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Full Length Article

The effect of tail paint formulation and heifer behavior on estrus detection

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ABSTRACT

Although heifers can have better conception rates than cows, they are still subject to poor estrus detection and economic losses from reduced reproductive efficiency. Tail paint has been successful in identifying estrus, but behaviors such a licking or rubbing have been believed to remove the paint and lead to false-positives. To investigate tail paint utilization and potential relationships among behaviors, eighteen Holstein heifers were randomly assigned to one of three treatments: a control tail chalk (**CON**), tail chalk with proprietary ingredient (**CHALK+**); and a spray formulation (**SPRAY**). Experimental design was a replicated 3×3 Latin square. Visual observations were performed in 30 min segments every 2 h from 6 AM to 6 PM. Ovaries were examined via ultrasound imaging on d 0, 7, and 9 of each period. The presence of follicles or a corpus luteum (CL) was recorded with their respective sizes. Heifers receiving SPRAY had a lower number of licks received per day and less tail paint removed regardless of day or follicle size when compared with CON or CHALK+. Rump lick received, chin rest received, anogenital sniff received, mount received, and both initiated and received behaviors for attempt to mount occurred more in heifers with large follicles regardless of day. Producers looking for heifers to breed should focus on those receiving rump lick, chin resting, anogenital sniff, mount, and attempt to mount. The use and combination of these estrus detection tools can improve reproductive efficiency in dairy operations.

1. Introduction

Similar to dairy cows, heifers experience reproductive challenges that contribute to economic losses due to delay of pregnancy resulting from poor estrus detection. It has been estimated that the US dairy industry loses approximately $300 million yearly to erroneous diagnosis and failure to detect estrus [1]. Although heifers usually have better conception rates than cows, with a mean rate of 57% in 2005 [2], failure in estrus detection and consequently breeding those animals, may lead to poor reproductive efficiency.

The use of tail paint as an estrus detection aid dates back to Victorian and New Zealand dairy farms in the late 1970's [3]. The paint strip method detects cows that are in estrus by indicating those which have been mounted, resulting in the tail paint being rubbed off. Using this estrus detection aid and visual observation, New Zealand herds had an AI rate > 90% [3]. Estrus detection efficiencies using a tail paint method have been reported to be >94% in heifers [4] and tail paint has been reported to have a higher sensitivity than heat mount detectors and activity monitors [5]. Tail paint has also been compared to other detection techniques such as visual observation and radiotelemetry, with no differences in efficiency or accuracy between the techniques [6–7]. One limitation of the tail paint system is the possibility of false-positives, when cows are detected by the tail paint to be in estrus but are not [8]. Tail paint has been shown to result in 5% false positives [3] which causes producers to doubt its efficacy for detecting estrus. Previous studies have involved enamel paint, tail chalk, and a combination of tail paint plus raddle marking [3,4,8]; however, few studies have compared multiple tail paint formulations. Therefore, this study did not try to analyze if tail paint is an adequate detection aid as has been frequently proven in literature, but instead aimed to compare different types of tail paint with behaviors typically used to detect estrus and those that may cause false-positives.

Behavioral studies have mainly focused on lactating dairy cow behavior, and most of the studies focusing on estrus behaviors in dairy heifers were done over two decades ago [9–11]. Traditionally, standing estrus has been defined as the period in which a cow makes no effort to escape when being mounted [12]. Thus,

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standing for mounting has been the primary sign of true estrus, but has been reported in very low frequencies in literature and can be easily overlooked [11,13]. In addition, it has been reported that some cycling animals have "silent heats" in which mounting behavior is not performed [14]. Therefore, observing other signs associated with estrus have generated higher estrus detection rates [15]. To aid the understanding of behaviors in dairy cattle, classifications have been made such as: estrus interactions, those which are associated with standing estrus in literature; antagonistic interactions, those that are aggressive or threatening to others; and social interactions, those that occur when an animal shows interest in another without any threatening, aggressive, or submission postures [11]. Social interactions (such as licking or rubbing behaviors) may lead to the removal of tail paint and consequently result in false-positives for estrus detection. Therefore, the main objective of this study was to compare the behaviors associated with 3 different types of tail paint formulations in Holstein heifers with an emphasis on social behaviors and the removal of tail paint.

2. Materials and methods

2.1. Animals and housing

The University of Illinois Institutional Animal Care and Use Committee approved all following experimental procedures. Eighteen (n = 18) Holstein heifers were balanced according to their age (13.7 ± 1.2 mo), BW 394 ± 32 kg, and BCS 3.43 ± 0.1, on a scale of 1 = emaciated to 5 = obese) and housed in free stalls with sand bedding and headlocks at the University of Illinois Dairy Cattle Research Unit (Champaign-Urbana, Illinois). All heifers received the same total-mixed ration fed once daily (~1200 h) to fulfill the requirements outlined by the 2001 National Research Council [16]. The experimental period was 6 wk.

