

# Identifying a Milk-Replacer and Weaning Strategy for Holstein Calves Using Automated Behavioral Measures of Lying and Environmental Enrichment Device Use

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## Summary

In dairy production, “weaning readiness” is often based on solid feed intake. The goal of this study was to determine weaning readiness using feed-intake, lying-behaviors, and the use of an environmental enrichment device (EED) in calves that underwent 1 of 4 milk-replacer and weaning protocols. Twenty-eight male Holstein calves ( $95 \pm 2.6$  lb BW at 1 d of age) were housed in individual pens and initially fed one type of milk replacer (25% crude protein (CP), 17% fat, 1.45 lb of dry matter (DM)) via nipple-buckets twice a day (AM and PM), and one type of textured calf starter (*ad libitum*; 20% CP and 37% starch). At age 3 days, calves were randomly assigned to one of the four nutrition-weaning strategies:

1. MOD-STEP - 1.46 lb per day of milk replacer; 2-step weaned, initiated at age 6 weeks, completed 3 days later;
2. HI-STEP - 2.4 lb per day of milk replacer; 2-step weaned, initiated at age 5 weeks and completed 1 week later;
3. HI-LATE - 2.4 lb per day of milk replacer; 2-step weaned, initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD - 2.4 lb per day of milk replacer; 5-step weaned, initiated at age 6 week and completed 2 weeks later.

Each calf’s pen had an EED, which included a dummy-nipple attached to a bottle and holder. A sensor and automated logger tracked each event (1 Hz) that the calf manipulated the EED (25 Hz sensitivity). Each calf was fitted with an accelerometer on the back leg to automatically measure lying behaviors. The device collected the y-axis (lie vs. stand) and z-axis (right or left percent during lying) of the calf every minute. For this experiment, 3-day sample periods were analyzed before and after weaning was initiated. In addition, the 3 days following weaning-completion were sampled.

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Feed intake among MOD-STEP calves increased by  $1.0 \pm 0.19$  lb after the first bottle was removed ( $P \leq 0.05$ ), and then by  $1.5 \pm 0.19$  SE lb after completion of weaning ( $P \leq 0.05$ ). The use of EED did not change among MOD-STEP calves ( $P > 0.05$ ), but after weaning, they increased their lying time, especially on their left side ( $P \leq 0.05$ ). These changes in lying-behaviors may indicate increased comfort and maturity of the rumen. On the contrary, calves in the HI-STEP treatment ate the least amount of feed overall ( $P < 0.05$ ), and they used the EED the most ( $P > 0.05$ ). Calves in the HI-STEP treatment showed reduced lying bouts after weaning ( $P \leq 0.05$ ), but no other lying-measures changed ( $P > 0.05$ ).

The HI-LATE calves had similar feed intake and EED use compared to MOD-STEP calves. These findings suggest that weaning age needs to be more than 8 weeks for calves fed 2.4 lb of milk replacer per day. Gradual weaning may also improve feed intake and reduce EED use. When calves were gradually weaned starting at age 6 weeks and completed at age 8 weeks, they had the same amount of solid feed intake as HI-LATE calves. More research is needed to determine if increased feed intake and reduced EED use are also indicators that cross-sucking is less likely to occur when calves are grouped after weaning.

## Introduction

Solid feed intake is commonly used by calf raisers to determine if a calf is ready to be weaned from milk or milk replacer. Before 2010, milk or milk replacer was fed at a minimal plane of nutrition (e.g., 20% CP, 20% fat, 1 lb of dry matter per day). This low plane of nutrition combined with a 2-step weaning process motivated calves to eat solid feed at the earliest age possible. The 2-step weaning strategy involved reducing the milk or milk-replacer by 1/2 for a few days, and once the calf ate a significant amount of feed, the second half of the liquid diet was removed. On average, most U.S. calves were weaned at weaning initiated at 6 weeks of age.

