The effect of milk feeding strategy and restriction of meal patterning on behavior, solid feed intake, and growth performance of male dairy calves fed via computer-controlled milk feeders

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ABSTRACT

Dairy calves are often offered milk allowance at a flat rate during the first 6 wk of life, although an initial high allowance followed by a stepwise reduction (step-down strategy) may correspond better to the calves' nutritional needs. The amount of milk offered per feeding is typically constant. However, during natural suckling, the daily number of milk meals (suckling frequency) declines with age, which may reflect increased consumption of solid feeds. Thus, allowing calves to pattern their meals on a computer-controlled feeder by placing less restriction on meal frequency may stimulate dairy calves' ingestion of solid feeds. This experiment investigated the effects of milk feeding strategy and milk feeding frequency on calves' feeding behavior, intake, and growth. Sixty-four male Holstein Friesian calves, purchased from conventional dairy farms, were housed in groups of 8 and fed milk replacer (MR) via computer-controlled milk feeders. From 14 d old, calves were assigned to either a conventional flat-rate milk allowance (CON: 6.5 L/d of MR from d 14 to 42; 4 L/d from d 43 to 49; 2 L/d from d 50 to 56 of age) or to stepwise reduction in milk allowance (STEP: 8 L/d of MR from d 14 to 28; 5 L/d from d 29 to 42; 4 L/d from d 43 to 49; 2 L/d from d 50 to 56 of age). Within each group of 8, 4 calves were randomly allocated to each of 2 milk feeding frequencies, either restricted portion size (RES: maximum milk portion size of 2.3 L/portion) or unrestricted portion size (UNRES). Concentrates, hay, and water were available *ad libitum*. Feeding behavior was recorded via video for 24 h on 26 and 40 d of age. On d 26, where STEP calves were offered 1.5 L/d more milk than CON calves, no difference was seen regarding the time spent eating concentrate (16.9 vs. 20.3

min/d). However, STEP calves spent more time eating concentrate on d 40, where these calves had 1.5 L/d less milk than CON (36.1 vs. 27.2 min/d). Thus, a lower daily milk allowance stimulated feeding on concentrate d 40, but not d 26. As predicted, UNRES calves spent more time eating concentrate (27.6 vs. 21.9 min/d) and more time eating hay (38.4 vs. 30.0 min/d) than RES calves. However, higher appetite for solid feeds by UNRES calves may not be explained by milk intake *per se*. Rather, this is likely due to their opportunity to have larger milk meals, which appeared to increase their appetite for milk (as indicated by longer time spent in the milk feeder; 64.0 vs. 48.8 min/d) as well as for solid feeds. Among CON calves, the UNRES treatment resulted in higher ADG 2 wk postweaning, but not among STEP calves. These findings partially support that relaxing restriction on milk portions helps calves to transition from milk to solid feeds before weaning off milk. However, more research is needed to determine how restriction on milk portions interacts with milk feeding strategy when a higher total milk allowance is offered.

Key words: step-down, feeding frequency, performance, behavior, automatic milk feeder

INTRODUCTION

In Europe and North America, male dairy calves are typically reared for veal or beef in specialized production units. For instance, in Denmark male calves are purchased from dairy herds at 2 to 3 wk of age and reared in specialized rosé veal calf herds. These calves are typically fed a daily milk allowance corresponding to approximately 10% of live weight (LW) until being weaned off milk at 7 to 8 wk of age. This milk allowance is considerably less than dairy calves' *ad libitum* intake, which is close to 20% of LW (de Passillé et al., 2008; de Passillé and Rushen, 2012). Before the age of 3 to 4 wk, the calf has limited capacity to digest solid feeds and relies primarily on nutrients from milk to meet the requirements for maintenance and growth

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(Diaz et al., 2001). It is now recommended that dairy calves this young are offered milk allowances close to ad libitum uptake because this increases ADG preweaning (Khan et al., 2011). For instance, Khan et al. (2007) fed calves a daily milk allowance of 20% of LW for the first 30 d before they stepped down to a conventional allowance of 10% of LW. Compared with calves fed 10% of LW throughout to weaning at 49 d, ADG both pre- and postweaning was higher in step-down calves, who ingested on average 7.7 L/d of milk compared with 4.6 L/d in conventionally fed calves. In replacement heifers, increased milk intake early in life is associated with earlier insemination and increased future milk production (Soberon and Van Amburgh, 2013).

For male dairy calves reared for beef or veal there is less incentive to increase milk allowance, but a higher proportion of the milk may be allocated during the first weeks of rearing. For instance, Daneshvar et al. (2017) fed individually housed male dairy calves a milk allowance of 7 L/d from d 1 to 35 followed by a step-down to 4 L/d, but this did not increase solid feed intake and ADG compared with control calves fed milk at a flat rate of 5.5 L/d. Concentrate intake was higher among pair-housed compared with individually housed calves (De Paula Vieira et al., 2010; Miller-Cushon and DeVries, 2016). In one study, this effect was only found when a high milk allowance was offered (Jensen et al., 2015), and social housing, through social facilitation of solid food intake, may further improve the effect of increasing milk allowance early in the milk feeding period.

During natural suckling, the calf takes larger and fewer milk meals with increasing age (Day et al., 1987), and a similar pattern was found among calves fed milk via a computer-controlled milk feeder when restriction on meal size and frequency was relaxed (Jensen, 2009). Allowing calves to reschedule their allowance into fewer and larger milk meals reduced the time spent at the milk feeder (Jensen, 2009), and may thus help change the calf's focus from milk to solid feeds. However, whether a stepwise reduction in milk allowance affects feeding behavior and solid feed intake has to our knowledge not been investigated. In a setup offering all calves the same total amount of milk replacer (MR), the objectives of the present study were, first, to investigate if feeding an enhanced milk allowance early followed by a stepwise reduction in milk allowance to below conventional levels later, stimulates feeding behavior and solid feed intake and results in higher LW gains compared with a conventional flat-rate milk feeding strategy. A secondary objective was to investigate the effect of placing no restriction on milk portion size and thus relaxing restrictions on milk meal frequency on feeding behavior, intake of solid food, and LW gain.

