviour scoring

Behaviour scoring was performed on the videos extracted from the DVR for one day each week. Saturday was chosen as the scoring day, as no research measurements were taken on this day and the only disturbance to the calves were those considered normal management (i.e. health checks by the farm manager or farm staff adding more concentrates to the feeder). Each calf was followed for eight weeks. Calf behaviour was scored using 10-minute scan sampling, from 00:00 h to 23:50 h, so that 144 observations were recorded. On two Saturdays, the farm staff spread straw in the calf pens (additional straw was added, pens were not completely cleaned out), disrupting the calves and preventing the view of the majority of the calves in the pen. When this occurred, no behaviour was recorded for that observation and the total number of scans per day was reduced to 143.

## 2.8. Data processing

### 2.8.1. Calculation of week of age

Week of age was determined for each calf at each behaviour observation date based on their age in days (Table 2). As calves entered the group pen at 3–4 d old, the first behaviour observation that they were in the group pen might have occurred at either week 1 or week 2 of age. Therefore, some calves have behaviour observations from week 1–8, while others have observations from week 2–9 (Table 2).

**Table 1**

Ethogram describing the different calf behaviours scored using 10-minute scan sampling from a 24-h video recording once a week for 8 weeks. The behaviours were split into two categories: posture and activity. Posture behaviours were always classified (144 combined observations). Activity behaviours were only recorded if they were occurring; if the calf was idle or sleeping, no activity behaviour was recorded.

Behaviour	Description
<i>Posture</i>	
Lying	Calf is resting either sternally or laterally with all four legs hunched close to body either awake or asleep; brisket is in contact with the ground
Standing	Calf is in a static upright standing position with weight placed on all four legs
<i>Activity behaviours</i>	
Drinking milk	Calf is standing in the automatic milk feeder drinking milk, with their mouth on the nipple.
Defecation/ urination	Calf defecates or urinates
Drinking water	Calf is standing at the water bowl with their nose/mouth partially submerged in the water.
Eating bedding	Calf is lying down on the straw and is making repeated lateral motions of the jaw with straw sticking out of their mouth. / Calf is lying down on the straw and is rooting their nose around in the bedding. / Calf is standing with their head near the ground making lateral motions of the jaw.
Eating concentrates	Calf is standing with their head within the flaps of the concentrate feeder. / Calf is standing with their head just outside the concentrate feeder while making lateral motions of the jaw
Eating forage	Calf makes a lateral motion of the jaw while standing at the hay feeder.
Grooming	Calf uses tongue to repeatedly lick own back, side, leg, tail areas
Oral manipulation of the pen structure	Calf licks, nibbles, sucks, or bites at the pen structure (barriers, walls, buckets, troughs etc.)
Other	Calf performs any other activity not mentioned above.
Play	Calf runs, jumps, changes direction suddenly, bucks, kicks hind legs, twists or rotates body. / Calf mounts or attempts to mount another calf. / Calf is engaged in head-to-head pushing with another calf. / Calf plays with an object in the pen.
Pacing	Calf repeatedly walks back and forth the same area in an active manner
Rumination/ chewing	Calf is lying down and making repetitive motions of the lower jaw in the lateral plane; calf is standing, not near the hay feeder, and making repetitive motions of the lower jaw in the lateral plane with their head with their head in a lateral position (not with head near floor)
Social interaction	Calf licks another calf in the same area multiple times. / Calf nudges another calf with its nose. / Calf sniffs another calf's head
Sniffing	Calf sniffs at their surroundings, including the ground, any part of the pen structure, or other calves bodies (not the head)
Scratching / rubbing / stretching	Calf scratches itself with one of their legs (generally hind legs). / Calf rubs itself on pen structure. / Calf stretches itself.
Tongue-rolling	Calf makes repeated movements with its tongue inside or outside its mouth.
Urine drinking / orally manipulate prepuce / cross sucking	Calf drinks the urine of another calf. / Calf attempts to suck the naval area of another calf. / Calf attempts to suck any body part of another calf.
Walking	Calf is actively moving from one point in the pen to another in an active walking motion.
Other	Calf performs any other activity not mentioned above.
<i>Out of frame</i>	
Out of frame	The calf's head is out of frame of the camera (i.e. in the corner of the pen or hidden behind another calf).

**Table 2**

Week and animal-based factor categories used in the analysis, including the number and percentage of total days, calves, and/or observations in each category. \*Birthweight is presented as percentage of projected mature bodyweight.

Animal-based factor	Mean (range)	Unit	Category levels	Category labels	Calves or days per level (n [%])	Observations per level (n [%])
Week	-	days	0–6	week 1	16 [5.9]	-
			7–13	week 2	35 [12.9]	-
			14–20	week 3	36 [13.2]	-
			21–27	week 4	35 [12.9]	-
			28–34	week 5	35 [12.9]	-
			35–41	week 6	35 [12.9]	-
			42–48	week 7	30 [11.0]	-
			49–55	week 8	37 [13.6]	-
			56–62	week 9	13 [4.8]	-
Breed	-	-	Holstein-Friesian (<25 % Jersey)	Holstein-Friesian	28 [71.8]	196 [72.1]
			Jersey (100 %) and Holstein-Friesian x Jersey (>25 % Jersey)	Purebred Jersey and Holstein-Friesian x Jersey	11 [28.2]	76 [27.9]
Birthweight*	6.0 (4.2–11.7)	%	<5.5	<5.5	16 [25.6]	111 [40.8]
			5.5–6.5	5.5–6.5	13 [33.3]	93 [34.2]
			>6.5	>6.5	10 [25.6]	68 [25.0]
Colostrum amount	9.5 (6.1–13.0)	%	<8.5	<8.5	13 [33.3]	91 [33.5]
			8.5–10	8.5–10	14 [35.9]	95 [34.9]
			>10	>10	12 [30.8]	86 [31.6]
Colostrum quality	25.3 (22.1–34.7)	%	<23	Adequate	11 [28.2]	76 [27.9]
			23–25	Moderate	15 [38.5]	106 [39.0]
			>25	High	13 [33.3]	90 [33.1]
Colostrum source	-	-	Dam	Dam	4 [10.3]	31 [11.4]
			Not dam	Not dam	32 [82.1]	225 [82.7]
			No data recorded	No data recorded	3 [7.7]	16 [5.9]
Calving score	-	-	No assistance	No assistance	28 [71.8]	195 [71.7]
			Assistance required	Assistance required	11 [28.2]	77 [28.3]
Dam parity	-	-	Primiparous	Primiparous	23 [59.0]	167 [61.4]
			Multiparous	Multiparous	16 [41.0]	105 [38.6]
Economic breeding index	241 (129–307)	€	<200	Average	7 [17.9]	51 [18.8]
			200–275	High	24 [61.5]	162 [59.6]
			>275	Elite	8 [20.5]	59 [21.7]
Average daily gain from birth	0.51 (–0.57–1.23)	kg/d	<0.4	<0.4	-	41 [15.7]
			0.4–0.6	0.4–0.6	-	162 [59.6]
			>0.6	>0.6	-	69 [25.4]
Average daily gain per week	0.54 (–0.29–1.35)	kg/d	<0.4	<0.4	-	88 [32.4]
			0.4–0.6	0.4–0.6	-	80 [29.4]
			>0.6	>0.6	-	104 [38.2]
Average daily pen temperature	10.1 (6.7–13.3)	°C	<9	<9	5 [41.7]	106 [39.0]
			9–11	9–11	2 [16.7]	71 [26.1]
			>11	>11	5 [41.7]	95 [34.9]
Minimum daily pen temperature	6.0 (2.0–11.0)	°C	<4	<4	3 [25.0]	58 [21.3]
			4–6	4–6	5 [41.7]	117 [43.0]
			>6	>6	4 [33.3]	97 [35.7]

