ALTERNATIVE BAIT MARKER SYSTEMS FOR WHITE-TAILED DEER

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Abstract: We compared alternative bait markers for a study of free-ranging white-tailed deer (*Odocoileus virginianus*) based on the following criteria: 1) detectability in fecal matter; 2) incorporation into corn bait; 3) palatability; and 4) cost. We used penned sheep (*Ovis aries*) as an experimental model to evaluate Microtaggants, metallic flakes, plastic chips, and rare earth elements as bait markers, and molasses and soy lecithin as marker adhesives. The metallic flake-soy lecithin combination best met our criteria. It was also successful in a field study evaluating supplemental feeding on deer behavior and activity in central Wisconsin. Metallic flakes were easily detected under field conditions, readily adhered to shelled corn bait, enabled assessment of deer activity at distinct feeding sites and could be used in studies of feeding behavior and movements of other free-ranging herbivores.

***Key words*:** bait, deer, fecal, Lanthanum, marker, metallic flake, Microtaggant, plastic chip

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INTRODUCTION

Bait markers are substances included in bait or food to identify animal consumers (Fisher 1999). Bait markers have been used to study feeding behavior (Fall and Johns 1987), animal movements (Tuyttens et al. 2000), rate of digestion (Hartnell and Satter 1979), spatial configuration of mammalian territories (Delahay et al. 2000), and effectiveness of pharmaceutical compounds and toxic bait delivery (Tobin et al. 1996, Murray and Poore 2004). Bait markers include dyes (Doenier et al. 1997), fluorescent pigments (Cittandino and Kravetz 2000), particulate markers (Johns and Thompson 1979, Fall and Johns 1987, Tobin et al. 1996, Levey and Sargent 2000), antibiotics (Taylor and Lee 1994, Van Brackle et al. 1994) and various chemicals (Hartnell and Satter 1979, Larson et al. 1981). Cowan et al. (1984) identified several criteria for the evaluation of effective bait markers: 1) simple marker detection; 2) unaltered palatability; 3) unaltered health of animal consumers; 4) a variety of distinct bait marker patterns; and 5) optimal period of bait marker persistence in animals. However, these criteria can impose practical limitations on the effectiveness of bait markers, and researchers should consider alternative bait marking techniques for conducting field studies on free-ranging wildlife species.

Our goal was to identify bait markers that could be detected easily in feces of free-ranging white-tailed deer (*Odocoileus virginianus*). We evaluated 4 bait markers based on marker detection, marker adhesion, palatability, and cost. We used domestic sheep (*Ovis aries*) as an experimental model

to compare marking systems for corn baits fed to free-ranging white-tailed deer. Based on these results, we used metallic flakes adhered to shelled corn with molasses and soy lecithin during a field study investigating deer behavior at supplemental feeding sites and potential deer movement among feeding sites.

METHODS AND RESULTS

Bait Markers

We evaluated 4 marking systems (3 particulate and 1 chemical) that we expected to withstand winter field conditions in Wisconsin: Microtaggant particles, metallic flakes, fluorescent plastic chips, and rare earth elements (REE). Microtaggant Identification Particles® (Microtrace, LLC, Minneapolis, MN) are microscopic plastic particles made of melamine plastic and coded by colored layers. Particles can be identified with a standard or handheld 100X microscope. A fluorescent particle layer was included in the Microtaggants to enhance detection using a black light. Microtaggant applications have included tracing animal movement through recovery of the markers incorporated with animal feed, identification of toxic waste sources, and tracing explosives used in criminal activities (Johns and Thompson 1979). The disadvantages of Microtaggants as a bait marker include high cost and necessity of microscopic detection.

We also evaluated Glowble® (Metalflake, Inc. Salem, NH) flakes, a silver polyester-film with color coating, developed for use in automotive paint finishes. These inert particles have been used in bait marking studies and were relatively unaffected by passage through digestive tracts of rats (Fall and Johns 1987, Tobin et al. 1996). Detection of metallic flakes in feces is possible with the unaided eye; however, enhanced detection can be facilitated by scat-washing procedures described by Burns et al. (1995).