MATERIALS AND METHODS

The experiment was carried out at the Danish Cattle Research Centre (DKC), Aarhus University Foulum, Denmark, and according to a protocol approved by the Danish Animal Experimentation Inspectorate (2016–15–0201–00975). The experiment included 2 blocks conducted from February to April 2017 and from June to August 2017, respectively. Within each block, the experimental period lasted 56 d (from 14 to 70 d of age).

Experimental Treatments and Design

A total of 64 male Holstein Friesian calves (32 per block) were purchased from 6 different dairy herds (4 herds for block 1 and 5 herds for block 2). Calves were removed from the dam within the first 24 h and were fed first colostrum and whole milk until purchase. Calves were transported to DKC at (mean $\pm$ SE) 10.9 $\pm$ 0.61 and 12.7 $\pm$ 0.78 d of age for block 1 and 2, respectively, and a LW of 47.1 $\pm$ 0.95 and 50.4 $\pm$ 0.85 kg for block 1 and 2, respectively. Within each block of 32 calves, 16 were assigned to each of 2 sub-blocks according to herd of origin, age, and LW (i.e., one sub-block included calves of higher LW and age, whereas the other sub-block included calves of lower LW and age). Within each of these 2 sub-blocks, 8 calves were randomly assigned to each of 2 pens; one pen was randomly assigned to conventional flat-rate milk allowance (**CON**: 6.5 L/d of MR from d 14 to d 42; 4 L/d from d 43 to d 49; 2 L/d from d 50 to d 56 of age), whereas the other pen was assigned to stepwise reduction in milk allowance (**STEP**: 8 L/d of MR from d 14 to d 28; 5 L/d from d 29 to d 42; 4 L/d from d 43 to d 49; 2 L/d from d 50 to d 56 of age; Figure 1). Within each pen of 8 calves, 4 calves were randomly allocated to each of 2 milk feeding frequencies, either restricted (**RES**: maximum milk portion size of 2.3 L) or unrestricted (**UNRES**: maximum milk portion size equal to the daily allowance). Thus, treatment 1 was randomly assigned to pen within sub-block, whereas treatment 2 was randomly assigned and applied via the milk feeder to individual calves within a pen (see Bello et al., 2016 for a discussion of the statistical implications of these 2 ways to assign animals in pens to treatment). Although calves varied in age at arrival, all calves were categorized as 12 d old at the day of arrival. The minimum milk portion size was 1.8 L for all calves. By manipulating the maximum portion size, we controlled milk feeding frequency. All calves in the experiment were offered a total of 224 L of MR throughout the experimental milk feeding period and calves did not receive any MR after d 56 of age.

Table 1. Analyzed (Eurofins A/S, Glostrup, Denmark) nutrient composition of milk replacer (MR), concentrate, and hay for each of the 2 blocks (1 and 2)

Item ¹	MR		Concentrate		Hay	
	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2
DM (%)	96.8	96.2	88.7	89.1	88.6	90.6
CP (% of DM)	22.3	23.8	23.6	22.3	14.3	14.3
CF (% of DM)	21.1	20.9	5.2	4.9	3.7	3.3
Starch (% of DM)			32.6	33.4		
Sugar (% of DM)	27.8	26.4			11.6	11.8
aNDF (% of DM)			16.0	16.2	52.6	50.9
iNDF (g/kg of NDF)			225.0	225.0	211.0	248.0
Ash (% of DM)	6.6	7.4	6.7	6.4	8.5	11.1
SFUC/100 kg	149.5	147.9	90.9	90.9	62.5	58.1
NE (MJ/kg)	11.80	11.67	7.17	7.17	4.93	4.58

¹CF = crude fat; aNDF = neutral detergent fiber; iNDF = indigestible neutral detergent fiber; NE = net energy. Scandinavian Feed Unit for Cattle: 1 SFUC = 7.89 MJ of NE.

Housing, Feeding, and Management

The 32 calves of each block were housed in 4 deep-bedded pens for 8 calves (4.20 m × 5.20 m; 2.7 m²/calf). The pens were located in a 4-sided, roofed, but uninsulated barn. Pen sides were made of tubular metal bars, allowing tactile contact between calves in neighboring pens (the 2 groups of the same sub-block). Each pen had one milk feeding stall with one teat and milk was offered via a computer-controlled milk feeder (Urban Calf Mom, Urban GmbH & Co. KG, Wüstring, Germany).

Throughout, calves were fed MR based on whey, vegetable oil, soy-protein concentrate (HP 110, Hamlet

Protein A/S, Horsens, Denmark), and 13% skim milk powder (e-lac aps, Broby, Denmark; Table 1), mixed at a rate of 140 g/L of water. The computer-controlled milk feeder was accessible throughout the 24 h but was nonfunctional from 0800 to 0900 h due to a pre-programmed cleaning procedure, and the 24 h period started at 0900 h. The feeder was placed at the front of each pen, which also had openings giving access to 2 water bowls, offering water for ad libitum intake. Pelleted concentrate (Kalvestart Hamlet, Vestjyllands Andel, A.m.b.a., Ringkøbing, Denmark; Table 1) was available in a trough while artificially dried grass hay (DLG, Fredericia, Denmark; Table 1) was available in a hayrack, both for ad libitum intake. Fresh straw bedding was added daily.

Procedures at Arrival

Immediately after arrival, all calves were guided to the milk feeder and encouraged to suck the artificial teat, where a portion of 2 L of MR was available. In subsequent visits to the feeder, calves were identified by an electronic ear tag ensuring milk allotment according to treatment. For the first 2 d after arrival, all calves were offered 6.5 L/d of MR in portions of a maximum of 2.3 L. Calves that had ingested less than 4 L during the previous 24 h were guided to the feeder by a stock-person at approximately 0800, 1000, and 1400 h. It was ensured that the calf had the teat in its mouth and that no other calves disturbed the calf for 5 min (see Jensen, 2007). At arrival at DKC (categorized as 12 d of age), all calves were injected 5 mL of vitamin supplement containing vitamin A, D, and E (ADEsan Vet, Boehringer Ingelheim Vetmedica, Ingelheim, Germany), and vaccinated against ringworm (2 mL of Bovilis Ringvac, MSD Animal Health, Copenhagen, Denmark) and bovine respiratory syndrome (5 mL of Bovilis Bovipast

Figure 1. Illustration of the milk allowance of calves assigned to a conventional flat-rate milk allowance (CON) and calves assigned to stepwise reduction in milk allowance (STEP), during the 70 d of the experimental period.