After 2010, calf raisers were encouraged by researchers to feed greater amounts of milk or milk replacer. For example, 2.4 lb per day of a formula with greater protein (e.g. 25% CP, 17% fat of DM) was considered a high plane of nutrition. Although accelerated preweaning body weight gain improved health after calves were group housed, researchers and early adopters of this strategy reported that the same 2-step weaning method did not motivate calves to consume solid feed before or during the two-step weaning strategy. One solution was to feed a “moderate” nutritional strategy with the same weaning protocol. An example of a moderate can include milk replacer that also has 25% CP and 17% fat, but lesser amount is offered (e.g. 1.5 lb/d of DM). Other solutions included feeding the high plane of nutrition and: 1) apply the 2-step weaning at a later age; or 2) apply a more gradual weaning method.

In addition to monitoring solid-feed intake, our lab proposed that two behavioral toolsets could be added to determine weaning readiness. Our laboratories and previous researchers used lying-behavior measures to monitor cattle comfort. As calves mature the number of lying bouts (i.e. naps) should decrease if they are comfortable with their environment but their overall time spent resting increases for rumination. In addition, as cattle age, the laterality of lying shifts to the left side. Some researchers suggest this lying-posture is used to make room for an active rumen.

Another major behavioral change among mature calves is decreased non-nutritive sucking (NNS). Early in life, NNS is important among most mammals for the neonate to develop the fine motor skills required for solid-food intake. However, as a ruminant grows, it should trade-off NNS behaviors for solid-feed intake. Our lab invented an environmental enrichment device (EED) that automatically measures how much a calf manipulates a dummy-nipple, which included NNS. Therefore, we proposed to use these three behavioral toolsets to determine weaning readiness of calves that underwent four different nutritional and weaning strategies.

## Experimental Procedures

Holstein bull calves (age 3–4 days) were purchased from 1 dairy, transported for 3.5 h, and received at the Nurture Research Center nursery (Provimi, Brookville, OH). All calves were under the approval of that institution's animal care and use committee and cared for according to the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010). Calves were housed in a barn with natural ventilation in individual pens (3.9 × 7.9 feet) with deep-bedded straw. Twenty-eight calves were randomly chosen out of a 96-animal project for this study. During their first 3 days of age, calves were fed 1.5 lb of one type of milk replacer (25% CP, 17% fat, DM) split into two feedings (6:00 AM and 3:30 PM). Water and a textured calf starter (20% CP and 37% starch) were provided *ad libitum* throughout the study. Solid feed (textured calf starter) intake was measured by weighing back the remaining solids at the end of each day.

Calves were randomly assigned to one of four nutrition/weaning protocols at 3 days of age (Figure 1). Calves in MOD-STEP treatment (Figure 1) were fed the same amount of milk replacer that they started with, then at age 6 weeks, a 2-step weaning was initiated by removing the PM feeding and was completed 3 days later by removing the AM milk feeding. A high plane of milk replacer nutrition (HI) was fed to all other calves. At age 4 days, these calves were stepped up to 2.4 lb of the same milk-replacer that they started with (Figure 1). At age 5 weeks, weaning was initiated for HI-STEP calves by removing the PM feeding and was completed one week later. At age 7 weeks, weaning was initiated for HI-LATE calves by removing PM feeding, and was completed one week later. Starting at age 7 weeks, both feedings of the HI-GRAD calves were reduced by three 20% increments every four days, then the PM feeding was withdrawn for three days until weaning was completed at 8 weeks by removing the AM feeding.

From age 6 days until 1 week after weaning was completed, calves were provided an EED, which consisted of a dummy nipple and bottle with sensor that logged events every time it was manipulated by the calf (20 Hz sensitivity of movement; 1 Hz collection-rate, HOBO State Data Logger UX90–001M). In addition, a 3-axis accelerometer (Onset Computer Corp., Bourne, MA) was attached medially to a hind leg of each calf for the same time period. These accelerometers recorded standing and lying (y-axis) and lateral lying percent (z-axis) behaviors in 1-minute intervals.

For the analysis, the behavior and starter intake data were sampled for 3 days prior to weaning initiation (pre), the first 3 days after weaning initiation (during), and first 3 days after weaning completion (after). For these data, a linear mixed model with fixed effects of time, treatment and treatment × time was fitted and analyzed by restricted

likelihood ANOVA using the MIXED procedure of SAS (v. 9.2, SAS Inst., Cary, NC). Treatment, time, and interaction differences of  $P < 0.05$  were considered significant at  $P \leq 0.05$ .