### 2.8.2. Temperature and relative humidity

For each temperature and humidity logger (two total, one from the feeding area and one from under the canopy; Fig. 1C), the average, standard deviation, minimum, and maximum daily temperature (°C) and average, standard deviation, minimum, and maximum daily relative humidity (%) for each day of the study were calculated in SAS (PROC MEANS). For each logger, the daily average, standard deviation, minimum, and maximum daily temperature-humidity index (THI) was also calculated using the formula from Kelly and Bond (1971):

$$THI = (1.8 * AT + 32) - (0.55 - 0.55 * RH) * ((1.8 * AT + 32) - 58)$$

where AT is the temperature (°C) and RH is relative humidity (expressed as a fraction). This formula is changed slightly from the original to use  $(1.8 * AT + 32)$  to change the formula to use Celsius (°C) rather than Fahrenheit (°F). The individual logger values for average, standard deviation, minimum, and maximum were then averaged to obtain pen-level average values for each variable (temperature, relative humidity, and THI; Fig. 2).

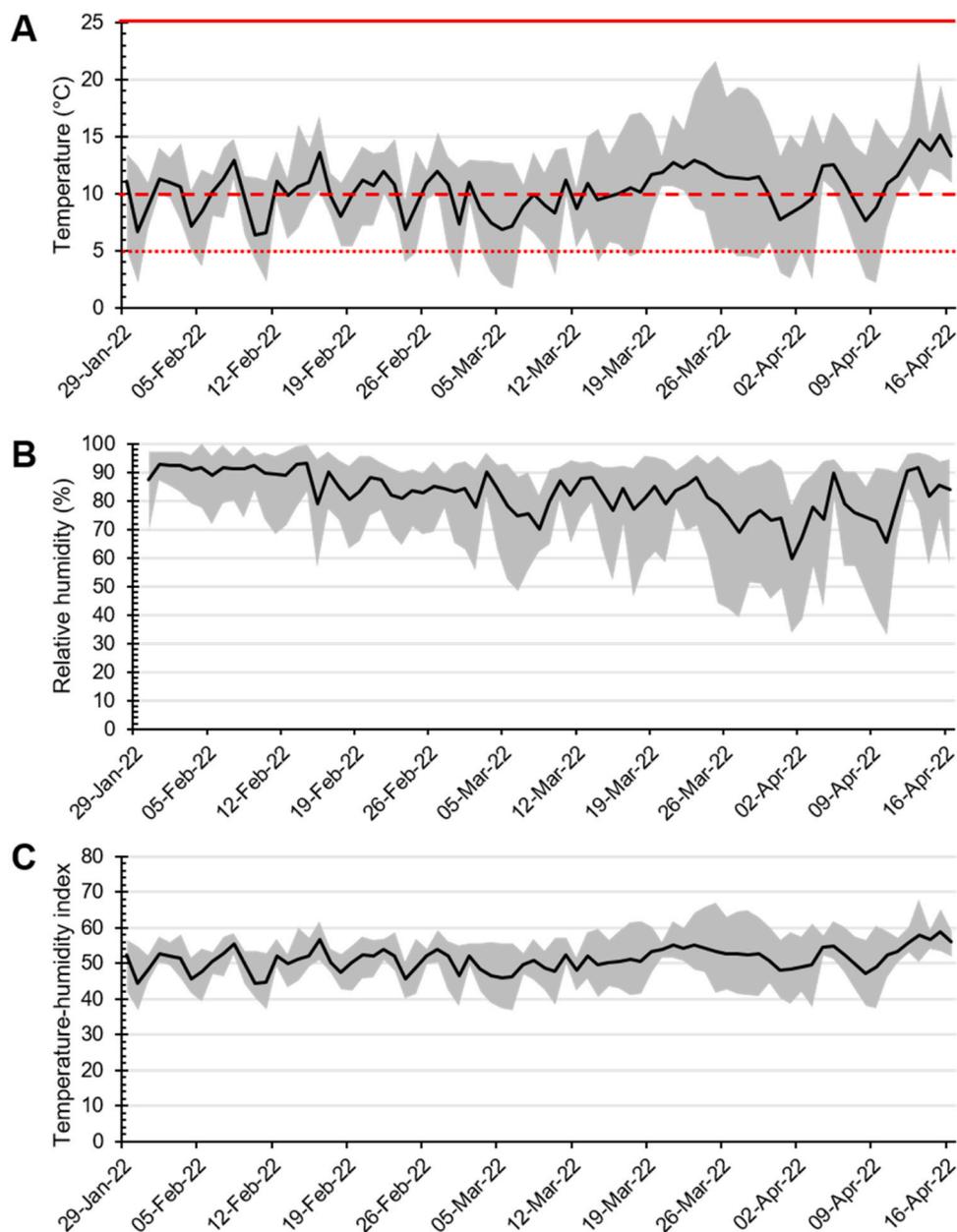
For the purpose of analysis, average daily temperature (avgT) and minimum daily temperature (minT) of each behaviour-scoring day were categorised into three levels (Table 2). The maximum temperature category was not included in the behaviour analysis, as it was always

within the range of thermo-neutral zone (TNZ; average maximum temperature =  $14.5 \pm 2.61^\circ\text{C}$ ). Relative humidity and THI were both initially categorised for the analysis, but were confounded with temperature and thus were not used as factors.

### 2.8.3. Animal-based factors

A number of animal-based variables were categorised into factors to be used in the behaviour analysis. Specific details on the animal-based categories can be found in Table 2, including the number of calves or days and the number of observations within each sub-category. Two breed categories were used due to the low number of purebred Jersey calves; Holstein-Friesian (<25 % Jersey) calves were compared with the combined category of Holstein-Friesian x Jersey (≥25 % Jersey) and purebred Jersey calves (Table 2). The Economic Breeding Index (EBI; see Berry et al., 2007 for more details) for each calf was extracted from the Irish Cattle Breeding Federation database. As average EBI in Ireland at the time of analysis (February 2024) was €160, calves were split into three EBI categories: average, high, and elite (Table 2).