RSP, MSD Animal Health). Calves were re-vaccinated at 28 and 42 d of age. Calves were weighed on the day of arrival and the following day, and an average LW was calculated to determine the initial LW.

Feed Intake and Growth

Daily intake of MR was recorded for individual calves by the computer-controlled milk feeder throughout the experimental period. Concentrate and hay intake was measured per pen (8 calves). The daily intake of hay and concentrates, respectively, were estimated by subtractingorts from the feed provided. For both concentrate and hay, orts were removed daily and replaced with fresh concentrate and hay. Two samples of MR, concentrate, and hay were collected on the first and the last day of the experimental period for each block, pooled within block, and then analyzed for chemical composition per block (Table 1). In addition, the number of rewarded visits for each calf and day was recorded via the computer-controlled milk feeder throughout the experimental period.

The LW of calves was recorded once weekly, except at the start of experimental period (d 14), at weaning (d 56), and at the end of the experimental period (d 70) where calves were weighed on 2 consecutive days to increase accuracy. Average daily weight gain, total DM and net energy intakes, and feed efficiency (feed efficiency = MJ of net energy/kg of LW gain) were calculated for each 2-wk period of the experiment, pre- and postweaning, and across the entire experimental period. The 2-wk periods were selected to follow the changes in milk allowance according to treatment at 28 and 42 d of age.

Calf Health and Recordings

Across the 2 blocks, 4 calves died, or were culled, due to severe respiratory disease, and 1 calf was culled due to severe ear infection. Two of these 5 calves died during the first week of experiment and were replaced with similar age calves born at DKC, and thus the experiment was completed with 61 calves (32 in block 1 and 29 in block 2; group size 7 or 8). However, one calf in block 2 was excluded from the experiment as an outlier due to very low performance (130 g/d of ADG wk 2–8). Thus, the statistical analyses included data from 60 calves.

All calves were monitored daily for general health and rectal temperature was measured if the calf showed disease symptoms. Calves that showed mild symptoms of diarrhea were given coal (Agrochemica Carbon Paste; Agrochemica GmbH, Bremen, Germany) orally

twice daily for minimum 5 d to prevent severe diarrhea and fever. In case of moderate symptoms, calves were bottle-fed 2 L of electrolyte solution (Hydrafeed, Hyprod Skandinavien A/S, Middelfart, Denmark) to prevent dehydration and to stabilize the gastrointestinal tract. In severe cases of diarrhea, calves were, in addition to the oral treatment, injected with 1 mL/40 kg of LW Loxicom (active ingredient: meloxicam; Norbrook, Newry, UK). Calves that had fever and signs of diarrhea or pneumonia were treated with antibiotics [1 mL/15 kg of LW Florkem (active ingredient: florfenicol; Ceva Santé Animale, Libourne, France) or Vetrinnoxin (active ingredient: amoxicillin; Ceva Santé Animale) and 1 mL/40 kg of LW Loxicom (active ingredient: meloxicam)]. All injectable drugs were chosen by the veterinarian and given on the day of diagnosis according to standard veterinary procedures. Calves with diarrhea and respiratory disease were observed in all pens. Six to 8 cases of respiratory disease were observed in each treatment group of 8 calves per block, 7 to 8 cases of oral coal treatment in each treatment group per block, 0 to 4 cases of electrolyte treatment in each treatment group per block, and 0 to 2 cases of antibiotic treatment for diarrhea in each treatment group per block.

Behavior

Four cameras were fitted above each pen (one camera covering the whole pen, one camera over the artificial teat, one camera over the concentrate trough, and one camera over the hay rack) and connected to one digital video recorder. Via these cameras, the behavior described in Table 2 was recorded by one observer using continuous recording (Martin and Bateson, 2017) for 24 h on 26 and 40 d of age [i.e., 2 d before the STEP calves stepped down from 8 to 5 L/d on d 28 of age, and 2 d before all calves stepped down to 4 L/d (Figure 1)].

Statistical Analysis

Milk feeding strategy was applied to pen within sub-block (i.e., pen was the experimental unit). For data that could be collected on individual calves (e.g., behavior, BW, milk intake), calf was the observational unit, whereas for data that could only be collected at pen level (e.g., intake of concentrates and hay), pen was the observational unit. Milk feeding frequency was applied to individual calves within pens and hence calf was both the experimental and observational unit (as defined by Bello et al., 2016). All statistical analyses were conducted using the SAS software (version 9.4, SAS Institute Inc., Cary, NC).

Daily Number of Rewarded Visits to the Computer-Controlled Milk Feeder

The daily number of rewarded visits in each of the six 7-d periods (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55) was transformed by the square root and analyzed for effect of milk feeding frequency separately for each of the 6 periods using a MIXED model. The model included the fixed effects of block, milk feeding strategy (**trt1**; CON, STEP), milk feeding frequency (**trt2**; RES, UNRES), and the interaction between milk feeding strategy and milk feeding frequency. Initial LW was included as a covariate. The model included the random and pen (nested within trt1), thus using pen as error term for the test of milk feeding strategy (St-Pierre, 2007). Sub-block was estimated to zero and therefore omitted from the model. The denominator degrees of freedom was estimated using the Kenward-Roger approximation.

Behavior Recorded from Video Recordings Preweaning

From the 24-h video recordings, the duration of time spent lying, being at milk feeder while sucking on the teat, being at milk feeder while manipulating the teat, being at milk feeder in total, eating concentrate, eating hay and drinking water, as well as the number of lying bouts, were calculated for each of the 2 d of observation. The variables were, if necessary to assume normality (being at the milk feeder in total, being at the milk feeder while manipulating teat, eating concentrate, eating hay, drinking water, as well as the number of lying bouts), transformed by the square root and analyzed by a MIXED model. The model included the fixed effects of block, milk feeding strategy (**trt1**; CON, STEP), milk feeding frequency (**trt2**; RES, UNRES), day of age, and

all 2-way interactions between milk feeding strategy, milk feeding frequency and day of age as fixed effects. The initial LW was included as a covariate. Sub-block, pen (nested within trt1), and calf were included as random effects. The denominator degrees of freedom was estimated using the Kenward-Roger approximation. The assumptions of normality were checked by visual inspection of the residuals, whereas the assumptions of variance homogeneity was checked by plotting the predicted values against the residuals.