## Results and Discussion

### *Solid Feed Intake*

Solid feed intake and automated behavioral data are presented in Table 1. The MOD-STEP calves increased their feed intake after weaning was initiated and again after weaning was complete (Figure 2;  $P \leq 0.05$ ). This behavior was similar to calves fed a low plane of nutrition. Therefore, calf raisers could use feed intake alone as a behavioral measure for calves with this nutrition-weaning strategy.

In the three days following weaning initiation, HI-STEP calves increased their solid feed intake compared to their pre-weaning measures, and again once weaning was finalized (Figure 2;  $P \leq 0.05$ ). However, overall, HI-STEP calves consumed the least amount of feed (Table 1;  $P \leq 0.05$ ). This finding replicated what other researchers and calf raisers had reported when they initiated weaning at age 5 weeks of age and completed weaning on or before a week later. Pre-weaning feed intakes were very low, which indicates that HI-STEP calves need to have more than just feed intake monitored to determine weaning readiness.

Calves in HI-LATE and HI-GRAD treatments did not significantly increase feed intake after weaning was initiated (Figure 2;  $P > 0.05$ ), but once weaning was completed for both groups, calves consumed the same amount of feed as MOD-STEP calves after weaning (Figure 2;  $P \leq 0.05$ ).

### *Lying Behaviors*

Total lying duration, left-sided lying, and number of lying bouts (lying for longer than 120 consecutive seconds) increased among MOD-STEP calves after weaning completion (Figure 3;  $P \leq 0.05$ ). These are all indications that the weaning method and feeding strategy influenced maturity in activity among MOD-STEP calves.

On the contrary, the only lying-behavior measure that changed among HI-STEP calves was a decrease in lying bouts significantly after weaning was completed (Figure 4;  $P \leq 0.05$ ). Calves in HI-LATE and HI-GRAD treatments did not change lying behaviors during the sample periods (Figures 3 and 4;  $P > 0.05$ ). Additionally, HI-GRAD calves had a slight, but not significant, reduction in lying time during weaning; no changes were observed in lying on their left or on number of lying bouts (Figures 3 and 4;  $P > 0.05$ ).

### *Environmental Enrichment Devices*

Calves in MOD-STEP did not change their EED use after weaning was initiated or completed (Figure 5;  $P > 0.05$ ). Over the entire experiment, calves in MOD-STEP treatment accounted for just 14.3% of the frequent EED users, which was similar to what was expected in the analysis (Table 2;  $\chi^2 = 63.59$ ,  $P \leq 0.001$ ). These data suggest that MOD-STEP calves may have traded their NNS behaviors for solid feed intake at an earlier age than calves in the other treatments. A similar finding was observed in

research comparing EED-use among low plane of nutrition fed calves vs. high plane of nutrition fed calves in a cohort of Holstein bull calves.

Calves in the HI-STEP treatment used the EED twice as much as calves in the MOD-STEP treatment (Table 1;  $P \leq 0.05$ ). They also made up 42.9% of the frequent-EED users (Table 2;  $\chi^2 = 63.59$ ,  $P \leq 0.001$ ). These findings suggest that HI-STEP calves did not trade off their NNS for increased feed intake.

Calves in HI-LATE used the EED as much as the MOD-STEP calves (Table 1). This finding contrasts with our previous research involving HI-LATE calves and EED-use. In this previous experiment, HI-LATE calves did not use the EED very often until weaning was initiated. Then, these HI-LATE calves used the EED three times the amount than the HI-LATE calves in the current experiment. In the current study none of the HI-LATE calves in this treatment could be classified as EED-frequent users (Table 2;  $\chi^2 = 63.59$ ,  $P \leq 0.001$ ). Furthermore, all calves in the high plane of nutrition treatments were treated the same until weaning initiation. For this experiment, we may have randomly selected a group of calves that have homogenous NNS temperament for the HI-LATE cohort. More research is needed with a greater number of calves per treatment to determine if EED-use is related to other toolsets for identifying temperament.

