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Holstein calves' preference for potential physical enrichment items on different presentation schedules

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ABSTRACT

Impoverished housing environments are thought to prevent motivated behaviors and may result in frustration. We first aimed to investigate an effective physical enrichment protocol to improve dairy calves' welfare and initially determine their use of various items. Thereafter, we aimed to determine dairy calves' preference for and ways of interacting with various items, and whether this was influenced by social housing. In experiment 1, at 21 ± 3 d of age, 27 individually housed calves were assigned alternately into 1 of 3 treatments: control (CON, no additional items), rotating enrichment (RE, one item each week on a rotating presentation schedule), and fixed enrichment (FE, 4 types of item at the same time). The items were stationary brushes, ropes, springs, nets filled with strawberry-scented hay, and dry teats. Calves' behaviors were observed from 4 to 7 wk of age using focal observations after feeding, followed by instantaneous scan sampling. Their behavioral responses to a novel object were assessed at 43 ± 1 d of age. In the instantaneous scans, calves in FE tended to interact with items more often than calves in RE. Calves in RE and FE expressed less non-nutritive oral behavior than those in CON. Latency to touch novel objects did not differ significantly between treatments. Calves in RE and FE interacted with nets filled with strawberry-scented hay more often than with other items in instantaneous scans. In experiment 2, 24 calves were assigned alternately into 8 individual pens and 8 pair pens at 2 d of age. All pens were provided with a stationary brush, plastic chain, net filled with strawberry-scented hay, and dry teat. Calves' behaviors were collected from 2 to 5 wk of age using instantaneous scan sampling. Calves interacted with nets filled with strawberry-scented hay more often than with other items. Pair housing reduced calves' interactions with items compared with individual housing. Individually and pair-housed calves' frequencies of overall interaction with items varied with time of day, with frequencies increasing to peaks at 0700, 1500, and 1900 h. Calves showed scratching, sniffing, sucking, butting, and hay intake toward nets filled with strawberry-scented hay and showed the first 3 behaviors toward stationary brushes, plastic chains, and dry teats. In conclusion, dairy calves are likely to prefer a fixed multi-item enrichment presentation schedule over a rotating schedule with a single enrichment item presented at one time. For the fixed multi-item enrichment presentation schedule, items were used more in individual pens than in pair pens, and a diurnal pattern was observed for use of the items. Nets filled with scented hay might be the most multifunctional and attractive item of the items tested.

Key words: animal welfare, dairy calf, social housing, environmental enrichment, behavior

INTRODUCTION

It is common to individually rear newborn dairy calves in relatively barren environments (Horvath et al., 2020). Such impoverished housing environments can restrict the expression of calves' natural behavior, which may lead to frustration (Mason and Burn, 2011). Environmental enrichment has been suggested to satisfy animals' species-relevant motivations and provide behavioral opportunities to control their environments (Van de Weerd et al., 2006).

Among the different environmental enrichment methods, adding items to animal enclosures has been implemented in many farm animals. Effective physical enrichment items are thought to have functional utilities (Newberry, 1995) and to facilitate the use of animals' behavioral skills (Mench, 1998). For example, in dairy cattle, mechanical brushes are effectively used to groom their bodies, particularly in places that are hard to reach. Use of brushes can keep animals clean and stimulate their grooming motivation and may reduce stress or frustration resulting from boredom (DeVries et al., 2007). For calves, brushes are consis-

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tently used to satisfy their natural grooming behavioral motivations (Pempek et al., 2017; Horvath and Miller-Cushon, 2019), which may help to compensate for the frequent grooming they would receive from their dam in natural conditions (Johnsen et al., 2015). Ropes may satisfy calves' oral manipulation (Zobel et al., 2017). Teats are used to release calves' sucking motivation and reduce their non-nutritive oral behaviors, including sucking peers or fixtures (Rushen and de Passillé, 1995; Jung and Lidfors, 2001; Veissier et al., 2002), as well as stimulate them to secrete more hormones related to satiety (insulin and cholecystokinin), which may help them relax (de Passillé et al., 1993). Providing part of the feed ration through a feed net is thought to promote calves to engage in feed collection and serve as a measure to increase the naturalness of feeding behavior (Mandel et al., 2016). Because calves can use their sensorial perceptions to choose palatable feeds (Baumont, 1996; Miller-Cushon et al., 2014), spraying heifers' preferred aroma of red berry (Meagher et al., 2017) on the feed ration may further stimulate their interest in the feed. However, it is still not clear which of the items mentioned above provide the most stimulation. Because social housing is growing in popularity (James and Machado, 2013) and animals housed in the same environment may mimic each other's behaviors (Galef, 1988), it is worth studying whether social housing can further increase the use of physical enrichment items.

In addition to satisfying these motivations, another characteristic of effective physical enrichment items is reliably attracting and sustaining an animal's interest (Jones et al., 1991). Ways of presenting items (e.g., alternately or simultaneously) have been suggested to play an important role in item use. Renewing items (replacing familiar items with novel items) maintains the novelty of items and has been shown to sustain animals' interest in enrichment for a protracted period (e.g., Trickett et al., 2009; Roy et al., 2019); thus, providing items to calves on a rotating presentation schedule may maintain their interest and provide experience with exposure to novel, harmless stimuli. However, some items can consistently satisfy animals' motivation to perform certain behaviors. For instance, heifers and adult cattle do not habituate to scratching enrichment devices and ropes (Wilson et al., 2002; Stanford et al., 2009). Therefore, calves may not habituate to these types of items and may use them intensively over long periods. Providing enrichments on a rotating schedule may not be a practical way to satisfy these motivations, given that farmers are unlikely to have several different items to target each and providing multiple items on a rotating schedule would increase labor. We therefore wished to compare the effects of multiple, fixed enrichments with a rotating single item enrichment protocol.