Plastic chips also have been used as a bait marker in studies of animal behavior (Delahay et al. 2000, Tuyttens et al. 2000) and rates of food passage (Remis 2000, Lambert 2002). We evaluated the use of plastic Light Chips® (Nocturn UV, Omaha, NE) composed of ultra violet reactants embedded in an acrylic layer.

Rare earth elements, are natural materials occurring at low levels in terrestrial environments. The REE lanthanum and samarium have previously been used in ruminant digestion studies. Feed or bait dosed with a lanthanum solution (10-40 ppm) is detectable in feces approximately 48 hours post-ingestion (Hartnell and Satter 1979, Shaver et al. 1986). Concentrations of REE in feces can be measured by mass spectrometry or Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Multiple REE can be used to provide marker variation (Hartnell and Satter 1979). Potential limitations of REE include non-visibility, cumbersome bait preparation, and costly analysis. Further product information for all our bait markers is included in Table 1.

We evaluated molasses and soy lecithin adhesives for particulate markers to shelled corn. Molasses is a common coating on corn supplied for feeding deer and is an animal attractant. Soy lecithin, composed primarily of phospholipids, glycolipids, and triglycerides, is safe for use in food products and is commonly used in gelatin capsules. Alcolec S® (American Lecithin Company, Oxford, CT.), the soy lecithin product used in our study, was used in a previous bait marking study in rats (Tobin et al. 1996).

Table 1. Evaluations of adhesive, marker type and concentration based on a 3-point Likert-based scale used in penned sheep study conducted at the Livestock Laboratory, University of Wisconsin, Madison, WI.

	1.0 % Molasses			1.0% Soy lecithin			
Criteria	0.3% Metallic flakes	0.3% Microtaggants	1.0% Plastic chips	0.5% Metallic flakes	0.1% Microtaggants	3.0% Plastic chips	0.3% Lanthanum
Marker size				0.44 × 0.38 mm	0.08-0.60mm	3.00 × 2.00 mm	NA
No. of marker variations				9 colors; some colors are a mixture of 2 flake colors	large number of unique codes available	9 colors	4; Lanthanum Samarium, Cerium, and Ytterbium most widely available
Marker recovery [a]	3	2	2	3	2	2	3
Marker adhesion [b]	1	1	1	3	3	2	NA [c]
Palatability [d]	3	amount consumed unknown	3	2	3	3	2
Marker cost/ 500 lb corn	$165	$735	$85	$275	$245	$255	$418 [e]

[a] Performance evaluation for marker recovery by aided or unaided eye: 1) not readily detectable 2) moderately detectable 3) easily detected
[b] Performance evaluation for marker adhesion: 1) none or few markers adhered to corn 2) approximately half adhered to corn 3) most or all adhered to corn
[c] Adhesive was not used in this treatment, degree of marker tenacity not measured
[d] Performance evaluation for palatability: 1) none of marked corn consumed 2) approximately half consumed 3) most or all consumed
[e] Value represents estimate based on Lanthanum costs alone, excluding analysis expense

Penned Sheep Study

We used 7 domestic sheep housed at the University of Wisconsin-Madison Livestock Laboratory to compare alternative bait markers and adhesives. We housed sheep individually during the feeding trial to evaluate consumption of marked shelled corn. We assigned 7 treatments to the 7 fasted sheep (n =1 for each treatment): 1) corn with Microtaggants (0.3% by weight) adhered with molasses; 2) corn with Microtaggants (0.1% by weight) adhered with soy lecithin; 3) corn with metallic flakes (0.3% by weight) adhered with molasses; 4) corn with metallic flakes (0.5% by weight) adhered with soy lecithin; 5) corn with plastic chips (1.0% by weight) adhered with molasses; 6) corn with plastic chips (3.0% by weight) adhered with soy lecithin; and 7) corn soaked in a lanthanum solution (Hartnell and Satter 1979). All treatments used an adhesive concentration of 1.0% by weight. To evaluate palatability, we provided each sheep 567 g of control (unmarked) corn and 567 g of marked corn, presented side by side. We measured total consumption of marked and control corn, determined by mass of remaining corn (g), 24 hours after feeding. Animals had *ad libitum* access to water and grain following initial feeding with bait markers.