Although HI-GRAD calves did not change their EED-usage after weaning was initiated (Figure 5), overall, they used the EED 1.6 times more than the MOD-STEP and HI-LATE calves (Table 1;  $P \leq 0.05$ ), and they made up 33.3% of the EED-frequent users (Table 2;  $\chi^2 = 63.59$ ,  $P < 0.001$ ). This finding is an indicator that the gradual weaning method may not help calves reduce NNS when fed a high plane of milk replacer.

## Conclusions

The strategy for milk replacer and weaning strategy needs careful consideration because early-life behavioral development sets the foundation for a stress- and immune-resilient adult. The MOD-STEP nutrition-weaning strategy may help calves extinguish NNS and adopt more nutritive oral behaviors. The HI-LATE strategy may also be considered, especially for calves housed in colder environments. However, calves in this program need to be weaned at a later age, which means they will consume overall more milk replacer. Therefore, the housing strategy must also be considered when adopting a new nutrition-weaning strategy. Calves placed in groups before they extinguish NNS are likely to cross-suck. Some preweaning housing types are built for Holstein calves that are less than 5 weeks of age, therefore late-aged weaning impedes space allowance. Previous research indicated that space allowance also influences starter intake and immune resilience. Solid feed intake served as a reliable and traditional indicator for determining weaning readiness. The lying-loggers are helpful for researchers, but must be worn by the calf, which is not practical devices for large calf raising operations. The EED fastens to the pen rather than the calf. The EED enhances the calves' environment and simultaneously provides a quantitative measure of weaning readiness.

**Table 1. Solid-feed intake<sup>1</sup> and automated behaviors of calves fed four different milk replacer programs before weaning, after weaning initiation, and after weaning completion**

	Treatment <sup>2</sup>				SEM <sup>3</sup>	P-values		
	MOD-STEP	HI-STEP	HI-LATE	HI-GRAD		TRT	Time	TRT × time
Number of calves	7	7	7	7	—	—	—	—
Solid feed, lb/day	2.4 <sup>a</sup>	1.2 <sup>b</sup>	2.3 <sup>a</sup>	2.1 <sup>a</sup>	0.19	<0.001	<0.001	<0.001
Lying time, min/day	1130	1079	1126	1072	18.7	0.067	0.017	0.001
Lie left, <sup>4</sup> %	591.2	552.7	571	534.9	20.64	0.261	0.156	0.005
Lying bouts, <sup>5</sup> no./day	24.5	23.1	25.1	23.9	1.66	0.845	0.036	<0.001
EED use, sec/day	153 <sup>c</sup>	307 <sup>a</sup>	102 <sup>d</sup>	253 <sup>b</sup>	21.8	0.036	0.101	<0.001
EED bouts, <sup>6</sup> no./day	3.2 <sup>c</sup>	11.1 <sup>b</sup>	7.3 <sup>b,c</sup>	22.0 <sup>a</sup>	3.55	0.006	<0.001	<0.001

<sup>a,b,c,d</sup>LS-means differ ( $P < 0.05$ ; Tukey-Kramer adjustment).

<sup>1</sup>Three-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After).

<sup>2</sup>Treatments were:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

<sup>3</sup>Largest standard error of the mean (SEM).

<sup>4</sup>While in the lying position, the time in min per d a calf leaned to the left.

<sup>5</sup>The number of times per day calves napped for 2 or more minutes.

<sup>6</sup>The number of times a calf moved from not touching to touching the environmental enrichment device (EED) for at least 2 sec with 1 sec interval.

**Table 2. Percent of calves classified as normal or frequent<sup>1</sup> environmental enrichment device (EED) users for each treatment during the sample periods<sup>2</sup>**

Treatment <sup>3</sup>	Normal				Frequent			
	n <sup>1</sup>	% <sup>4</sup>	Expected <sup>5</sup>	Residuals	n	%	Expected <sup>5</sup>	Residuals
MOD-STEP	6	85.7	90	2.35	1	14.3	10	-2.35
HI-STEP	4	57.1	90	-5.66	3	42.9	10	5.66
HI-LATE	7	100.0	90	6.22	0	0.0	10	-6.22
HI-GRAD	5	66.7	90	-2.90	2	33.3	10	2.90

<sup>1</sup>Total number of calves that were categorized as normal (< 400 × per day) or frequent (> 400 × per day) EED users.