The first aim of the present study (experiment 1) was to investigate an effective physical enrichment protocol to promote dairy calves' use of the items (stationary brushes, ropes, springs, dry teats, and nets filled with strawberry-scented hay), to improve their welfare and initially determine dairy calves' use of various items. In experiment 1, we hypothesized that (1) compared with providing a single item on a rotating presentation schedule, providing the items in combination throughout the period would stimulate more interactions with items and result in a greater reduction of non-nutritive sucking because items eliciting oral manipulation would always be present; (2) the rotating presentation schedule of a single enrichment item would be most effective in reducing fear of novelty through more frequent exposure to novel stimuli; (3) nets filled with strawberry-scented hay would be used more often than other items because they might offer more complex stimulation and provide extrinsic reinforcement (hay consumption). The second aim of the study (experiment 2) was based on the first aim to determine dairy calves' preference for and ways of interacting with various items in the effective physical enrichment protocol, and whether this was influenced by social housing. It would contribute to our understanding of mechanisms underlying the improvement of dairy calves' welfare by these items. For experiment 2, we hypothesized that (1) nets filled with strawberry-scented hay would be used more often than other items in both individual and pair pens; (2) compared with individual housing, pair housing would promote calves' interactions with items because it could promote social facilitation; (3) calves' interactions with items would show a circadian rhythm associated with feeding patterns because they might be more active around feeding times; and (4) calves would show different interaction behaviors toward individual items to satisfy their diverse behavioral motivations.

MATERIALS AND METHODS

Experiments 1 and 2 were conducted at the Centre for Dairy Research, University of Reading (Reading, UK). Both experiments were approved by the ethics administrator at the university and the departmental ethics coordinator. All procedures complied with the guidelines for the *Ethical Treatment of Animals in Applied Animal Behavior and Welfare Research* (Sherwin et al., 2017).

Experiment 1

Animals, Housing, and Feeding. Twenty-seven female calves (pure registered Holsteins) with birth weights (mean ± SD) of 40.43 ± 5.94 kg were indi-

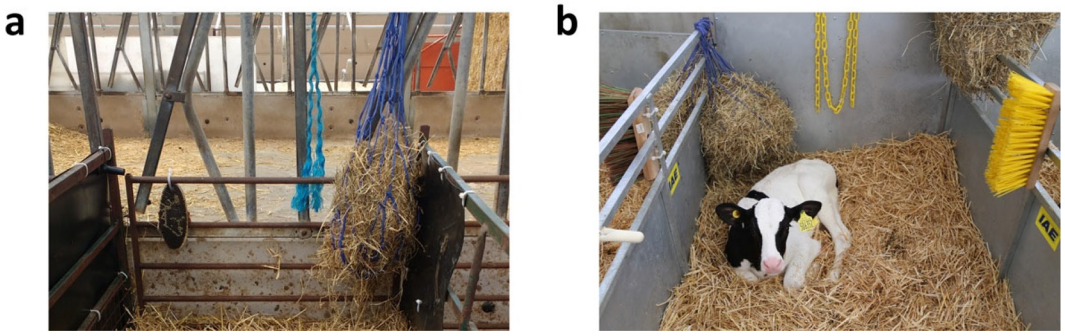

Figure 1. Physically enriched individual pen in (a) experiment 1, including stationary brush, rope, dry teat (black), and net filled with strawberry-scented hay, and (b) experiment 2, including stationary brush, plastic chain, dry teat (white), and net filled with strawberry-scented hay.

vidually housed from 3 ± 3 d of age to 49 ± 3 d of age in individual pens (2.0 m^2 each). Calves could have limited tactile contact with their neighbors above the panels and through the gaps between the panels. At 21 ± 3 d of age, calves were assigned alternately into 1 of 3 treatments: (1) control (**CON**), providing no additional items for the full study period; (2) rotating enrichment (**RE**), providing one type of item each week on a rotating presentation schedule for 4 wk; or (3) fixed enrichment (**FE**), providing 4 types of item at the same time for 4 wk. These protocols were chosen as practical ways that farms might implement enrichment. The items given in this experiment were stationary brushes (170 mm long, 65 mm wide, a combination of horse hair and pig bristles; Robinsons Equestrian), either ropes (nylon rope, 20 mm in diameter) or springs (flexible nylon tubing, 5 mm in diameter; Altec Extrusions Ltd.), dry teats (black rubber teat, 25 mm in diameter, 100 mm long; Tanner Trading Ltd.), and nets (Fine Mesh Haynet, 760 mm long, 4-kg capacity; Robinsons Equestrian) filled with strawberry-scented hay; the strawberry flavoring (Sainsbury's) was sprayed on ryegrass hay in nets every 2 d. Items were secured on the bars of the front or side panel, 800 mm away from the floor (Figure 1a). In RE, all 9 calves received stationary brushes, dry teats, and nets filled with strawberry-scented hay; 4 received ropes and 5 received springs as their fourth enrichment. In FE, all 9 calves received stationary brushes, dry teats, and nets filled with strawberry-scented hay; 5 received ropes and 4 received springs. Ropes and springs were hung vertically and were of similar length when hanging untouched; these last enrichments were not considered to have any specific biological relevance but could be orally manipulated and were tested as potential practical items that might provide general enrichment. All items were checked daily and cleaned if needed; hay nets were refilled if substantial amounts of hay were missing. The concrete floor of every pen was bedded with deep straw. Calves were fed milk replacer twice per day at 0800 and 1500 h using teat buckets. Calves were fed 6 L of milk replacer per day until 7 ± 3 d of age and after 28 ± 3 d of age. Calves were fed 8 L of milk replacer per day between 7 ± 3 d of age and 28 ± 3 d of age. Calves also had ad libitum access to concentrate, plain hay, and water throughout the study period.

Home Pen Behaviors. Calves' behavioral interactions with items and non-nutritive oral behaviors (defined in Table 1) were directly observed and recorded by observers when calves were 4, 5, 6, and 7 wk of age. Calves were observed 3 times per week for 2.5 h during an undisturbed period of the afternoon (1200 to 1425 h) using instantaneous scan sampling at 5-min intervals, with observers slowly walking down the aisle and recording the behavior before they reached the individual's pen. Calves were also continuously observed twice per week for 10 min immediately after morning milk feeding using focal sampling, with feeding being staggered such that one observer watched only 1 or 2 calves at a time.