Live Weight, Growth Rate, and Feed Intake

The LW, ADG, DMI, and feed conversion efficiency (**FCE**) were analyzed separately for each of 4 2-wk periods (d 14 to 27, d 28 to 41, d 42 to 55, and d 56 to 69), for the preweaning period (d 14 to 55) and for the entire experimental period (d 14 to 69).

When analyzed as one observation per calf, LW and ADG were analyzed by a MIXED model including the fixed effects of block, milk feeding strategy (**trt1**; CON, STEP), milk feeding frequency (**trt2**; RES, UNRES), and the 2-way interaction between milk feeding strategy and milk feeding frequency. The initial LW was included as a covariate. Pen (nested within trt1) was included as a random effect. Sub-block was estimated to zero and therefore omitted from the model. The Kenward-Roger method was used to estimate the denominator degrees of freedom. The assumptions of normality were checked by visual inspection of the residuals whereas variance homogeneity was checked by plotting the predicted values against the residuals.

When analyzing LW and ADG for the milk feeding period or the entire experimental period using weekly data, the MIXED model described above was extended with week (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, d 49 to 55, d 56 to 62, and d 63 to 69),

Table 2. Description of the behavioral recordings (ethogram)

Posture	Description
Upright	Standing or moving around
Lying	Lying on chest with head raised, or lying flat on one side
Behavior while upright	
At milk feeder, sucking on teat	Sucking on the teat (the teat is fully or partly in the calf's mouth) while standing in the milk feeder
At milk feeder, manipulating the teat	Actively pushing, butting, or biting on teat (not sucking on teat) while standing in the milk feeder
At milk feeder, inactive	Inactively standing in the milk feeder (not sucking on teat, not manipulating the teat)
Eating concentrate	Standing by the concentrate trough with either the muzzle in the trough or chewing concentrate with the head raised over the trough
Eating hay	Standing by the hay rack and trough with the muzzle touching the hay or rack, or with the muzzle in hay trough below the hay rack, or chewing hay with the head near hay rack or hay trough
Drinking water	Standing with muzzle in water bowl either drinking water, pressing water valve, or moving/spilling water
No activity	No activity or any activity other than elements defined above

i.e., data for 6 wk, or data for 8 wk, respectively, as a repeated measure, as well as the 2-way interactions of week with trt1 and trt2, respectively. Pen (nested within trt1) was included as a random effect and the dependence between repeated observations (i.e., week) on the same calves were modeled as autoregressive of first order [AR(1); Littell et al., 1996]. The Kenward-Roger method was used to estimate the denominator degrees of freedom.

Solid feed intake (i.e., concentrates and hay) was recorded per pen (either 7 or 8 calves per pen). Milk intake was also analyzed per pen to be comparable to solid feed intakes. Thus, feed intakes (concentrates, hay, and milk, respectively) were analyzed by a GLM model that included 8 observations (one per pen). The model included the fixed effects of milk feeding strategy (trt1; CON, STEP), block, and sub-block nested within block.

When analyzing feed intake for the milk feeding period or the entire experimental period using weekly data [i.e., including data for 6 wk from d 14 to 55 for milk (in this case milk intake per pen), hay or concentrate intake, or including data for 8 wk from d 14 to 69 for hay or concentrate intake, respectively] the MIXED model included the fixed effects of block, milk feeding strategy (trt1; CON, STEP), week, and the 2-way interaction between trt1 and week. Pen (nested within trt1) was included as a random effect and the dependence between repeated observations (i.e., week) on the same pen were modeled as autoregressive of first order [AR(1); Littell et al., 1996]. The Kenward-Roger method was used to estimate the denominator degrees of freedom.

RESULTS

The average frequencies of rewarded visits per day to the milk feeder in each of the periods d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55 are shown in Figure 2. No significant effects of milk feeding strategy or milk feeding frequency were found for the periods from d 14 to 20 or d 21 to 27, whereas during the remaining periods, UNRES calves had fewer rewarded visits than RES calves (d 28 to 34: $F_{1,49.7} = 25.45$; $P < 0.001$; d 35 to 41: $F_{1,49.3} = 29.85$; $P < 0.001$, d 42 to 48: $F_{1,49.6} = 72.38$; $P < 0.001$, and d 49 to 55: $F_{1,49} = 63.19$; $P < 0.001$).

Behavior Recorded from Video Recordings on 26 and 40 d of Age

There were no interactions between milk feeding strategy and milk feeding frequency for any of the recorded variables and no main effects of milk feeding strategy (Table 3). However, the following interactions between

milk feeding strategy and day of age were found for time spent manipulating the teat and time spent eating concentrate. On 26 d of age, CON calves (offered 6.5 L/d at this age) spent more time manipulating the teat than STEP calves (offered 8 L/d at this age), whereas on 40 d of age (CON and STEP were offered 6.5 and 5 L/d, respectively) no difference was seen (interaction between milk feeding strategy and day of age, $F_{1,57} = 4.06$; $P < 0.05$; Figure 3). Regarding time spent eating concentrate, no difference was detected on 26 d of age, whereas on 40 d of age STEP calves spent more time eating concentrate than CON calves (interaction between milk feeding strategy and day of age, $F_{1,57} = 7.87$; $P < 0.01$; Figure 4).