As Irish dairy calves are, on average, smaller than other strains in other countries (mature bodyweight approximately 550 kg; Murphy et al., 2023), instead of using calf birthweight, to make our results more transferable we instead calculated calf birthweight as a proportion of projected mature bodyweight. Each calf's projected mature bodyweight



**Fig. 2.** Temperature (A; °C), relative humidity (B: %), and temperature-humidity index (C) of the calf shed during the study period. The black line represents the average daily temperature, while the grey shaded area represents the temperature or relative humidity range for each day (top and bottom of the shaded area represent the minimum and maximum temperature or relative humidity that was recorded that day). The solid red line represents the high critical temperature threshold for a calf's thermo-neutral zone (Drackley, 2008). The dashed line represents the low critical temperature threshold for a calf's thermo-neutral zone, when the calf is under 21 d old (Drackley, 2008). The dotted line represents the low critical temperature threshold for a calf's thermo-neutral zone, when the calf is over 21 d old (Drackley, 2008). The average temperature and relative humidity between two different data loggers, one placed under the canopy in a calf group pen and one placed at the front of a pen in the feeding area, are presented. Behaviour observations were made each Saturday during the study period, which are the dates denoted at the bottom on the graphs.

could be calculated from its value for the Maintenance sub-index of the EBI; a value of €0 for Maintenance gives a projected mature bodyweight of 641 kg, with each €5 increase or decrease resulting in a lower or higher projected mature bodyweight, respectively (Teagasc, 2024). Projected mature bodyweight (kg) was thus calculated using the formula: projected mature bodyweight =  $(-5 * \text{Maintenance}) + 641$ . Each calf's birthweight as a percentage of their projected mature bodyweight was then calculated ( $(\text{birthweight} / \text{projected mature bodyweight}) * 100$ ) and categorised into three categories (Table 2).

Quality of the colostrum the calf received (all colostrum given to calves measured  $\geq 22\%$  on a Brix refractometer; median = 24.2%) was categorised into three categories (Table 2). As calves were given a

standardised amount of colostrum (3 L), we calculated how much colostrum was given as a proportion of their birthweight ( $(\text{colostrum amount} / \text{birthweight}) * 100$ ), which was then categorised into three categories (Table 2). Whether the calf received colostrum from its dam or another cow was included as a factor (colostrum source), as was dam parity (primiparous or multiparous).

A composite, 2-level calving difficulty score was created based on calving data recorded by the farm staff, where 0 = no assistance required, single birth, and normal presentation and 1 = assistance required and/or multiple-calf birth and/or backwards presentation. No calves on the experiment were born by Caesarean section. Weekly ADG (ADGw) and ADG from birth (ADGb) were calculated based on the

values from the preceding and following weeks. Both ADGw and ADGb were categorised into three categories, based on their respective average values for each (Table 2).

#### 2.8.4. Behaviour proportions

Behaviour proportions were independently calculated for each calf on each behaviour scoring date. The proportion of time spent lying was calculated by taking the number of observations the calf spent lying divided by the total number of scans on that scoring date. The number of scans each calf spent out of frame on each behaviour scoring date was calculated by summing the number of occurrences. Activity-based behaviours were calculated as the number of occurrences of that particular behaviour divided by the total number of scans where the calf was visible.

#### 2.8.5. Exclusion criteria

Calves were excluded from the behaviour analysis based on their health status, as the aim of this study was not to investigate the behaviour of sick calves:

- If a calf received an antibiotic or NSAID injection, the behaviour observation immediately preceding and following the antibiotic treatment were removed. If a calf received the antibiotic treatment on the behaviour scoring date, only that behaviour observation was excluded.
- If a calf received an antibiotic or NSAID injection due to dehorning (1 calf), only the behaviour event following the treatment was excluded (not the behaviour observation preceding the injection). For all other calves, the behaviour observation following the dehorning event was included in the dataset on the basis that it was a normal management practice and occurred 4 d previously.
- If a calf was removed from their group pen (for any length of time) and placed into an individual or group sick pen, then the behaviour observations immediately preceding and following the movement were removed (these mostly coincided with antibiotic/NSAID injections).
- If a calf had a high health score (cumulative score >7 and/or a score of 3 in  $\geq 1$  category and/or a score of 2 in  $\geq 3$  categories), then the behaviour observation closest to the health scoring date was removed, regardless of whether it was before or after.
- After all of the exclusions were made, if an individual calf only had  $\leq 3$  behaviour observations remaining (out of 8 total) then that calf was removed from the analysis altogether (5 calves removed, leaving 39 calves included in the analysis). This left 15 calves with 8 observations, 15 calves with 7 observations, three calves with 6 observations, five calves with 5 observations, and one calf with 4 observations.

## 2.9. Statistical analysis

### 2.9.1. Behaviour prevalence data

Behaviour data were expressed as a proportion of total (visible) scans. The mean, minimum, maximum, and standard deviation of each of the 20 different behaviours for each week of the observation period were calculated in SAS (PROC MEANS). For ease of understanding, all proportions were converted to percentages (percentage of total scans) for the tables and graphs. Due to their low number of occurrences, three behaviours (pacing, tongue-rolling, and urine drinking/orally manipulating the prepuce/cross sucking) were combined to create an abnormal behaviour category.

### 2.9.2. Analysis

Analysis of each behaviour or behaviour category was done in SAS using generalised linear mixed models (PROC GLIMMIX) with a binomial distribution, a logit link function, and the Kenwood-Rogers method of determining denominator degrees of freedom. The model consisted of

the fixed effect of week, and then animal-based factors were kept in the model based on significance in a two-step procedure. In step 1, for each behaviour, each animal-based factor (described above and in Table 3) was tested in an interaction effect with week and separately as a fixed effect. In step 2, all animal-based factors (whether alone or as an interaction) that had a p-value <0.10 were then added to a multi-factorial model for that behaviour. Fixed effects in these multi-factorial models with p-values >0.10 were then removed using backwards selection (the effect with the highest p-value was removed each time) until all remaining effects (other than week) had a p-value <0.10 (see Table 3 for the fixed effects included in the model for each behaviour). No interaction with week was included in the final model for any behaviour as none of the interactions were found to have a p-value <0.10. The random effects were calf nested within pen and the residual. Week was also included as a random repeated measure, acting upon the subject of the calf, using a first-order autoregressive lag 1 or compound symmetry covariance structure (Table 3). In the final models, p-values <0.05 were considered significant. Due to the nature of the logit function used in the generalised linear mixed models, only raw statistical means ( $\pm$  standard deviation) are reported throughout.