2.2. Experimental design and treatments

The experiment was performed using a 3 × 3 replicated Latin square design with 3 animals per square and 6 total squares for 3 periods of 14 d each. The heifers were randomly assigned to one of 3 treatments in each period: control (**CON**), a commercially available chalk formulation; a chalk formulation with an added proprietary ingredient designed to discourage licking (**CHALK+**); or a commercially available formulation with the same ingredients as CON but with a spray paint consistency (**SPRAY**). All treatments were orange in color (All Weather PaintStick, LA-CO Industries, Elk Grove, IL). Treatments were refreshed once a day before feeding time. Old treatments were completely removed at the end of each period prior to application of the new treatment. Treatments were evaluated once per day before re-application to score the degree of tail paint removal (**TPR**). If no paint was removed from the previous day, the score was 0; if less than half was removed, the score was 1; and a score of 2 was given if more than half or all was removed (Fig. 1).

2.3. Estrus synchronization and follicle size

An Ovsynch protocol was used starting on d 0 of each period (d 0: GnRH: 2 mL of Factrel, Zoetis, Florham, NJ); d 7: PGF_{2a}: 5 mL of Lutalyse, (Pfizer Animal Health, New York City, NY); d 9: GnRH to stimulate periods of high and low interactions. The protocol was not used for timed AI, but as an attempt to stimulate groups of heifers for increased estrus behaviors. A protocol was used with a second shot of GnRH to increase the proportion of heifers showing estrus in d 10 [17–21]. All injections were given intra-muscularly in the rear leg. Ovaries were examined via ultrasound imaging using the Ibex Pro portable ultrasound (E.I. Medical Imaging, Loveland, CO) with L6.2 transducer (8-5 MHz 66-mm linear array, 12 cm scan depth) on d 0, 7, and 9 of each period. The transducer was inserted into the rectum and placed over the broad ligament and uterine horns to examine the ovaries. Both the right and the left ovaries were examined and images were captured to determine if structures were present. The presence of follicles or corpus luteum (CL) was recorded and Image J (U.S. National Institutes of Health, Bethesda, MD) was used to measure follicle size. All follicles were measured using an image with a known length of millimeter, measuring the pixels of the known length, and calibrating the scale from pixels to millimeter. Hormone injections and ultrasound were done prior to daily feeding.

2.4. Behavior observation

Each day, behavior was observed for 30-min every 2 h from 6 AM to 6 PM, for a total of 7 time-points per day. A total of 13 behaviors were observed, adapted from Sveberg et al. [13]. The following behaviors were not observed during this trial: avoid, threat, chase away, flehmen, bellow, follow, lean head, side mount, and head mount. Notes were taken to identify which heifer was the initiator or the receiver, with the exception of play rub, where the initiator and receiver could not be clearly distinguished. Definitions of all behaviors can be seen in Table 1. In attempt to give a more clear definition, we modified the following behaviors from Sveberg et al. [13]: winner, the initiator wins in an antagonistic interaction over a resource (such as feed or water) or an interaction in which the behavior cannot be defined, and the receiver (the loser) moves away or changes position. In addition, we included the following behavior and definition to fit the objectives of the study: paint lick (the initiator consistently licks the tail paint of the receiver). Videos were watched retrospectively by one person to verify the observations and record any missed behaviors. The behaviors were recorded as counts of occurrences.

2.5. Statistical analyses

All statistical analyses were performed using SAS (v9.4; SAS Institute Inc., Cary, NC). Behavior counts were summed for each 30-min time-point with 7 variables per day and TPR had just one variable per day: the score for the degree of product removal. For all analyses, the experimental unit was heifer. The frequencies of traits for all observation time-points in 3 periods were analyzed using PROC FREQ and graphs for 4 behaviors related directly to identifying how heifers respond to the tail paint treatments were generated (Fig. 2). The following behaviors were considered related to the treatments: paint lick, social lick, rump lick, and anogenital sniff. Paint lick was selected because it directly related to licking behavior and TPR. The other behaviors were selected because they may have been mistaken for paint lick or could have demonstrated heifers showing interest in the treatments. In addition, the frequency graphs shown were only for the received behaviors because the treatments on the receiving heifer were affected.

Behaviors were analyzed with a Poisson distribution in PROC GLIMMIX. The model contained heifer as a random effect and the fixed effects of period, treatment (when applicable), and week. Least squares means were calculated for tail paint treatments of related behaviors and a Tukey's adjustment was used for controlling multiple comparisons error rate. The incidence rate ratio was also determined for the aforementioned behaviors. The incident rate ratio represents the change in the first treatment when compared to the second treatment in terms of a percentage increase or decrease; with the percentage determined by the amount the rate ratio was above or below 1. The PROC MEANS procedure was used to estimate the mean frequency per week of the

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