Interactions were also found between milk feeding frequency and day of age. On 26 d of age, UNRES calves spent more time in the milk feeder in total than RES calves, whereas no difference was seen on d 40 (interaction between feeding frequency and day of age, $F_{1,57} = 6.33$; $P < 0.05$; Figure 5). Similarly, on d 26, UNRES calves spent more time manipulating the teat than RES calves, whereas on 40 d of age no difference was seen (interaction between feeding frequency and day of age, $F_{1,57} = 8.51$; $P < 0.01$; Figure 6). In addition, milk feeding frequency affected lying time, time spent eating concentrate, and time spent eating hay in that UNRES calves spent less time lying down, more time eating concentrate, and more time eating hay than RES calves (Table 4).

Irrespective of treatments, calves had more lying bouts and spent less time sucking the teat, less time in the milk feeder, more time eating concentrate, and more time eating hay on 40 d of age than on 26 d of age (Table 5).

Figure 2. The frequency of rewarded visits per day to the milk feeder (means $\pm$ SEM) of calves on milk feeding frequency treatment with restricted milk portion size (RES) and unrestricted milk portion size (UNRES) in each of the periods d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55. Back-transformed means and SEM are given (data were square root-transformed before analysis).

Figure 3. The daily time spent manipulating the teat (means $\pm$ SEM) of calves on the conventional flat-rate milk feeding strategy (CON) and the step-down milk feeding strategy (STEP) on 26 and 40 d of age. For each day, different letters (a, b) indicate significant differences ($P < 0.05$) between treatments on that day. Back-transformed means and SEM are given (data were square root-transformed before analysis).

Live Weight and Growth Rate

No significant difference was observed between treatment groups in LW before the start of the experiment (approximately 14 d of age). However, numerical differences of up to 2 kg between the 4 treatment groups were observed, and LW at d 14 (start of experiment) was included as a covariate in the analyses of LW, ADG, and feed intake.

No main effects were observed for the milk feeding strategy (CON vs. STEP), milk feeding frequency (RES vs. UNRES), or their interaction on LW pre- or postweaning or at the end of the experiment (70 d of age). Estimates for the interaction between milk feeding strategy and milk feeding frequency are presented in Table 6.

An interaction was observed between milk feeding strategy and milk feeding frequency for ADG on d 28 to 41 (Table 6); only among calves on the RES treat-

Figure 4. The daily time spent eating concentrate (means $\pm$ SEM) of calves on the conventional flat-rate milk feeding strategy (CON) and the step-down milk feeding strategy (STEP) on 26 and 40 d of age. For each day, different letters (a, b) indicate significant differences ($P < 0.05$) between treatments on that day. Back-transformed means and SEM are given (data were square root-transformed before analysis).

Figure 5. The daily time spent at the milk feeder (means $\pm$ SEM) of calves on milk feeding frequency treatment with restricted milk portion size (RES) and unrestricted milk portion size (UNRES) on 26 and 40 d of age. For each day, different letters (a, b) indicate significant differences ($P < 0.05$) between treatments on that day. Back-transformed means and SEM are given (data were square root-transformed before analysis).

ment did STEP calves have a higher ADG than CON calves between d 28 and 41. An interaction was also observed between milk feeding strategy and milk feeding frequency for ADG postweaning (d 56 to 69); the UNRES treatment resulted in higher ADG than RES treatment among CON calves, but not among STEP calves (Table 6). No main effect of the milk feeding strategies was seen for ADG pre- and postweaning or for ADG during the entire experiment from d 14 to 69 (main effects not shown).

When LW was analyzed as 7 repeated observations across the 6 wk of milk feeding (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, d 49 to 55), only the effect of week was significant (data not shown, $F_{6,339} = 282$; $P < 0.001$). The same was evident when ADG measured weekly was analyzed as 6 repeated observations across the 6 wk (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55), as only the effect of week was significant (data not shown, $F_{5,253} =$

Figure 6. The daily time spent manipulating the teat (means $\pm$ SEM) of calves on milk feeding frequency treatment with restricted milk portion size (RES) and unrestricted milk portion size (UNRES) on 26 and 40 d of age. For each day, different letters (a, b) indicate significant differences ($P < 0.05$) between treatments on that day. Back-transformed means and SEM are given (data were square root-transformed before analysis).

Table 3. Behavior (means $\pm$ SEM) of calves fed by the conventional flat-rate milk feeding strategy (CON) and the step-down milk feeding strategy (STEP)

Milk feeding strategy	CON (mean)	$\pm$ SEM	STEP (mean)	$\pm$ SEM	Numerator, denominator (df)	F-value	P-value
Lying (min/24 h)	1,039	19.8	1,086	19.7	1,3.3	7.28	0.067
Lying ¹ (frequency/24 h)	4.28 (18.3)	0.11	4.39 (19.3)	0.11	1,3.0	1.06	0.380
Sucking teat (min/24 h)	25.9	3.44	20.9	3.43	1,3.1	1.87	0.263
Manipulating teat ^{1,2} (min/24 h)	4.38 (19.2)	0.29	4.06 (16.4)	0.29	1,54	0.64	0.429
Total time at milk feeder ¹	7.84 (61.5)	0.37	7.15 (51.1)	0.37	1,3.2	2.32	0.221
Eating concentrate ¹ (min/24 h)	4.86 (23.7)	0.27	5.06 (25.6)	0.27	1,3.1	0.33	0.604
Eating hay ¹ (min/24 h)	6.31 (39.9)	0.48	5.36 (28.7)	0.48	1,3.2	3.63	0.148
Drinking water ^{1,3} (min/24 h)	3.87 (15.0)	0.21	3.66 (13.4)	0.21	1,4.4	0.49	0.521

¹Transformed by square root. Back-transformed mean values are indicated in parentheses in the line below.

²The random effects of sub-block and pen were estimated to zero and taken out of the model.

³The random effect of sub-block was estimated to zero and taken out of the model.

37.4; $P < 0.001$). The estimated mean ADG for each week was 0.35, 0.53, 0.64, 0.89, 0.72, and 1.12 (SEM: 0.06) kg/d for d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55, respectively.

Feed Intake

Average total milk intake from d 14 to 55 was numerically 1.1% lower for UNRES compared with RES (210 vs. 212 L), but this was not significant ($F_{1,7} = 0.39$; $P < 0.56$). In contrast, the average total milk intake of CON was 5% higher than that of STEP (217 vs. 206 L; $F_{1,3} = 11.2$; $P < 0.05$). On average, calves consumed 211 L of

MR, which was 6% less than the total allowances (224 L; data not shown).