### 2.9.3. Checking for confounding factors

For animal-based factors that were obviously confounded (i.e. ADGw and ADGb; minT and avgT), only one was chosen to be included in the final model for each behaviour using backwards selection; of the obviously confounded variables, whichever one had the lowest p-value was selected to remain in the model. To check the potential confounding of ADG and breed, the non-categorised ADG from birth and weekly ADG were analysed in a generalised linear mixed model (as described in Section 2.9.2), using all of the other animal-based factors as fixed effects. The fixed effects were removed one-by-one using backwards selection; the fixed effect with the highest p-value was removed each time, until all remaining variables (other than week) had  $P < 0.10$ . Breed was removed from the weekly ADG models before all variables had  $P < 0.10$ , signalling that breed and ADGw were not confounded. Breed was significantly correlated to ADG from birth; therefore, only one of the two was selected to be in the final model for each behaviour, using the method described above.

## 3. Results

### 3.1. Temperature and relative humidity

In the calf shed during the study period (29 January to 16 April, 2022), average temperature was  $10.3 \pm 2.43^\circ\text{C}$ , average relative humidity was  $82.6 \pm 8.13\%$ , and average THI was  $51.1 \pm 4.08$  (average daily values can be found in Fig. 2). On behaviour scoring days (Saturdays), the average temperature in the calf shed was always below the lower critical temperature of the TNZ for calves under 21 d of age ( $15^\circ\text{C}$ ; Drackley, 2008) and was always above the lower critical temperature of the TNZ for calves over 21 d of age ( $5^\circ\text{C}$ ; Drackley, 2008).

### 3.2. Prevalence of behaviours in healthy calves

The statistical prevalence ( $\pm$  standard deviation) of each behaviour per week is presented in Table 4 and Fig. 3.

### 3.3. Effect of age and other variables on normal pre-weaned calf behaviour

Due to the nature of the backwards selection used in the analysis, not all variables were included in each behaviour model (see Table 3 for which variables were included in each behaviour model); therefore, we only report on the variables that were included in each model for each behaviour.

**Table 3**

Covariance structure and fixed effects included in the generalised linear mixed models (SAS; PROC GLIMMIX) used to analyse the proportion of time calves spent performing specific behaviours. Week was always included in each behaviour model as a repeated measure, regardless of its significance. Each covariate was tested individual as a fixed effect and in an interaction with week for each behaviour model. Covariates where  $P < 0.10$  were then included in the model, and were removed if  $P > 0.10$ , one at a time using backwards selection, until all included fixed effects had  $P < 0.10$ . No interactions were included in the final model. The covariance structure used for the repeated measure in each model is also included. '✓' denotes that the variable was included in the model ( $P < 0.05$ ) and '✓\*' denotes that the variable was included in the model but not a significant effect ( $P > 0.05$ ).

Behaviour model	Covariance parameter	Week	ADGb	ADGw	Breed	Calving score	Colostrum quality	Colostrum source	Dam parity	avgT	minT
lying	ar(1)	✓	-	-	✓	-	✓*	-	-	-	✓
abnormal	cs	✓*	-	-	-	-	-	-	✓	-	-
drinking milk	cs	✓*	-	-	-	-	-	-	-	-	-
defecate & urinate	cs	✓*	-	-	-	-	-	✓*	-	-	-
drinking water	ar(1)	✓	-	-	-	-	-	-	-	-	✓
eating bedding	ar(1)	✓	-	-	-	-	-	✓	✓	-	✓
eating concentrate	ar(1)	✓	-	✓*	✓*	-	-	✓	-	-	✓
eating forage	cs	✓	-	-	-	-	-	-	-	-	✓
grooming	ar(1)	✓*	-	-	✓*	-	-	-	-	-	-
oral manipulation of pen structure	ar(1)	✓	-	-	-	-	-	-	-	-	✓
play	ar(1)	✓	-	✓	-	-	-	-	✓	-	-
ruminant / chewing	ar(1)	✓	-	-	-	-	-	✓	-	✓	-
social interaction	ar(1)	✓*	-	✓	-	✓	-	-	-	-	✓*
sniffing	ar(1)	✓*	-	-	-	-	-	✓*	-	✓	-
stretch/ rub/ scratch	ar(1)	✓*	-	-	-	-	-	-	-	-	✓
walking	ar(1)	✓*	✓*	-	-	-	-	-	-	-	✓

**Table 4**

Average prevalence (%) of calf behaviours (mean  $\pm$  standard deviation) from scan sampling observations. Calves were scanned every 10 minutes over a 24 h period (144 total scans) once a week for 8 weeks. Activity behaviours were calculated out of the total number of scans – the number of out of frame observations for each individual calf at each scoring period (each week). Cross-suckling represents the behaviour category urine drinking/orally manipulate the prepuce/cross-suckling. Abnormal represents the combination of tongue-rolling, pacing, and urine drinking, cross suckling, and oral manipulation of prepuce. Dehorning occurred at an average age of  $17 \pm 10.7$  d (0–6 d old = 0 calves; 7–13 d = 16 calves; 14–20 d = 22 calves; 21–27 d = 4 calves; 28–34 d = 0 calves; 35–41 d = 2 calves).