Novel Object Tests. Fear can be elicited by events' characteristics of novelty and presentation method (Forkman et al., 2007). Currently, fear is often assessed through response to novelty (neophobia) in novel object test (Meagher et al., 2016). In this experiment, novel object tests were carried out when calves were 43 ± 1 d of age. Calves were tested sequentially in birth order. One calf at a time was walked to a testing pen (3.33 m^2) with solid sides such that calves were visually isolated from other subjects but still had some auditory contact. After 5 min to habituate to this environment, a novel object (black and white rubber disks hanging from a string at approximately calf eye level or just above) was then extended into the pen on a rod. The duration of latency to touch the object and the frequencies of vocalizations and retreats from the object over a 10-min test period were video recorded as indicators of fear.

Table 1. Ethogram of behavioral interactions with items and non-nutritive oral behaviors

Category	Behavior	Definition
Items including stationary brushes, ropes, springs, plastic chains, dry teats, and nets filled with strawberry-scented hay	Item scratching[1]	Putting head, neck, or body in contact with the items and slightly moving back and forth or up and down
	Item sniffing[1]	Putting muzzle in contact with or less than one muzzle length from the items with neck not relaxed
	Item sucking[1]	Licking, sucking, or biting the items
	Item butting[1]	Standing and butting head against the items in a playful manner
	Hay intake[1]	Chewing hay from nets filled with strawberry-scented hay
Non-nutritive oral behaviors	Non-nutritive sucking	Licking, sucking, or biting any fixture except the items
	Cross-sucking	Sucking or biting toward ear, mouth, navel, or other body parts of other calves

[1]Behaviors toward items were only recorded in experiment 2.

Experiment 2

Animals, Housing, and Feeding. Twenty-four male calves (pure registered Holsteins) with birth weights (mean ± SD) of 43.90 ± 4.80 kg were included in this experiment from 2 to 42 d of age. At 2 d of age, calves were assigned alternately into 1 of 2 treatments: (1) physically enriched individual pens (**IP**; n = 8, 2.4 m^2 each), 1 calf in each pen with 1 stationary brush (330 mm long, 72 mm wide, plastic bristles; O'Donovan Engineering Co. Ltd.), 1 plastic chain (25 mm diameter), 1 dry teat (white rubber teat, 25 mm diameter, 100 mm long; Tanner Trading Ltd.), and 1 net filled with strawberry-scented hay; or (2) physically enriched pair pens (**PP**; n = 8, 4.8 m^2 each), 2 calves in each pen with 2 stationary brushes, 2 plastic chains, 2 dry teats, and 1 net filled with strawberry-scented hay. Items were secured on the bars of the side or back panel, 800 mm away from the floor (Figure 1b). All items were checked daily and cleaned if needed; hay nets were refilled if substantial amounts of hay were missing. Calves could have limited tactile contact with their neighbors through the gaps between the panel bars. The concrete floor of every pen was bedded with deep straw. Calves were fed milk replacer twice a day at 0700 and 1500 h using teat buckets. Calves were fed 5 L of milk replacer per day until 14 d of age, followed by 6 L of milk replacer per day between 15 and 42 d of age. Calves also had ad libitum access to concentrate, plain hay, and water throughout the study period.

Home Pen Behaviors. Calves' behavioral interactions with items (defined in Table 1) were recorded by closed circuit cameras (Transit-PTZ, Revader Security Ltd.) for 14 h (0600 to 2000 h) twice weekly when calves were 2, 3, 4, and 5 wk of age. Calves were observed during daytime because they are more active during this time period (e.g., Zobel et al., 2017). Video recordings were played using Windows Media Player (Microsoft Corp.) and data recorded using Excel (version 16.53, Microsoft Corp.) by one observer watching the video recordings using instantaneous scan sampling at 5-min intervals.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics (version 27.0.1.0, IBM Corp.). Significant differences were declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Experiment 1. Use ratios (**UR**; defined as frequency or duration of calves' interaction behaviors toward items divided by frequency or duration of all behaviors) of overall items, UR of individual items, and ratios of non-nutritive oral behaviors for every calf collected using instantaneous scans. Continuous focal observations were first calculated by averaging UR of overall items, UR of individual items, and ratios of non-nutritive oral behaviors across the 12 testing days due to the very large number of zeroes.

Generalized linear mixed models were used to compare UR of overall items in different physical enrichment protocols. For both instantaneous scans and continuous focal observations, the fixed factors were enrichment treatments (RE and FE) and calves' birth weight. The random factor was age span (i.e., how many weeks of life were included in the data for that calf). The Satterthwaite method was used to calculate degrees of freedom.

Generalized linear mixed models were used to compare UR of stationary brushes, ropes, springs, dry teats, and nets filled with strawberry-scented hay. For instantaneous scans, the subject was calves' ID number; the repeated measure was individual items. The fixed factors were individual items, enrichment treatments (RE and FE), interactions between both factors, and calves' birth weight. The random factor was calves' ID number. For continuous focal observations, the subject was calves' ID number; the repeated measure was individual items. The fixed factors were individual items, enrichment treatments (RE and FE), interactions be-

tween both factors, and calves' birth weight. The random factor was calves' ID number and age span. The Satterthwaite method was used to calculate degrees of freedom, and a post hoc test of least significant difference (**LSD**) was carried out to identify differences among individual items.

Generalized linear mixed models were used to compare ratios of non-nutritive oral behaviors in enriched and nonenriched environments. For instantaneous scans, the fixed factors were all treatments (CON, RE, and FE) and calves' birth weight. For continuous focal observations, the fixed factors were all treatments (CON, RE, and FE) and calves' birth weight. The random factor was age span. The Sattherwaite method was used to calculate degrees of freedom, and LSD was used to identify differences among all treatments (CON, RE, and FE).