Intakes of concentrate and hay were measured per pen and can thus only be reported for milk feeding strategy (CON vs. STEP). Table 7 presents intake of MR, concentrate, and hay, intake of total DM, and FCE (MJ of net energy/kg of gain) pre- and postweaning and for the entire experimental period (d 14 to 69) for CON and STEP treatments, respectively.

When milk intake was analyzed as repeated observations across the 6 wk of milk feeding (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55), a significant milk feeding strategy by week interaction

Table 4. Behavior (means $\pm$ SEM) of calves on milk feeding frequency treatment with restricted milk portion size (RES) and unrestricted milk portion size (UNRES)

Milk feeding frequency	RES (mean)	$\pm$ SEM	UNRES (mean)	$\pm$ SEM	Numerator, denominator (df)	F-value	P-value
Lying (min/24 h)	1,079	19.2	1,047	19.2	1,49.9	4.93	0.031
Lying ¹ (frequency/24 h)	4.42 (19.5)	0.10	4.25 (18.1)	0.10	1,49.4	2.69	0.107
Sucking teat (min/24 h)	23.88	3.06	22.94	3.06	1,49.4	0.22	0.634
Manipulating teat ^{1,2} (min/24 h)	3.49 (12.2)	0.29	4.95 (24.5)	0.29	1,54	12.35	<0.001
Total time at milk feeder ¹	6.99 (48.8)	0.35	8.00 (64.0)	0.35	1,49.7	6.53	0.014
Eating concentrate ¹ (min/24 h)	4.68 (21.9)	0.25	5.25 (27.6)	0.25	1,49.7	4.70	0.035
Eating hay ¹ (min/24 h)	5.48 (30.0)	0.44	6.20 (38.4)	0.44	1,49.6	4.40	0.041
Drinking water ^{1,3} (min/24 h)	3.66 (13.4)	0.19	3.88 (15.0)	0.19	1,49.7	0.79	0.377

¹Transformed by square root. Back-transformed mean values are indicated in parentheses on the line below.

²The random effects of sub-block and pen were estimated to zero and taken out of the model.

³The random effect of sub-block was estimated to zero and taken out of the model.

Table 5. Behavior (means $\pm$ SEM) of calves on 26 and 40 d of age

Behavior	26 d of age (mean)	$\pm$ SEM	40 d of age (mean)	$\pm$ SEM	Numerator, denominator (df)	F-value	P-value
Lying (min/24 h)	1,053	18.69	1,073	18.69	1,57	3.14	0.082
Lying ¹ (frequency/24 h)	4.23 (17.9)	0.10	4.44 (19.7)	0.10	1,57	6.16	0.016
Sucking teat (min/24 h)	26.72	2.99	20.09	2.99	1,57	19.3	<0.001
Manipulating teat ¹ (min/24 h)	4.28 (18.3)	0.243	4.16 (17.3)	0.243	1,57	0.19	0.662
Total time at milk feeder ¹	7.79 (60.7)	0.312	7.19 (51.6)	0.312	1,57	6.06	0.017
Eating concentrate ¹ (min/24 h)	4.31 (18.6)	0.238	5.62 (31.5)	0.238	1,57	38.3	<0.001
Eating hay ¹ (min/24 h)	5.43 (29.5)	0.43	6.25 (39.0)	0.43	1,57	10.1	0.002
Drinking water ¹ (min/24 h)	3.62 (13.1)	0.17	3.92 (15.4)	0.17	1,57	2.8	0.100

¹Transformed by square root. Back-transformed mean values indicated in parentheses on the line below.

($F_{5,18.9} = 59.6$; $P < 0.001$) was observed as expected, corresponding to the development of the milk allowance curve and the realized milk intake over the weeks (data not shown). An effect of week ($F_{5,18.9} = 637$; $P < 0.001$) and milk feeding strategy ($F_{1,4.77} = 14.4$; $P < 0.02$) were also observed, showing the overall 5% lower milk intake in STEP compared with CON, but milk intake was similar in the 2 blocks. The overall 5% lower milk intake by STEP calves compared with CON calves was due to a lower milk intake than allowed (6.7 vs. 8.0 L/d during d 14 to 27).

When intakes of concentrate, hay, and DM were analyzed as repeated observations across the 6 wk pre-weaning (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, and d 49 to 55) or the entire 8 wk of the experiment (d 14 to 20, d 21 to 27, d 28 to 34, d 35 to 41, d 42 to 48, d 49 to 55, d 56 to 62, and d 63 to 69), there was no interaction between milk feeding strategy and week (data not shown). Only the main effect of week was significant for concentrate ($F_{7,28.7} = 50.3$; $P < 0.001$), hay ($F_{7,40.2} = 16.7$; $P < 0.001$), and DMI ($F_{7,30.9} = 53.4$; $P < 0.001$), and tended to be different

Table 6. Live weight (LW) and ADG for calves fed by the conventional flat-rate milk feeding strategy (CON) and the step-down milk feeding strategy (STEP)¹

Item	Treatment (trt)				SEM	Numerator, denominator (df)	trt1 × trt2	
	CON		STEP				F-value	P-value
	RES	UNRES	RES	UNRES				
LW ² (kg)								
Start, d 14	48.1	48.9	50.2	47.9	1.85	1,49.3	1.66	0.21
d 28 ³	55.0	55.5	54.7	54.8	0.82	1,53	0.07	0.80
d 42 ³	64.4	66.2	67.2	65.7	1.23	1,53	1.99	0.16
Weaning, d 56 ³	76.9	77.0	79.7	78.2	1.78	1,53	0.20	0.66
Final, d 70	93.1	96.7	99.3	96.2	2.49	1,48.2	2.74	0.11
ADG ² (kg/d)								
d 14 to 27 ³	0.45	0.47	0.42	0.43	0.06	1,53	0.02	0.90
d 28 to 41	0.67 ^a	0.77 ^{ab}	0.89 ^b	0.78 ^{ab}	0.07	1,48.4	5.27	0.03
d 42 to 55	0.94	0.80	0.95	0.94	0.08	1,48.5	0.88	0.31
Preweaning, d 14 to 55 ³	0.69	0.68	0.75	0.72	0.04	1,53	0.16	0.70
Postweaning, d 56 to 69	1.16 ^a	1.41 ^b	1.38 ^{ab}	1.28 ^{ab}	0.08	1,48.2	9.90	0.003
Overall, d 14 to 69	0.80	0.86	0.91	0.86	0.04	1,48.6	2.47	0.13

^{a,b}Different letters in a row indicate significant differences ($P < 0.05$) between mean values.