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	P-value
Out of Frame	3.9 $\pm$ 3.50	3.1 $\pm$ 3.23	4.2 $\pm$ 3.79	6.8 $\pm$ 5.86	6.6 $\pm$ 5.31	6.6 $\pm$ 5.12	7.3 $\pm$ 5.02	7.4 $\pm$ 5.49	7.3 $\pm$ 6.16	NA
<b>Behaviours</b>										
Drinking milk	0.4 $\pm$ 0.48	0.7 $\pm$ 0.62	0.6 $\pm$ 0.86	0.4 $\pm$ 0.58	0.6 $\pm$ 0.64	0.5 $\pm$ 0.62	0.5 $\pm$ 0.61	0.5 $\pm$ 0.48	0.6 $\pm$ 0.75	0.136
Abnormal	0.6 $\pm$ 0.71	0.7 $\pm$ 1.09	0.7 $\pm$ 1.22	0.9 $\pm$ 1.05	0.6 $\pm$ 0.93	1.0 $\pm$ 1.47	0.8 $\pm$ 1.27	0.9 $\pm$ 1.19	0.4 $\pm$ 0.60	0.713
Cross-suckling	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.14	0.0 $\pm$ 0.17	0.2 $\pm$ 0.50	0.6 $\pm$ 1.40	0.4 $\pm$ 0.77	0.6 $\pm$ 1.12	0.3 $\pm$ 0.52	-
Pacing	0.0 $\pm$ 0.18	0.0 $\pm$ 0.16	0.0 $\pm$ 0.12	0.0 $\pm$ 0.12	0.0 $\pm$ 0.00	0.0 $\pm$ 0.16	0.0 $\pm$ 0	0.0 $\pm$ 0.16	0.1 $\pm$ 0.19	-
Tongue-rolling	0.5 $\pm$ 0.68	0.7 $\pm$ 1.08	0.6 $\pm$ 1.12	0.9 $\pm$ 1.07	0.5 $\pm$ 0.81	0.4 $\pm$ 0.77	0.4 $\pm$ 0.82	0.3 $\pm$ 0.68	0.1 $\pm$ 0.19	-
Social interaction	5.7 $\pm$ 3.17	5.8 $\pm$ 2.8	5.5 $\pm$ 3.40	6.8 $\pm$ 4.53	5.2 $\pm$ 3.00	5.7 $\pm$ 3.44	4.7 $\pm$ 3.64	4.3 $\pm$ 2.87	3.9 $\pm$ 2.34	0.100
Sniffing	1.2 $\pm$ 0.73	1.6 $\pm$ 1.27	1.8 $\pm$ 1.55	2.0 $\pm$ 1.82	1.7 $\pm$ 1.58	2.1 $\pm$ 1.79	2.2 $\pm$ 2.00	2.7 $\pm$ 1.60	3.0 $\pm$ 2.64	0.058
Defecation and urination	1.7 $\pm$ 0.95	2.4 $\pm$ 1.24	2.2 $\pm$ 1.41	2.5 $\pm$ 1.36	2.6 $\pm$ 1.82	2.6 $\pm$ 1.74	2.3 $\pm$ 1.65	1.9 $\pm$ 1.29	1.8 $\pm$ 1.60	0.631
Grooming	3.6 $\pm$ 1.48	3.2 $\pm$ 1.86	2.5 $\pm$ 1.48	2.8 $\pm$ 1.56	2.4 $\pm$ 1.57	2.3 $\pm$ 1.29	2.9 $\pm$ 1.97	3.2 $\pm$ 1.62	2.8 $\pm$ 2.24	0.085
Stretching, rubbing, scratching	0.4 $\pm$ 0.68	0.5 $\pm$ 0.62	0.4 $\pm$ 0.59	0.3 $\pm$ 0.46	0.4 $\pm$ 0.69	0.3 $\pm$ 0.62	0.6 $\pm$ 0.64	0.7 $\pm$ 0.94	0.3 $\pm$ 0.39	0.421
Walking	1.8 $\pm$ 1.10	1.6 $\pm$ 1.14	1.6 $\pm$ 1.21	1.6 $\pm$ 1.14	1.5 $\pm$ 1.05	2.0 $\pm$ 1.54	1.5 $\pm$ 1.26	1.8 $\pm$ 1.13	2.4 $\pm$ 1.68	0.657

### 3.3.1. Posture

The proportion of time a calf spent lying was affected by age (Fig. 3A), breed ( $P = 0.056$ ), colostrum quality ( $P = 0.018$ ), and minT ( $P < 0.001$ ). Lying time was highest in week 1 and had decreased by week 3 where it remained similar from weeks 3–9 (Fig. 3A). Calves fed colostrum of  $<23$  % quality ( $73.1 \pm 6.46$  %) spent less time ( $P = 0.022$ ) lying than calves given colostrum of  $>25$  % quality ( $75.8 \pm 6.03$  %); calves given moderate quality (23–25 %) colostrum did not differ from either ( $74.9 \pm 5.04$  %). Calves spent less time ( $P < 0.001$ ) lying when minT were  $>6^\circ\text{C}$  ( $72.3 \pm 5.78$  %), compared to when minT were  $<4^\circ\text{C}$  and  $4$ – $6^\circ\text{C}$ , which were similar ( $76.0 \pm 5.45$  %).

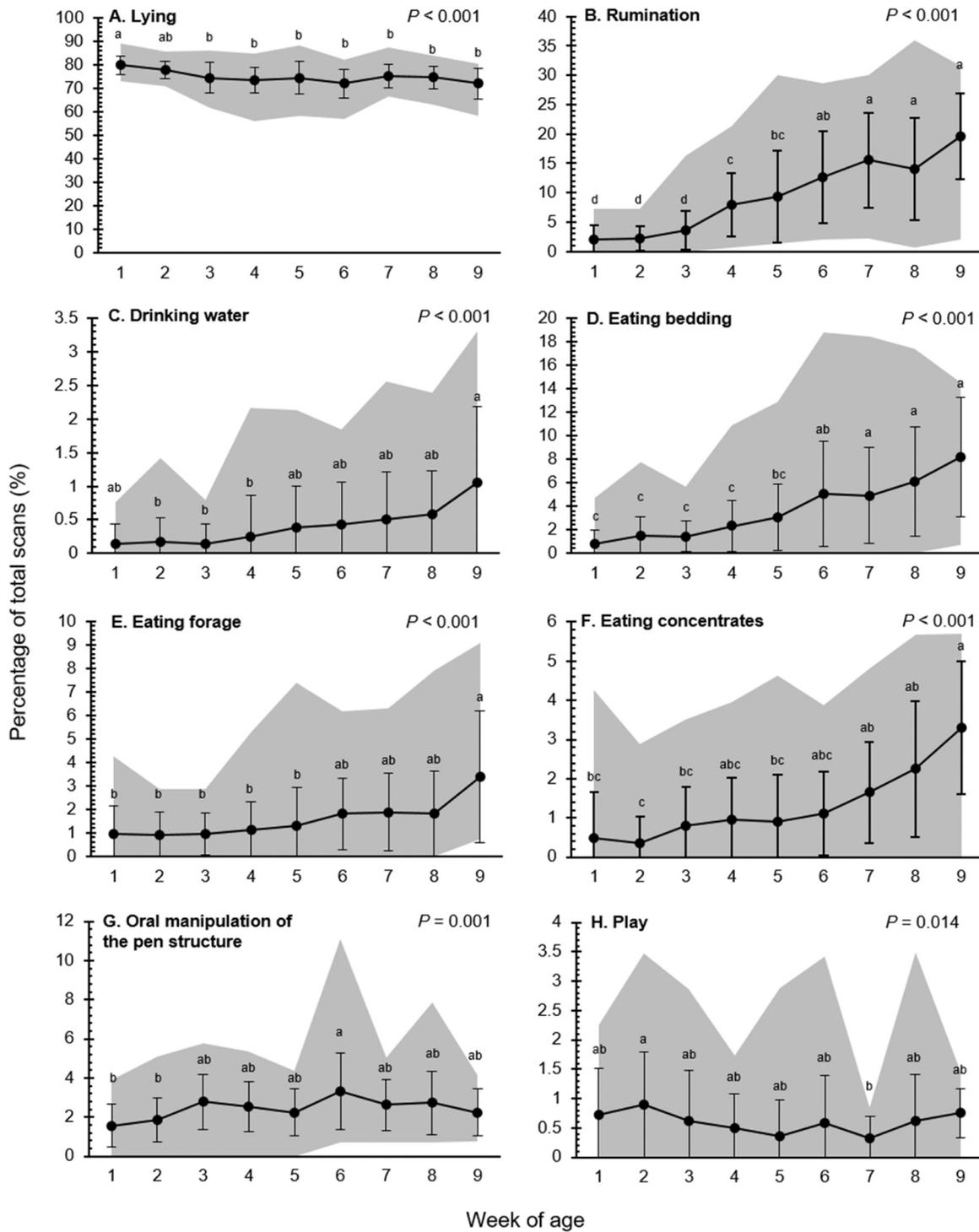
### 3.3.2. Activity-based behaviours

The proportion of time calves spent ruminating was affected by age (week; Fig. 3B), colostrum source ( $P = 0.001$ ), and avgT ( $P = 0.025$ ). Rumination time was consistently low for weeks 1–3, but had increased by week 4 (21–27 d of age); rumination time in weeks 7–9 was similar and higher than the rest of the weeks (except week 6; Fig. 3B). Calves given colostrum from their dam ( $14.3 \pm 10.51$  %) spent more time ruminating ( $P < 0.001$ ) than calves given colostrum from other cows ( $8.6 \pm 7.89$  %). Calves spent less time ruminating when avgT were