Seven of the 27 calves (2 from CON, 3 from RE, 2 from FE) could not be observed for the full period in continuous focal observations. To determine the interobserver reliability, 2 observers (**O**; O1 and O2) observed 22 calves together for 1 hour using instantaneous scan sampling at 5-min intervals. Another observer (O3) also observed the calves with the 2 observers in the first 30 min of the observation. The reliability between every pair of observers was compared using Cohen's kappa (**κ**). According to Landis and Koch (1977), O1 and O2 had substantial reliability ($\kappa = 71.97\%$; $P < 0.001$), O1 and O3 had almost perfect reliability ($\kappa = 87.27\%$; $P < 0.001$), and O2 and O3 had substantial reliability ($\kappa = 78.18\%$; $P < 0.001$).

Latencies to touch the novel object were non-normal, and a log transformation was therefore applied so that the assumptions of parametric tests were met. Data were then analyzed for differences between housing treatments using ANOVA. One calf from the FE treatment was not recorded because of a recording error with the camera. The details for vocalizations and retreats from the object are reported in the Supplemental Material (https://data.mendeley.com/datasets/3ttdsjj2pn/1; Zhang et al., 2022).

Experiment 2. The UR of individual items for every calf in every testing week was collected using instantaneous scans. A generalized linear mixed model was used to compare UR of stationary brushes, plastic chains, dry teats, and nets filled with strawberry-scented hay and to determine the effect of pair housing. The subjects were pen number and calves' ID number; the repeated measures were calves' week of age and individual items. The fixed factors were individual items, pair housing (IP or PP), interactions between both factors, calves' birth weight, milk refusal during the testing days, and average temperature of the barn during the testing days. The random factors were pen number, calves' ID number, and calves' week of age. The Satterthwaite method was used to calculate degrees of freedom, and the least significant difference (LSD) test was used to identify differences among individual items.

The UR of overall items across hours for every testing day was collected using instantaneous scans. The data of every calf between 0600 and 1959 h were categorized into fourteen 1-h periods: 0600 h (i.e., 0600 to 0659 h), 0700 h (i.e., 0700 to 0759 h), and so on. Descriptive statistics were run to calculate means of UR of overall items for every 1-h period for the 8 testing days.

Calves' behavioral ratios toward stationary brushes, plastic chains, dry teats, and nets filled with strawberry-scented hay were defined as times of calves' interaction behaviors toward these individual items divided by total number of times observed. Descriptive statistics were run to calculate means of behavioral ratios and coefficient of variations for the 8 testing days.

Because of navel inflammation in one calf from PP, behavioral interactions with the items at 3, 4, and 5 wk of age for this calf were discarded before analysis. Due to a technical problem, behavioral interactions with the items for 2 calves (1 from IP, 1 from PP) at 2 wk of age were not recorded. To determine the interobserver reliability, another observer who was blind to the hypothesis under test watched the video recordings of 4 calves by randomly choosing 1 wk of data from 2, 3, 4, and 5 wk of age for each calf. The reliability between the 2 observers was compared using Cohen's κ, which indicated substantial reliability ($\kappa = 71.80\%$; $P < 0.001$).

RESULTS

Experiment 1

Use of Overall Items in RE and FE. In the instantaneous scans, calves in FE tended to spend more time interacting with overall items than those in RE ($F_{1,15} = 3.51$, $P = 0.081$; Figure 2a). In the continuous focal observations after feeding, no significant differences in interacting with overall items were found between calves in RE and FE ($F_{1,15} = 0.356$, $P = 0.560$; Figure 2b).

Non-nutritive Oral Behaviors. In the instantaneous scans, calves in RE and FE spent less time expressing non-nutritive oral behaviors than those in CON ($F_{2,23} = 8.34$, $P = 0.002$; Figure 3a). In the continuous focal observations post-feeding, calves in RE and FE tended to spend less time expressing non-nutritive oral behaviors than those in CON ($F_{2,23} = 2.69$, $P = 0.089$; Figure 3b).

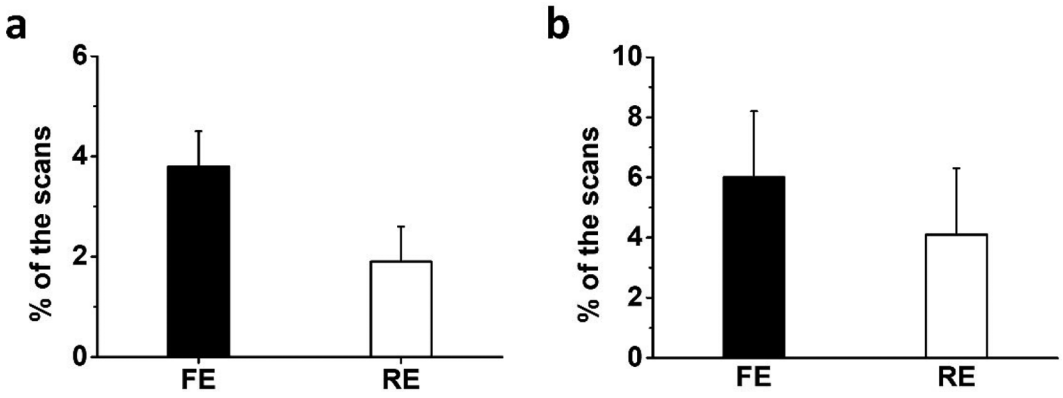

Figure 2. Least squares means (±SEM) of use ratios of overall items for calves in pens with fixed enrichment (FE, n = 9 pens) and rotating enrichment (RE, n = 9 pens) in experiment 1 collected using (a) instantaneous scans in the afternoon (30 times/d), and (b) continuous focal observations after morning feeding (10 min/d).