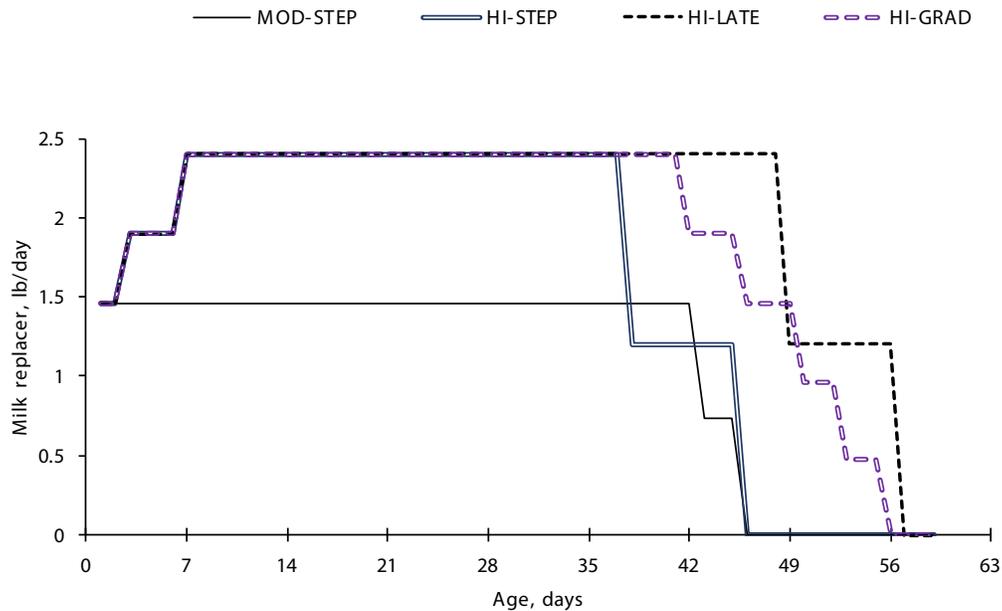
<sup>2</sup>Three-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After).

<sup>3</sup>Treatments were:

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

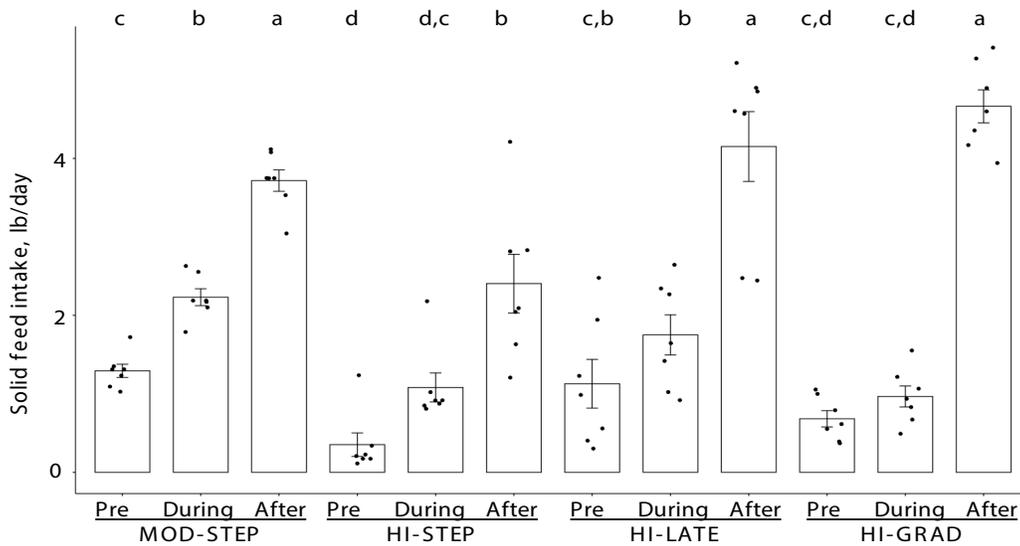
<sup>4</sup>Percent of calves in each treatment that were categorized as normal or frequent EED users.

<sup>5</sup>Expected percentage of calves for each group (normal and frequent) according to chi-square analysis ( $\chi^2 = 63.59$ ,  $P < 0.001$ ).