¹For LW and ADG, the results are further divided into whether calves were subject to restricted milk portion size (RES) or unrestricted milk portion size (UNRES) within the 2 treatments CON and STEP.

²LW d 14 was included as a covariate in the analyses of LW (except at d 14) and ADG, as there was unintentionally up to 2.1 kg difference (NS) in LW between the 2 $\times$ 2 treatment groups at 14 d of age.

³For these analyses the covariance for the effect of pen could not be estimated in MIXED, and thus the F-test for the effect of trt1 was made using the pen variation from a similar GLM model and the test option (see Statistical Analysis description).

for FCE ($F_{7,16.7} = 2.14$; $P < 0.10$), whereas the effect of milk feeding strategy (CON vs. STEP) and block was nonsignificant.

DISCUSSION

Distributing the same milk allowance according to a step-down strategy, rather than at a conventional flat rate, resulted in calves spending more time feeding on concentrate after the step-down in milk allowance, but not before. Thus, a lower daily milk allowance stimulated concentrate intake on d 40, but not on d 26, supporting the suggestion that allocating a higher proportion of the total milk allowance during the first 4 wk of a calf's life corresponds better to the calves' nutritional needs. Calves with no restriction on meal frequency spent more time eating concentrate and hay. However, only among calves fed according to a flat-rate milk feeding strategy, calves with no restriction on meal frequency also had a higher ADG postweaning. Thus, the higher appetite indicated by the increased time spent feeding among calves with no restrictions on meal frequency only resulted in higher gain for calves fed according to a conventional milk feeding strategy.

Calves on a high milk allowances ingest little solid feed preweaning (Jasper and Weary, 2002; Rosenberger et al., 2017) and research focus on strategies to stimu-

late intake of solid feed and to maintain high ADG when daily milk allowance is reduced during and after weaning is important. In the present study, distributing the same milk allowance according to a step-down strategy rather than a flat-rate strategy stimulated the calves to spend more time feeding on concentrate on d 40. On d 40, the daily milk allowance of STEP calves was 1.5 L lower than that of CON calves, whereas the opposite was the case on d 26. Thus, a lower daily milk allowance stimulated concentrate intake on d 40, but not on d 26, supporting that it is better to allocate a higher proportion of the milk early in calves' life, because calves generally do not ingest much concentrate before 3 to 4 wk of age (Khan et al., 2011) and because calves cannot digest much concentrate before this age (Kristensen et al., 2007). Thus, young calves are unable to completely compensate for a low milk intake during the first weeks of life.

The higher time spent eating concentrates at d 40 suggests that the STEP calves (fed 5 L/d at this age) were hungrier than CON calves (fed 6.5 L/d at this age). However, another sign of hunger is the time spent manipulating the empty teat after the milk is ingested (Herskin et al., 2010). On d 26 time spent manipulating the empty teat was higher in CON calves (fed 6.5 L/d at this age) compared with STEP calves (fed 8 L/d at this age), despite the fact that STEP calves did not

Table 7. Daily intake of milk, concentrates, hay, and total DM, and feed conversion efficiency (FCE) for calves fed by the conventional flat-rate milk feeding strategy (CON) and the step-down milk feeding strategy (STEP)

Item	Treatment (trt)		SEM	df	F-value (trt1)	P-value (trt1)
	CON	STEP				
Milk ¹ (L/d)						
d 14 to 27	6.0	6.7	0.11	1,3	16.9	0.03
d 28 to 41	6.5	5.0	0.03	1,3	1155	0.001
d 42 to 55	3.0	3.0	0.01	1,3	0.93	0.41
Prewaning, d 14 to 55	5.2	4.9	0.06	1,3	11.2	0.05
Concentrate (kg/d)						
d 14 to 27	0.079	0.064	0.010	1,3	0.97	0.40
d 28 to 41	0.31	0.51	0.051	1,3	7.40	0.08
d 42 to 55	1.02	1.32	0.149	1,3	1.98	0.26
Prewaning, d 14 to 55	0.47	0.63	0.067	1,3	2.65	0.21
Postweaning, d 56 to 69	2.51	2.74	0.093	1,3	2.84	0.20
Overall, d 14 to 69	0.98	1.16	0.073	1,3	2.89	0.19
Hay (kg/d)						
Prewaning, d 14 to 55	0.11	0.10	0.017	1,3	0.27	0.64
Postweaning, d 56 to 69	0.27	0.21	0.036	1,3	1.38	0.33
Overall, d 14 to 69	0.150	0.13	0.022	1,3	0.65	0.48
DMI (total; kg/d)						
Prewaning, d 14 to 55	1.21	1.31	0.061	1,3	1.15	0.37
Postweaning, d 56 to 69	2.48	2.62	0.072	1,3	1.95	0.26
Overall, d 14 to 69	1.53	1.63	0.062	1,3	1.43	0.32
FCE (MJ of net energy/kg of ADG)						
Prewaning, d 14 to 55	18.5	17.8	0.46	1,3	1.10	0.38
Postweaning, d 56 to 69	15.2	15.7	0.60	1,3	0.26	0.65
Overall, d 14 to 69	17.2	17.0	0.15	1,3	1.04	0.39

¹The milk intake does not include the 2 first days of adaptation after arrival.

drink the whole daily allowance between 14 and 28 d of age (i.e., 6.7 vs. 8.0 L/d). There is concern that limited milk feeding cause undernutrition and negative affective states of hunger in young calves (Jensen, 2004; De Paula Vieira et al., 2008), as indicated by the time they spent manipulating an empty teat after milk ingestion. This supports that an initial high allowance followed by a stepwise reduction is a better strategy than a conventional flat-rate milk feeding. At d 40 there was no difference in time spent manipulating the teat irrespective of the difference in milk allowance between STEP and CON calves at this age. This is likely because STEP calves were able to compensate for the lower milk allowance by increasing the intake of solid feed on d 40, again supporting that this milk feeding strategy better corresponds to their nutritional needs.