Response to Novelty. Latencies (in seconds) to make contact with the novel object did not differ significantly between treatments. The back-transformed means (95% CI) were as follows: CON 45.1 s (19.8–103.6), FE 40.3 s (16.7–97.4), and RE 33.2 s (14.4–76.2) ($F_{2,23} = 0.15$, $P = 0.863$; n = 26). The results for vocalizations and retreats from the object are reported in the Supplemental Material (https://data.mendeley.com/datasets/3ttdsjj2pn/1; Zhang et al., 2022).

Calves' Preference for Individual Items. In the instantaneous scans, calves in RE and FE interacted with nets filled with strawberry-scented hay more often than with stationary brushes, ropes, springs, or dry teats ($F_{4,16} = 4.97$, $P = 0.008$; Figure 4a). In contrast, in the continuous focal observations post-feeding, calves in RE and FE spent similar amounts of time interacting with the individual items ($F_{4,15} = 1.22$, $P = 0.343$; Figure 4b).

Experiment 2

Calves' Preference for Individual Items and Effect of Pair Housing. There were no interactions between items and pair housing with respect to the incidence of uses of individual items ($F_{3,90} = 2.01$, $P = 0.119$). Calves interacted with nets filled with strawberry-scented hay more often than with stationary brushes, plastic chains, or dry teats, and calves interacted with stationary brushes more often than with plastic chains ($F_{3,92} = 35.81$, $P < 0.001$; Figure 5). Pair housing (PP) reduced calves' interactions with overall items compared with individual housing ($F_{1,84} = 6.14$, $P = 0.015$; Figure 6).

Hourly Distributions of Calves' Interaction with Overall Items. The frequency of calves' interaction behaviors with overall items changed throughout the day (Figure 7), peaking in the hours beginning at

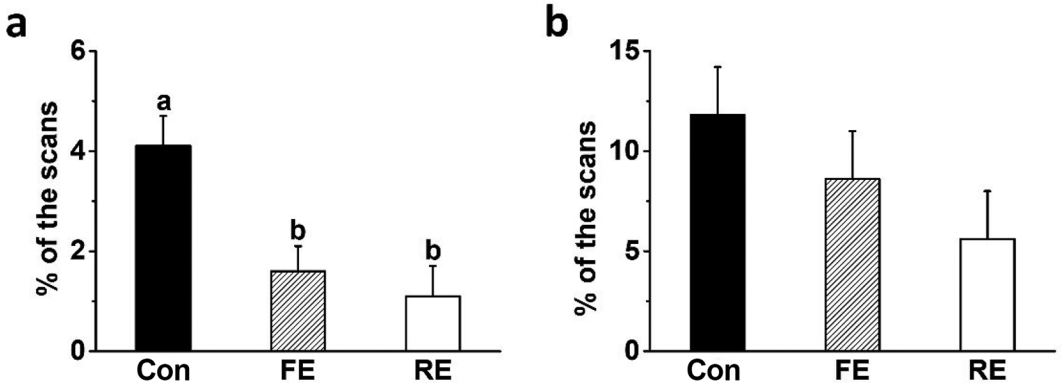

Figure 3. Least squares means (±SEM) of ratios of non-nutritive oral behaviors for calves in pens without additional enrichment (control, CON, n = 9 pens), with fixed enrichment (FE, n = 9 pens), and with rotating enrichment (RE, n = 9 pens) in experiment 1 collected using (a) instantaneous scans (30 times/d, afternoon) and (b) continuous focal observations (10 min/d, morning). Different letters (a, b) indicate significant differences between treatments at $P \leq 0.05$.

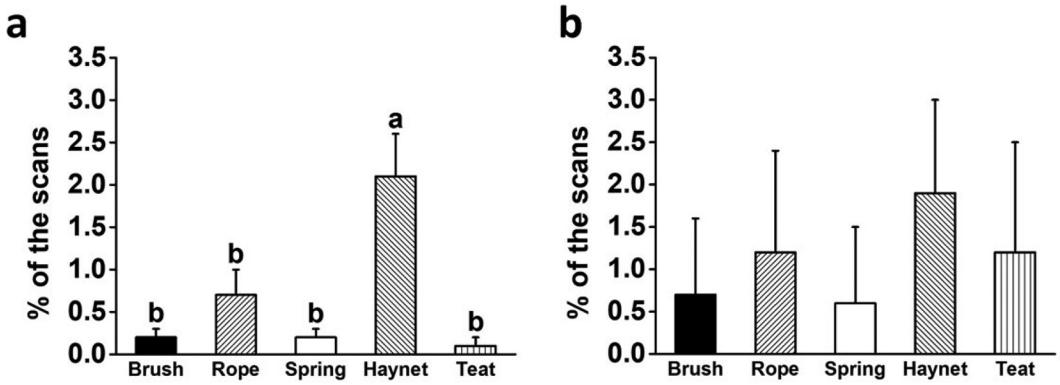

Figure 4. Least squares means (±SEM) of use ratios of stationary brushes, ropes, springs, dry teats, and net filled with strawberry-scented hay (haynet) for calves with rotating enrichment (n = 9 pens for stationary brushes, dry teats, and net filled with strawberry-scented hay; n = 4 pens for ropes; n = 5 pens for springs) and fixed enrichment (n = 9 pens for stationary brushes, dry teats, and net filled with strawberry-scented hay; n = 5 pens for ropes; n = 4 pens for springs) in experiment 1 collected using (a) instantaneous scans (30 times/d, afternoon) and (b) continuous focal observations (10 min/d, morning). Different letters (a, b) indicate significant differences between treatments at $P \leq 0.05$.

0700, 1500, and 1900 h, and falling to the lowest incidences at 1200 and 1600 h.

Calves' Interaction Behaviors Toward Individual Items. For stationary brushes (Table 2), calves spent the longest time scratching on them, with the rest of the time spent sucking and sniffing them. Calves sucked plastic chains for the longest time, followed by sniffing and scratching them. For nets filled with strawberry-scented hay, calves spent the longest time consuming hay from them, with the rest of the time sniffing, sucking, scratching, and butting the net. Calves sucked dry teats for the longest time, followed by sniffing and scratching them.