$9$ – $11^\circ\text{C}$  ( $7.7 \pm 7.32$  %) compared to when avgT were  $<9^\circ\text{C}$  ( $9.8 \pm 7.81$  %;  $P = 0.025$ ); avgT were  $>11^\circ\text{C}$  ( $9.8 \pm 9.43$  %) rumination time did not differ.

There was no effect of calf age ( $P = 0.136$ ) on the proportion of time spent drinking milk (Table 4), and it was not affected by any other variable (Table 3). The proportion of time calves spent drinking water was affected by age (Fig. 3C) and minT ( $P = 0.019$ ). Calves spent more time drinking water in week 9 compared to weeks 2, 3, and 4 (Fig. 3C). The minT categories did not differ significantly in the post-hoc analysis.

The proportion of time calves spent eating bedding was affected by age (Fig. 3D), colostrum source ( $P = 0.032$ ), dam parity ( $P = 0.013$ ), and minT ( $P < 0.001$ ). The proportion of time spent eating bedding increased over time (Fig. 3D); calves spent less time eating bedding in weeks 1–4 and had increased by week 6; time spent eating bedding was highest during weeks 7–9. Calves fed colostrum from their dam ( $5.5 \pm 4.75$  %) spent more time ( $P = 0.004$ ) eating bedding than calves fed colostrum from another cow ( $3.2 \pm 3.63$  %). Calves born from primiparous dams ( $4.0 \pm 3.78$  %) spent more time ( $P = 0.004$ ) eating bedding than calves from multiparous dams ( $2.7 \pm 3.82$  %). Calves spent more time eating bedding when minT were  $>6^\circ\text{C}$  ( $4.0 \pm 4.78$  %) compared to when minT were  $<4^\circ\text{C}$  ( $2.7 \pm 2.87$  %;  $P = 0.003$ ) and  $4$ – $6^\circ\text{C}$  ( $3.5 \pm 3.33$  %;  $P =$



**Fig. 3.** Average prevalence (%) of each calf behaviour (mean  $\pm$  standard deviation) from scan sampling observations made over time during the pre-weaning period in group-housed dairy calves. Calves were scanned every 10 minutes over a 24 h period (144 total scans) once a week for 8 weeks. Lying was calculated out of the total number of scans. Activity behaviours were calculated out of the total number of scans – the number of out of frame observations for each individual calf during each scoring period (each week). P-values were generated through generalised linear mixed models, using week as the fixed effect. The grey bands represent the range in observations for each week (minimum to maximum) and the error bars represent standard deviation. Dehorning occurred at an average age of  $17 \pm 10.7$  d (0–6 d old = 0 calves; 7–13 d = 16 calves; 14–20 d = 22 calves; 21–27 d = 4 calves; 28–34 d = 0 calves; 35–41 d = 2 calves).

0.045), which were similar.

There were effects of age (Fig. 3E) and minT ( $P = 0.002$ ) on the proportion of time calves spent eating forage. Time eating forage increased over the weeks, with calves in week 9 spending more time eating forage than calves in weeks 1–5 (Fig. 3E). During days when minT were  $<4^{\circ}\text{C}$  ( $0.8 \pm 0.91\%$ ), calves spent less time eating forage than on

days when minT were  $4\text{--}6^{\circ}\text{C}$ ; ( $1.7 \pm 1.78\%$ ;  $P = 0.002$ ) and  $>6^{\circ}\text{C}$  ( $1.5 \pm 1.57\%$ ;  $P = 0.005$ ).

There were effects of age (Fig. 3F), colostrum source ( $P = 0.044$ ), and minT ( $P = 0.045$ ) on the proportion of time calves spent eating concentrates. Calves spent more time eating concentrates as they aged (Fig. 3F); time spent eating concentrates was highest in week 9 and

lowest in week 2. Calves that received colostrum from their dam ( $2.0 \pm 1.71\%$ ) spent more time ( $P = 0.044$ ) eating concentrates than calves that received colostrum from another cow ( $1.1 \pm 1.36\%$ ). Calves spent more time ( $P = 0.028$ ) eating concentrates when minT were  $>6^{\circ}\text{C}$  ( $1.4 \pm 1.49\%$ ) compared to when they were  $<4^{\circ}\text{C}$  ( $0.8 \pm 1.11\%$ ); when minT were  $4\text{--}6^{\circ}\text{C}$  ( $1.3 \pm 1.43\%$ ) did not differ from either.

There was no effect of age on the proportion of time spent performing abnormal behaviours (Table 4), but there was an effect of dam parity ( $P < 0.001$ ). Calves born from primiparous cows ( $0.6 \pm 1.00\%$ ) spent less time ( $P < 0.001$ ) performing abnormal behaviours compared to calves born from multiparous cows ( $1.1 \pm 1.26\%$ ). The proportion of time calves spent orally manipulating the pen structure was affected by age (Fig. 3G) and minT ( $P = 0.037$ ). Calves spent more time orally manipulating the pen structure during week 6 compared to weeks 1 and 2 (Fig. 3G). Calves spent more time orally manipulating the pen structure when minT were  $>6^{\circ}\text{C}$  ( $2.8 \pm 1.66\%$ ) compared to when minT were  $4\text{--}6^{\circ}\text{C}$  ( $2.3 \pm 1.34\%$ ;  $P = 0.037$ ); minT  $<4^{\circ}\text{C}$  ( $2.5 \pm 1.29\%$ ) were similar to both.