**Figure 1. Milk replacer timeline for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:**

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

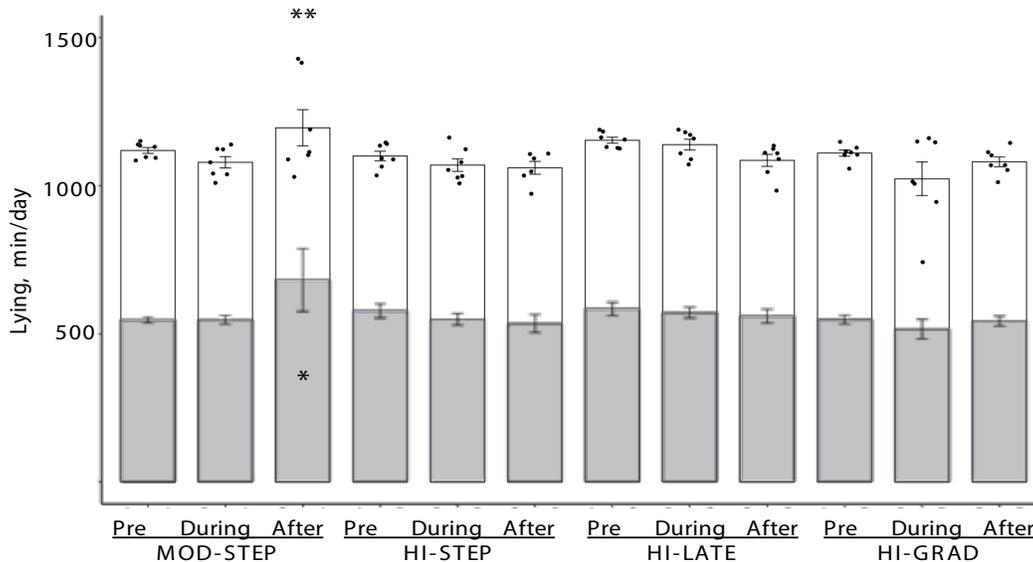


**Figure 2. Solid feed intake for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:**

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, and TRT × TIME were < 0.01.

<sup>a,b,c,d</sup>LS Means differ (*P* < 0.05; Tukey-Kramer adjustment). For each plot, the box represents the average for each treatment group during each time period, the lines represent the standard error of the mean, and the dots represent the distribution of individual calves.

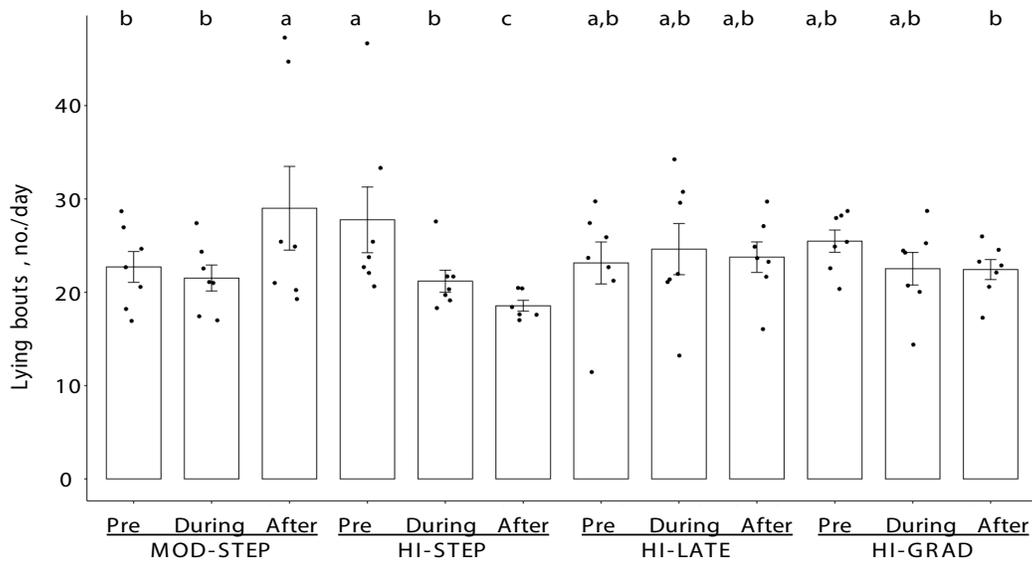


**Figure 3. Lying duration for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:**

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, and TRT × TIME were < 0.01.