DISCUSSION

Our results suggest that multi-item FE and RE of a single item at a time were both effective protocols to reduce calves' non-nutritive oral behaviors, although FE tended to attract more single-item interactions than RE. Calves had a preference for nets filled with strawberry-scented hay, which seemed to be the most multifunctional item and stimulated a larger range of behavior types. Compared with IP, the PP treatment reduced calves' interactions with the items. Calves had a diurnal pattern of interaction with items in 3 peak periods (0700, 1500, 1900 h) every day.

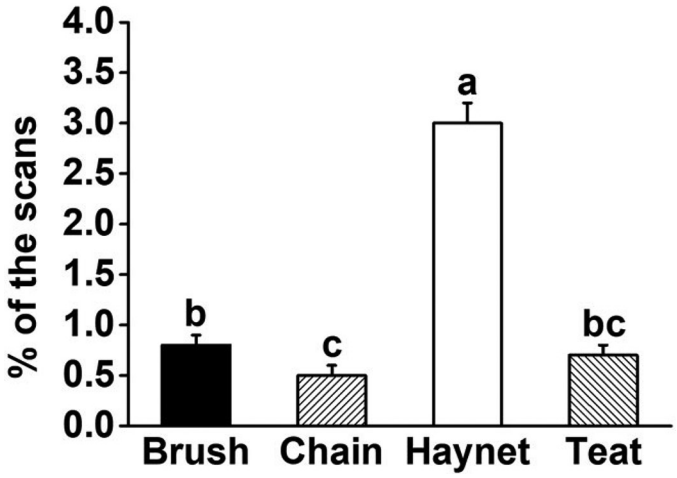

Figure 5. Least squares means (±SEM) of use ratios of stationary brushes, plastic chains, nets filled with strawberry-scented hay (haynet), and dry teats for calves in individual pens (n = 8 pens) and pair pens (n = 8 pens) in experiment 2 collected using instantaneous scans (168 times/d). Different letters (a–c) indicate significant differences between treatments at $P \leq 0.05$.

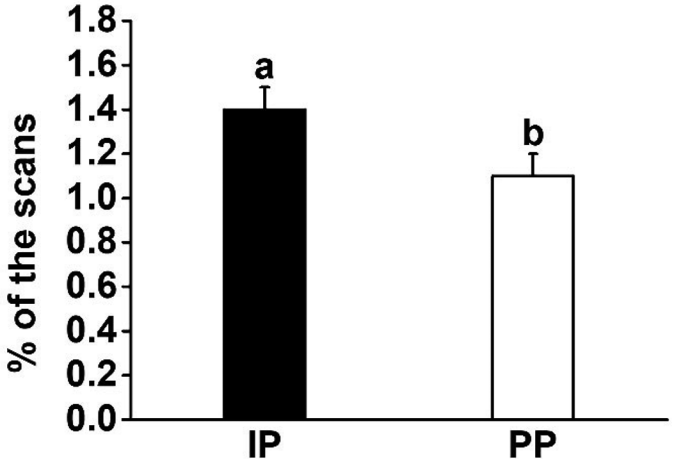

Figure 6. Least squares means (±SEM) of use ratios of overall items for calves in individual pens (IP, n = 8 pens) and pair pens (PP, n = 8 pens) in experiment 2 collected using instantaneous scans (168 times/d). Different letters (a, b) indicate significant differences between treatments at $P \leq 0.05$.

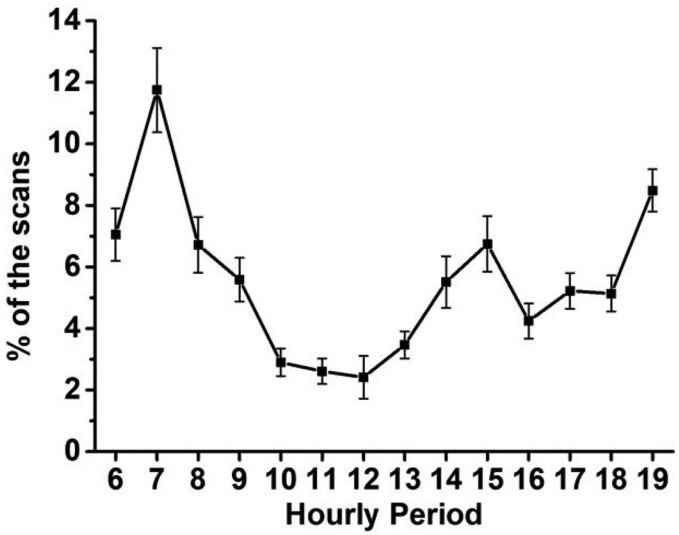

Figure 7. Means (±SEM) of use ratios of overall items by hourly period for calves in individual pens (n = 8 pens) and pair pens (n = 8 pens) in experiment 2 collected using instantaneous scans (168 times/d). Observation spanned from 0600 to 1959 h.

Enrichment Protocols

In experiment 1, all items provided were used no matter whether they were available alternately or simultaneously, which might indicate that both enrichment protocols are valuable for calves. Although neither protocol significantly reduced fear of novelty according to our measure, latencies were numerically lower in both enrichment protocols compared with controls, so there may be a welfare benefit that the present study did not have adequate power to detect. Because calves in FE tended to spend more time interacting with items than calves in RE, some properties of the enrichment protocol other than novelty may be more effective in maintaining calves' attention. This is in agreement with Trickett et al. (2009), who found that providing ropes and wood together for pigs elicited higher item interaction than providing the items in rotation. This may be explained as various items having different properties, which may be attractive in different and additive ways; by having all available at once, the calves make use of all or several of them throughout the day. In experiment 2, calves showed different principal behaviors toward stationary brushes, plastic chains, dry teats, and nets filled with strawberry-scented hay. The items might satisfy their intrinsic behavioral motivations of grooming, sucking, and feed intake, which is restricted or redirected in barren housing conditions (de Passillé, 2001; Khan et al., 2011; Zobel et al., 2017). Thus, compared with providing an individual item, providing those items in combination may lead to a cumulative effect. Moreover, calves' behavioral motivations of grooming, sucking, and feed intake may not be affected by the novelty of stimuli. For example, Horvath and Miller-Cushon (2019) suggested that brushes were consistently used by calves across weeks (4–7 wk of age). Hammell et al. (1988) indicated that calves having access to dry teats usually sucked them after milk feeding. Horvath and Miller-Cushon (2017) showed that calves consumed increasing amounts of hay with increasing age. Therefore, calves are likely to continue using these items and not lose interest due to habituation. Although we cannot exclude the possibility that having multiple items in the pen increases the chance a calf will interact with one at any given moment regardless of the item time (i.e., that providing 4 brushes would also increase interaction compared with a one-at-a-time rotating schedule), it seems likely that the increased overall use of enrichments in this treatment is due to their ability to satisfy these different, ongoing motivations.