There were effects of age (Fig. 3H), dam parity ( $P = 0.001$ ), and ADGw ( $P = 0.014$ ) on the proportion of time calves spent playing. Calves spent more time playing in week 2 compared to week 7 (Fig. 3H). Calves from primiparous dams ( $0.7 \pm 0.81\%$ ) spent more time ( $P = 0.005$ ) playing than calves from multiparous dams ( $0.4 \pm 0.53\%$ ). Calves with ADGw  $<0.4\text{ kg/d}$  ( $0.5 \pm 0.64\%$ ) spent less time playing than calves with ADGw  $>0.6\text{ kg/d}$  ( $0.7 \pm 0.80\%$ ;  $P = 0.015$ ), calves with ADGw  $0.4\text{--}0.6\text{ kg/d}$  ( $0.6 \pm 0.75\%$ ) were similar ( $P = 0.871$ ) to calves with ADGw  $>0.6\text{ kg/d}$  and tended ( $P = 0.053$ ) to spend more time playing than calves with ADGw  $<0.4\text{ kg/d}$ . The proportion of time calves spent socially interacting was not affected by age (Table 4), but was affected by calving score ( $P = 0.032$ ) and ADGw ( $P = 0.035$ ). Calves that had a normal calving (no issues;  $1.9 \pm 1.53\%$ ) spent less time ( $P = 0.038$ ) socially interacting than calves that had a difficult calving ( $2.4 \pm 2.07\%$ ). Calves with ADGw  $>0.6\text{ kg/d}$  ( $2.6 \pm 1.97\%$ ) spent more time socially interacting than calves with ADGw of  $0.4\text{--}0.6\text{ kg/d}$  ( $1.6 \pm 1.31\%$ ); calves with ADGw  $<0.4\text{ kg/d}$  ( $1.8 \pm 1.58\%$ ) did not differ from either.

There was an effect of avgT ( $P = 0.026$ ) on the proportion of time calves spent sniffing (Table 4). Calves spent more time ( $P = 0.013$ ) sniffing when avgT were  $9\text{--}11^{\circ}\text{C}$  ( $6.8 \pm 3.38\%$ ) compared to when avgT were  $>11^{\circ}\text{C}$  ( $4.5 \pm 2.76\%$ ); avgT  $<9^{\circ}\text{C}$  ( $5.2 \pm 3.67\%$ ) did not differ from either. The proportion of time calves spent defecating and urinating was not significantly affected by age (Table 4) or any animal-based factor (Table 3; animal-based factors that were included in these respective models were  $P < 0.10$ , but not  $P < 0.05$ ). There was a tendency for the proportion of time calves spent grooming to be affected by age (Table 3) and was not affected by any other animal-related factors (Table 3). There was no effect of age on the proportion of time calves spent scratching, rubbing, or stretching (Table 4), but there was an effect of minT ( $P = 0.022$ ). The proportion of time spent scratching, rubbing, and stretching was less when minT were  $<4^{\circ}\text{C}$  ( $0.3 \pm 0.49\%$ ) compared to minT between  $4$  and  $6^{\circ}\text{C}$  ( $0.6 \pm 0.76\%$ ;  $P = 0.045$ ); calves did not differ from either when minT  $>6^{\circ}\text{C}$  ( $0.4 \pm 0.58\%$ ). The proportion of time a calf spent walking was not affected by age (Table 4) but was affected by ADGb ( $P = 0.038$ ) and minT ( $P = 0.001$ ). Calves with ADGb  $<0.4\text{ kg/d}$  ( $1.2 \pm 0.91\%$ ) spent more time walking than calves with ADGb from  $0.4$  to  $0.6\text{ kg/d}$  ( $1.8 \pm 1.28\text{ kg/d}$ ;  $P = 0.030$ ); calves with ADGb  $>0.6\text{ kg/d}$  ( $1.8 \pm 1.24\text{ kg/d}$ ) tended ( $P = 0.071$ ) to differ from calves with ADGb  $<0.4\text{ kg/d}$ . Calves spent more time walking when minT were  $>6^{\circ}\text{C}$  ( $2.0 \pm 1.40\%$ ) compared to when minT were  $<4^{\circ}\text{C}$  ( $1.5 \pm 1.20\%$ ;  $P = 0.012$ ) or between  $4$  and  $6^{\circ}\text{C}$  ( $1.5 \pm 1.05\%$ ;  $P = 0.010$ ).

#### 4. Discussion

The observation and quantification of normal calf behaviour was explored in this experiment, and led to our establishment of a behaviour

baseline for group-housed, pre-weaned dairy calves, reared under conventional management conditions in Ireland. Our results can be used as a comparison in future studies and can help guide areas where calf welfare and management can be improved.

##### 4.1. Effect of age on calf behaviour

In this study, calves spent the most time lying in week 1 ( $\sim 80\%$ ) and had reduced their lying time by week 3; from then they remained relatively stable for the rest of the study ( $\sim 74\%$ ). This was expected, as it is generally accepted that young calves spend the majority of their time lying (Vitale et al., 1986; Hutchison et al., 1962), and lie down for a larger proportion of their day compared to 6-month-old calves ( $38\text{--}47\%$ , season-dependent; Tripon et al., 2014) or mature cattle ( $38\text{--}50\%$ , system-dependent; Tucker et al., 2021). The difference observed in lying time between pre-weaned calves ( $<12$  weeks) and older calves/cows can primarily be attributed to the difference in sleep required; calves spend the majority of their time lying down sleeping (calves aged 2–3 d slept around 20 h/d; Hänninen et al., 2008a), but this has been shown to decrease with age (calves aged 97 d were asleep 25 % of all observations, approximately 6 h/d; Hänninen et al., 2008b).

Calves increased the proportion of time they spent ruminating, eating concentrates, drinking water, eating bedding, and eating forage as they aged. The observed changes in feeding behaviour were characteristic of rumen development, a critical process in calf development. Calves are born with a non-functional rumen and thus initially rely on milk digestion via the abomasum to meet their nutrient needs (Khan et al., 2016); the first rumination event typically occurs from 2 to 3 weeks old and rumination time increases from there (Swanson and Harris, 1958; Noller et al., 1959; Wang et al., 2022). In this study, the proportion of time calves spent ruminating and consuming solid feed (eating bedding, forage, and concentrates) were low in weeks 1–3, similar to what has been found previously (Swanson and Harris, 1958; Noller et al., 1959; Wang et al., 2022). From weeks 4–6, calves gradually increased the amount of time they spent ruminating and consuming solid feed, likely because calves were still nutritionally reliant on milk (at maximum amount of 6 L/d). However, gradual weaning started after 6 weeks (42 d; reduction in milk  $\sim 0.14\text{ L/d}$  from 42 to 54 d), which coincided with the observed increase in rumination and solid feed consumption, thus emphasising the critical importance of weaning gradually.

Calves likely have an innate need to consume solid feed to initiate rumen development. The solid feeds provided in this study were concentrates and barley straw/hay, while barley straw was used as the bedding material. Calves in this study spent more time eating bedding than they did eating forage. For their behaviour to be classified as eating forage, calves had to stand (unless they were eating it while laying directly under the feeder) and often were observed clustered around the forage feeder, in line with social facilitation. In contrast, calves could eat bedding while standing or lying down, from almost anywhere in the pen. Calves were also frequently observed eating bedding while sniffing and slowly walking across the lying area of the pen, in a motion that mimicked grazing (Werner et al., 2018). This suggests that their motivation to eat bedding may be spurred by their curiosity, by an innate need to consume roughage, or by a combination thereof. As such, calves may benefit from additional or alternative methods of forage provision, such as outdoor access to pasture, which may also help to meet these and other behavioural needs. In addition, if calves are consuming bedding, it should be kept clean and dry.