In the present study, UNRES calves spent more time eating concentrate and hay than RES calves. This supports the suggestion that allowing calves to ingest the same daily milk allowance in fewer and larger meals as they grow older may shift calves' focus from milk to solid feed. However, the UNRES calves also spent more time manipulating the teat and spent more total time at the milk feeder on d 26 and these behaviors have been related to hunger (Jensen, 2004; De Paula Vieira et al., 2008). Taken together, these findings suggest that calves with no restriction on meal frequency generally experienced a higher feeding motivation (possibly reflecting hunger) than calves fed the same milk allowance in portions of 1.8 to 2.3 L.

It should be emphasized that the results obtained relate to feeding a MR primarily containing whey and soy-protein concentrates as protein sources and only 13% skim milk powder. It has been reported that feeding fewer MR portions results in a more complete casein hydrolysis as well as curd formation and a better digestibility compared with more frequent milk portions (Okada et al., 2010). Due to differences in curd formation and protein-peptide outflow of the abomasum from casein-based MR (and whole milk) compared with whey-based MR (Caugant et al., 1992), satiety signaling to stimulate concentrate intake may well be affected by the type of protein source of the milk fed. However, to elucidate such an influence of milk protein source on the feeding behavior aspects reported herein, studies are still warranted.

Among CON calves, UNRES milk feeding frequency resulted in higher ADG postweaning compared with a RES milk feeding frequency. However, among calves fed according to a stepwise reduction in milk allowance, no effect of milk feeding frequency was found. The total milk intake of UNRES calves was 1% lower than that of RES calves, but this insignificant difference cannot explain the difference in ADG. Rather, this

is likely due to the lack of restriction on milk feeding frequency, resulting in fewer and larger meals as calves grow older, increasing ADG through increased solid feed intake. In the present study, a difference in meal frequency between RES and UNRES was not detected until d 28. This coincides with the age where STEP calves were reduced from 8 to 5 L/d. Previously it has been found that a high milk allowance was required for calves to distribute milk to fewer and larger meals, whereas calves on a low milk allowance ingested the milk as soon as it became available to them (Jensen, 2009). Furthermore, calves fed *ad libitum* ingested their milk in 7 meals when 6 wk of age compared with 2 daily milk meals in calves fed at 5 L/d (Miller-Cushon et al., 2013). These findings suggest that applying the UNRES method makes little sense in combination with a low daily milk allowance, which probably explains why ADG were numerically higher with UNRES among CON calves (fed 6.5 L/d) and numerically lower with UNRES among STEP calves (fed 5 L/d) between d 28 and 41. Therefore, although a step-down milk feeding strategy, as well as relaxing restriction on milk feeding frequency, both have stimulating effects on solid feed intake and ADG, they did not stimulate intake and gain when used in combination. This is because the low daily milk allowance after the step-down (i.e., 5 L/d for 28-d-old calves) fed in the present study was so small that the calves ingested it as soon as it became available to them. This also explains why in the present study we only found a stimulating effect of UNRES on ADG among CON calves postweaning.

The positive effects obtained with applying step-down milk feeding strategies have in many studies been confounded with a higher total milk allowance compared with the applied conventional flat-rate milk feeding strategies (e.g., Khan et al., 2007; heifer dairy calves). In other studies, step-down strategies during weaning have been associated with less total milk intake compared with more gradual weaning procedures (e.g., Welboren et al., 2019; male dairy calves). In the present study, the step-down milk feeding strategy (STEP) used the same total milk allowance as the conventional flat-rate strategy (CON), but rescheduled a higher daily allowance to male dairy calves for the first 14 d after being transported to and having settled in at the fattening unit at the age of 14 d. We only found stimulating effects on ADG postweaning of the UNRES treatment among CON calves, but our results need to be interpreted in relation to the generally low total milk allowance for the male dairy calves of the present study as compared with the milk allowances currently recommended to heifer calves. In replacement heifer calves offered a higher total milk allowance, the 2-way interactions may have been different from that

obtained herein, and more research is needed to determine the interactive effects of milk feeding strategy and milk feeding frequency on feeding behavior, intake, and gain to optimize calf rearing protocols.

During the first weeks, the actual milk intake among STEP calves was only on average 6.7 L/d and much lower than the allowance of 8 L/d. Similar results were reported by Rosenberger et al. (2017). This is somewhat surprising as the maximum ad libitum intake at this age is usually higher (e.g., Rosenberger et al., 2017). Thus, it deserves further study to uncover if the computer-controlled milk feeders due to their programming are unintentionally reducing early milk intake (e.g., because calves do not learn to drink). Failure to learn to drink when milk is available may also explain the overall 5% lower total milk intake in STEP compared with CON calves. This difference in intake was due to STEP calves ingesting less milk than their allowance between 14 and 27 d of age. Young calves have more difficulties learning to use a computer-controlled milk feeder and require more human assistance (Jensen, 2007; Medrano-Galarza et al., 2018). Ensuring that milk feeders are set up so that calves can figure out how to access the allowance allotted to them deserves more research attention, especially when calves are introduced to the teat-feeders while very young, when coming from bucket feeding, or following transport. Had STEP calves drunk their allocated milk allowance on d 14 to 27, these calves would have been expected to have a higher ADG during this period and possibly an even higher concentrate intake on d 28 to 55.

CONCLUSIONS

The results support that allocating a higher proportion of the total milk allowance during the first 4 wk of calves' lives corresponds better to the calves' nutritional needs. Placing no restriction on milk portion size and thus relaxing restrictions on milk meal frequency may help calves transition from milk to solid feeds before weaning off milk, but a step-down milk feeding strategy and fewer restrictions on milk feeding frequency do not work well together when the total milk allowance is low and adapted to male calves reared for meat or veal, as in the present study.

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