Non-nutritive oral behaviors, including non-nutritive and cross-sucking, are nonfunctional and potentially harmful for calves (Le Neindre, 1993; Jensen, 2003) and are considered detrimental to calves' health and welfare (Babu et al., 2004). In experiment 1, calves in FE and RE expressed less non-nutritive oral behavior than calves in CON, which might indicate that the items used in both enrichment protocols could attract calves' attention and effectively reduce their undesirable behaviors. Because calves with FE and RE showed similar frequencies of non-nutritive oral behavior, FE did not show a cumulative effect on reducing non-nutritive oral behaviors. The finding is consistent with previous studies. Horvath et al. (2020) found that the provi-

Table 2. Descriptive statistics for behavioral ratios toward enrichment items in 24 calves averaged over 8 testing days each from 0600 to 1959 h in experiment 2

Item	Behavior	Ratio of all scans[1] (%)	CV[2] (%)
Stationary brushes	Scratching	0.3565	60.59
	Sniffing	0.1565	62.02
	Sucking	0.2427	66.31
Plastic chains	Scratching	0.0195	172.12
	Sniffing	0.1297	100.07
	Sucking	0.3475	83.31
Nets filled with strawberry-scented hay	Butting	0.0613	144.19
	Hay intake	2.5450	41.96
	Scratching	0.1885	76.40
	Sniffing	0.5885	66.06
	Sucking	0.2569	116.29
Dry teats	Scratching	0.0161	194.00
	Sniffing	0.0900	139.19
	Sucking	0.5850	91.07

[1]Ratio = times of an interaction behavior/times of all behaviors in calves × 100%.

[2]Coefficient of variation provides a measure of the dispersion of the means of each calf over the 8 testing days (the higher the %, the higher the variance between individual calves).

sion of brushes, hay, or brushes and hay all reduced teat-directed sucking, but all treatments showed similar pen-directed non-nutritive oral behaviors. Haley et al. (1998) also indicated that hay provision reduced the duration of teat-directed sucking. The results may indicate that the provision of an alternative outlet for oral behaviors to accommodate a greater range of behavioral expression can only partly satisfy calves' sucking motivations and cannot fully eliminate non-nutritive oral behaviors (Horvath et al., 2020). Future research should study other ways such as milk feeding methods to further reduce calves' undesirable behaviors.

Because calves fed ad libitum milk can drink around 9 L of milk per day (Jasper and Weary, 2002), the amount of milk replacer provided in both experiments was restricted to some degree. Limited milk provision has been reported to negatively affect calves' behavior. For instance, calves fed limited milk spent less time on locomotor play than calves fed more milk (Krachun et al., 2010; Jensen et al., 2015). However, the amount of milk consumed per se does not necessarily affect non-nutritive sucking; Rushen and de Passillé (1995) reported that halving the amount of milk that calves drink during a meal did not increase the amount of non-nutritive sucking that occurs after the meal. The performance of sucking behavior itself is more effective in reducing the underlying sucking motivation (de Passillé, 2001). When calves take longer to suck their milk; for example, due to use of teats with reduced milk flow rates (Haley et al., 1998), they do less non-nutritive sucking (Haley et al., 1998; Jongman et al., 2020). Because limited milk provision may lead to shorter milk durations, which can reduce time for secretion of cholecystokinin and other hormones to provide negative feedback during a meal, calves with limited milk provision may finish their meal before negative feedback occurs and thus show increased non-nutritive sucking (De Paula Vieira et al., 2008). Therefore, the calves in the current studies may have performed more oral behaviors toward the enrichments than calves on ad libitum schedules would; however, there was no obvious difference in their use between the calves in these 2 studies despite having different milk allowances.

The results of UR of overall items in continuous focal observations are inconsistent with the results in instantaneous scans in experiment 1. This may be because of the special testing time of the continuous focal observations. The test of continuous focal observations was implemented for 10 min immediately after morning milk feeding for every calf. Calves have a strong sucking motivation during this period (Loberg and Lidfors, 2001), which may suppress other behavioral motivations. Because calves with FE and RE were fed identical amounts of milk replacer through teat buckets, they might spend similar amounts of time interacting with items in their pens after milk feeding to release sucking motivation.