The two other behaviours that changed as the calves aged were oral manipulation of the pen structure and play. Oral manipulation of the pen structure is considered a normal, exploratory behaviour (Bertelsen and Jensen, 2019), but also may be considered an abnormal behaviour when expressed beyond normal levels of exploration (i.e., an indication of frustration or lack of satiety; Webb et al., 2015). The proportion of the other measured abnormal behaviour was very low, as was expected with

the selected group of calves. Oral manipulation of the pen structure peaked during week 6, which coincided with the start of gradual weaning and likely reflected the calves' frustration with their reduced milk intake. Previous studies have shown that calves on lower milk allowances perform fewer play behaviours (Das et al., 2000; Krachun et al., 2010) and calves on more restricted diets play more after eating (Vitale et al., 1986; Das et al., 2000). Conversely, the increase in oral manipulation of the pen structure may be due to calves switching from locomotor to object play. In calves, play can be classified into three sub-types: social, object, and locomotor (Ahloy-Dallaire et al., 2018). Locomotor play (i.e. running, jumping, mounting, and head butting) can be negatively affected by low space allowances (Jensen et al., 1998; Jensen and Kyhn, 2000; Færevik et al., 2008). Although all replicates (replicates 1 and 2 = 2.13 m<sup>2</sup>/calf; replicate 3 = 3.01 m<sup>2</sup>/calf) had a space allowance over the minimum required (1.5 m<sup>2</sup> required for calves <150 kg, 1.7 m<sup>2</sup> recommended; European Union, 2009), 20 m<sup>2</sup>/calf is required for them to express their full extent of locomotor play in group-housing systems (European Food Safety Authority Panel on Animal Health and Animal Welfare et al., 2023); this is unattainable in most indoor systems. The amount of space required to exhibit locomotor play may also increase with growth. As locomotor play may have been restricted by space as calves grew, in order to express their play behaviour urge, they may have switched from locomotor to object play and this may have been classified as oral manipulation of the pen structure in our ethogram (i.e., a calf excessively orally manipulating a loose chain on a gate). This suggests calves may benefit from environmental enrichment in their pens (i.e., designated objects to play with; Zhang et al., 2021).

#### 4.2. Effect of environmental temperature on calf behaviour

Environmental temperature influenced calf behaviour to almost the same extent as age (and thus expected rumen development); the cold minT (<4°C) was linked to a generic decrease in activity (i.e. feeding behaviours and walking). Low temperatures often result in dairy cows and calves modifying their behaviour by seeking out shelter or warmer areas (Borderas et al., 2009; Hänninen et al., 2003; Sawalrah et al., 2016), increasing their lying time (Tripon et al., 2014; Tucker et al., 2021), huddling together while lying (Bøe and Havrevoll, 1993), and adopting a more tucked or nestled lying posture to reduce their surface area (Hänninen et al., 2003; Lago et al., 2006), all of which help to reduce the lower critical TNZ threshold (Webster, 1984). It appears that during cold temperatures, the calves' need to moderate their own body temperature might outweigh their needs to be active and perform social or feeding behaviours. Colder temperatures may also reduce appetites (Arnold, 2020). Therefore, the risk low temperatures may have on calf behaviour and rumen development, and thus growth, should be emphasised to farmers. This is especially relevant in areas with year-round calving and areas with seasonal-calving during colder weather. During extended periods of cold temperatures, farmers may need to wean calves later to promote solid feed intake pre-weaning or improve their temperature management (i.e., providing more bedding material or installing heaters).

#### 4.3. Effect of animal-based factors on calf behaviour

As mentioned previously, some animal-based factors seemed to have caused some variation in the proportion of time calves spent performing specific behaviours. Calves with higher ADGw performed more play and social interactions; this may indicate that they had more energy available to play and interact, which allowed them to be in more positive affective states (Altmann and Gotlib, 1988; Ahloy-Dallaire et al., 2018). Colostrum source affected behaviour, as calves provided with colostrum from their own dam spent more time ruminating, eating concentrates, and eating forage. Calves given maternal colostrum are provided with maternal leukocytes and cytokines (Godden et al., 2019), which have

been suggested to be better at priming the mucosal innate immune system in piglets (Bandrick et al., 2011). This may promote faster rumen development, by decreasing the time required to reach a stable commensal population within the gastrointestinal tract (Amin and Seifert, 2021). Calves with a more difficult calving spent more time socially interacting with other calves. This was not expected, as calving dystocia can cause injuries to the calf (Murray and Leslie, 2013), causing pain and deterring social interactions. Perhaps these calves used tactile social interactions as a method of self-soothing, which has previously been found in calves after disbudding (Adcock and Tucker, 2021). Calves with multiparous dams spent a higher proportion of time performing abnormal behaviours and a lower proportion of time eating bedding and playing than calves born from primiparous dams. Dam parity can influence foetal calf growth and metabolism (Duncan et al., 2023), which might influence future growth and performance. Although this experiment was not set up to explicitly test each of these fixed effects (it was designed to observe and quantify), our results suggest that they did have an effect on behaviour, and thus should be explored further.

## 5. Conclusion

We have observed and quantified how the behaviour of group-housed dairy calves changed during the pre-weaning period, to establish a baseline of normal calf behaviour under conventional management conditions in Ireland. Calves started exploring solid feeds (forage, bedding, and concentrates) immediately after entering the group pens, emphasising that calves should be provided access to solid feeds as soon as possible after birth, as solid feeds help meet calves' behavioural needs and their consumption promotes rumen development and growth. Time spent ruminating and consuming solid feeds increased at the start of weaning, accentuating the importance of a gradual weaning process to allow calves to slowly shift their reliance on milk to solid feed. Calves appeared to have an innate drive to consume forage, and consumed both the provided forage in the feeder and their bedding. Farmers should ensure that forage feeders remain full; if calves are consuming their bedding, it should be kept clean and dry to prevent health issues. Environmental enrichment (i.e., objects for calves to play with) may also improve calf welfare, by increasing play opportunities in group pens and decreasing the performance of negative oral behaviours. Low ambient temperatures (<4°C, below TNZ) caused calves to modify their behaviour by increasing the proportion of time spent lying and decreasing all other activities, highlighting the importance of temperature management in calf housing (i.e., using ventilation, heaters, or sheltered areas to keep the ambient temperature within the calves' TNZ). The behaviour baseline of dairy calves during the pre-weaning period will be useful for future studies to use as a comparison.

#### CRedit authorship contribution statement

**Emer M. Kennedy:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Formal analysis. **Sarah Elizabeth McPherson:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marie McFadden:** Validation, Resources, Investigation. **Laura Webb:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Eddie Bokkers:** Writing – review & editing, Supervision, Conceptualization. **Anna Flynn:** Validation, Investigation.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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