\*\*Total Lying duration differs (*P* < 0.05; Tukey-Kramer adjustment); \*Lying percent on the left side differs (*P* < 0.05; Tukey-Kramer adjustment). For each plot, the whole box represents the average lying time for each treatment group during each time period; the gray box represents the percent of time spent lying more to the left than the right. The lines represent the standard error of the means. The dots represent the distribution of individual calves for total lying duration.

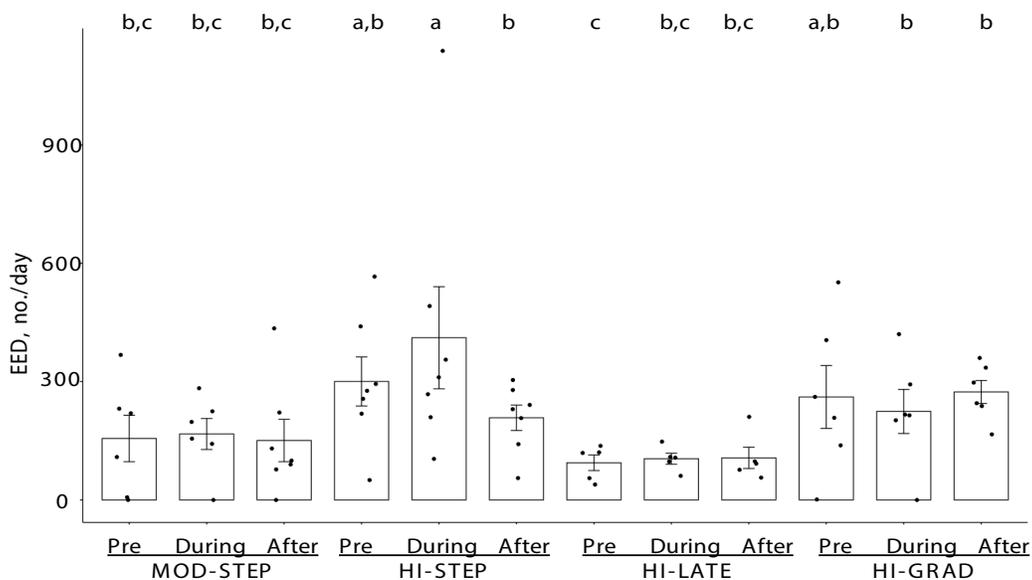


**Figure 4. Number of lying bouts for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:**

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, TRT × TIME were < 0.01.

<sup>a,b,c</sup>LS Means differ (*P* < 0.05; Tukey-Kramer adjustment). For each plot, the box represents the average for each treatment group during each time period, the lines represent the standard error of the mean, and the dots represent the distribution of individual calves.



**Figure 5. Environment Enrichment Device (EED) use for Holstein bull calves (n = 28) that were placed into 4 nutrition-weaning treatments:**

1. MOD-STEP (1.46 lb per day of milk replacer); 2-step weaned; initiated at age 6 weeks, completed 3 days later;
2. HI-STEP (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 5 weeks and completed 1 week later;
3. HI-LATE (2.4 lb per day of milk replacer); 2-step weaned; initiated at age 7 weeks and completed 1 week later; and
4. HI-GRAD (2.4 lb per day of milk replacer); 5-step weaning initiated at age 6 weeks; both feedings of the HI-GRAD calves were reduced in three 20% increments every four days, then the PM feeding was withdrawn for three additional days. Weaning was completed at age 8 weeks, by removing the AM feeding.

For this experiment, 3-day sample periods were collected before (Pre) and after the first step of weaning (During), as well as after the last step of weaning (After). *P*-values for TRT, TIME, and TRT × TIME were < 0.01.

<sup>a,b,c</sup>LS Means differ (*P* < 0.05; Tukey-Kramer adjustment). For each plot, the box represents the average for each treatment group during each time period, the lines represent the standard error of the mean, and the dots represent the distribution of individual calves.