Calves' Interaction Behaviors Toward Individual Items

The intensity of behavioral interactions with items reveals their significance to an animal's key motivations (Van de Weerd and Day, 2009). In experiment 2, as predicted, calves expressed behaviors reflecting different key motivations toward individual items, spending the highest proportion of time scratching stationary brushes, sucking plastic chains and dry teats, and consuming hay from nets filled with strawberry-scented hay. The findings are in agreement with previous studies. Toaff-Rosenstein et al. (2017) reported that weaned heifers were motivated to use brushes to scratch their head and body. Veissier et al. (2002) showed that bucket-fed and teat-fed calves were motivated to suck dry teats after milk feeding. The circadian pattern found in experiment 2 also fits with this reported pattern of sucking motivation. Mandel et al. (2016) suggested that providing part of feed rations through feed nets could prolong feeding behavior. In addition to these key behaviors toward specific items, the same behaviors were performed to some degree using other items. For instance, calves showed scratching behavior toward plastic chains, dry teats, and nets filled with strawberry-scented hay. Calves expressed sucking behavior toward stationary brushes and nets filled with strawberry-scented hay. Calves also sniffed all the items. This may be partly due to calves' exploratory motivation, which can be stimulated when animal is in novel situations with restricted fear (Murphy, 1978). In experiment 2, calves spent the second highest proportion of time scratching nets filled with strawberry-scented hay (following stationary brushes), which may indicate that the hay net is an effective item to satisfy calves' scratching motivation. Calves spent a high proportion of time sucking stationary brushes and nets filled with strawberry-scented hay, in addition to plastic chains and dry teats. This may indicate that all the items were outlets for calves' sucking motivation and thus the items need to be kept clean to protect calf health and welfare. Calves spent more time sniffing nets filled with strawberry-scented hay than other items. This may indicate that besides exploratory motivation, calves' preferred aroma of red berry (Meagher et al., 2017) plays an important role in attracting their attention. Calves also showed butting behavior toward nets filled with strawberry-scented hay. Because butting has been considered as a play behavior (Jensen et al., 1998), its expression may indicate that nets filled with strawberry-scented hay can stimulate calves' play

motivation. Therefore, the items used in experiment 2 may be multifunctional, which can satisfy multiple behavioral motivations in calves.

Calves' Preference for Individual Items

In experiments 1 and 2, as predicted, calves interacted with nets filled with strawberry-scented hay more often than with other items. According to Table 2, nets filled with strawberry-scented hay could stimulate 5 types of interaction behaviors, whereas other items could only stimulate 3 types of interaction behaviors. This finding may indicate that nets filled with strawberry-scented hay have more characteristics than other items to stimulate calves' behavioral motivations. As different characteristics of an item may be synergistic and capture more interests of animals (Bracke et al., 2006), the multiple characteristics of nets filled with strawberry-scented hay may explain the increased interaction. Another potential reason is that these hay nets provided extrinsic reinforcement, which occurs when the performance of behavior leads to a consequence that is external to the behavior itself and increases the likelihood that the behavior will recur (Tarou and Bashaw, 2007). In dairy cattle, red berry flavoring was previously found to be a preferred aroma to increase the palatability of feeds (Meagher et al., 2017). Therefore, in experiment 2, spraying strawberry flavoring onto hay in nets may increase its palatability and promote an external outcome of hay consumption. It also suggested that compared with independently using aroma, in which cattle lose interest within days of using it (Wilson et al., 2002), using their favorite aroma for items that can lead to external outcomes may be a better presentation method. In contrast, stationary brushes, plastic chains, and dry teats did not result in external outcomes. Those items may be considered to provide intrinsic reinforcement, which occurs when simply performing a behavior increases the probability that the behavior will occur again (Hughes and Duncan, 1988). Tarou and Bashaw (2007) suggested that extrinsic reinforcement generally has a longer lasting attraction to animals than intrinsic reinforcement because the external outcome can increase the likelihood that the behavior will be performed again. Therefore, nets filled with strawberry-scented hay were used more often than other items.

Effect of Pair Housing

Galef (1988) defined social facilitation as "the initiation of a particular response while observing others engaged in that behavior." In experiment 2, we predicted that in PP, when one calf interacted with an item, the other one could observe the process and initiate a particular response toward an identical item; thus, pair-housed calves interacted with overall items more often than calves in the IP group. However, the results determined that pair housing reduced calves' interactions with overall items compared with individual housing, which is contrary to the prediction. It may be because the unrestricted social contact in pair pens takes up part of calves' active time and suppresses their interactions with overall items. Preweaning calves rest for large parts of the day (Horvath et al., 2020), and thus they may have limited time to show active behaviors. For pair-housed calves, they are attracted to each other and show unrestricted social contact (Jensen and Larsen, 2014). Compared with individually housed calves, they may spend part of their active time expressing social behaviors and spend less active time interacting with items overall. Another potential explanation is that in pair pens, one calf was dominant over a preferred item. Although 2 sets of items were provided to calves housed in every pair pen in experiment 2 to ensure that both calves in the same pens could interact with every type of item at the same time, stationary brushes and dry teats were fixed on opposite panels of the pens. This suggests that when one calf observed the companion calf interacting with a dry teat or a stationary brush, the calf might not see the other available identical item and not be triggered to interact with it.

Hourly Distributions of Calves' Interaction with Overall Items

In experiment 2, overall items were used most around milk feeding times, as well as at 1900 h. Similarly, Zobel et al. (2017) found that use bouts of rotating brushes and hanging ropes peaked around milk feeding times, and around 1800 and 1900 h. Miller-Cushon et al. (2013) showed increased hay consumption around milk feeding times. Pempek et al. (2017) reported that artificial teats, rubber chains, and calf lollies (pipes containing molasses) were used most in the 3 h following milk feeding. Therefore, milk feeding times are considered periods of increased activity for most behaviors (Horvath et al., 2020), such as sucking and nursing behaviors (Pempek et al., 2017). Because cattle are most active at sunrise and sunset (Albright, 1993), 1900 h may be another active time for calves. The patterns of use of overall items may be related to the redirection of motivations to engage in particular behaviors that cannot be satisfied in the environment.

CONCLUSIONS

Compared with RE, FE with multiple items might be a better protocol to improve dairy calves' welfare because it promoted more total item interactions and reduced non-nutritive oral behaviors. Dairy calves had a diurnal pattern with 3 peak periods for interacting with the items every day, 2 of which coincided with feeding times (0700 and 1500 h). The net filled with scented hay might be the most multifunctional and attractive of the items tested, given that dairy calves showed the most types of behavior toward it and had the most frequent interaction with it. Interactions with items were reduced by pair housing, suggesting that provision of the items to individually housed dairy calves may be more important for their welfare than to calves housed together.

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