A bag placed on each animal allowed collection of feces prior to feeding and at 12-hr intervals for 60 hours post feeding. We examined recovered pellets for marker detection. We visually evaluated metallic flakes and plastic chips in 5g of feces from each 12 hr fecal sample. Fecal samples collected prior to feeding and 48 hr post-feeding were analyzed for REE (lanthanum) at the University of Wisconsin Soils and Plants Analysis Lab using ICP-MS. We evaluated fecal samples from the Microtaggant treatments using a handheld microscope and a black light for fluorescence. We misted all treatments with water for 1 hour to evaluate marker adhesion. The bait markers and adhesives evaluated in this study had no adverse effects on animals or potential human consumers. Animal care and experimental procedures were approved by the University of Wisconsin Animal Care and Use Committee (Protocol A1139). We evaluated marker recovery, marker adhesion, and palatability of the treatments on a 3-point Likert-based performance scale described in Table 1.

The amount of corn consumed by the sheep fed the 0.3% Microtaggant-molasses treatment could not be determined as the feed dish (marked and control) was overturned. All remaining sheep consumed the entire control (unmarked) ration. The 0.1% Microtaggant-soy lecithin, 0.3% metallic flake-molasses, 1.0% plastic chips-molasses, and 3.0% plastic chips-soy lecithin treated corn was completely consumed within 24 hours. The 0.5% metallic flake-soy lecithin treated corn (34.1% remaining) and the Lanthanum treated corn (41.5% remaining) were not entirely consumed 24 hours post feeding.

We detected Microtaggants in fecal samples by 36 hours post-feeding. However, we found that undigested corn fluoresced under black light and could be misidentified as fluorescent layers of Microtaggants. Detection of Microtaggants was therefore limited to using a handheld microscope under natural light. In both metallic flake treatments, flakes appeared 36 hour post-feeding and were easily identified on the surface of the fecal pellets of the sheep

fed the higher concentration (0.5%). Despite the lack of fluorescence of the metallic flakes, the reflectance of the flakes facilitated visual detection. We observed fluorescent plastic chips within 26 to 48 hours post-feeding, but detection was difficult because chips were concealed within the fecal pellets. In addition, many chips remained in the feed dish post-feeding indicating either avoidance by the sheep or failure of the adhesive. We readily detected lanthanum in the fecal samples despite the incomplete consumption of the treated corn. The 48 hours fecal sample demonstrated an elevated concentration (1,322.6 ppm) of lanthanum compared with the background sample collected prior to feeding (0.3 ppm).

When adhesive-marker combinations were exposed to water mist for 1 hour, molasses failed to adhere the markers to the corn. In contrast, the soy lecithin adhesive was much more effective and all markers remained adhered when exposed to water. Two sheep consumed all of the corn with molasses adhesive, whereas the feed dish of the remaining sheep was overturned. Two of 3 sheep consumed the entire ration of corn with the soy lecithin. Considering the performance and cost of all the treatments, the metallic flake marker with soy lecithin treatment most adequately met the criteria for our field study (Table 1).

Free-Ranging Deer Study

Based on the findings from the sheep study, we further evaluated the metallic flake bait marker during a winter feeding study of free-ranging white-tailed deer at Sandhill Wildlife Research Area in central Wisconsin. Supplemental feeding occurred from December 2004 to March 2005. We applied metallic flakes and soy lecithin adhesive to shelled corn at 0.5% and 1.0% by weight, respectively. We subsequently coated marked corn in molasses to disguise the soy lecithin flavoring as 1 sheep in the penned study failed to consume all of the corn treated with soy lecithin. Before providing marked corn at sites, alfalfa hay was provided for a 2-week period to encourage habituation to the feeding sites. Six consecutive feeding trials consisted of a 12-day feeding period followed by an 8-day non-feeding period. We used uniquely colored metallic flake markers to detect animal movement among 4 different feeding sites separated by approximately 3.2 km.

We recorded deer use and consumption of corn at feeding sites using motion sensing digital cameras. We calculated deer-use minutes per trial at each site (deer-use minutes = number of deer × total minutes in feeding area) (Beringer et al. 2003). We conducted pellet surveys using transects extending 600 m in cardinal directions from each feeding site before (fall 2004) and after (spring 2005) experimental feeding trials. We established 4 m^2 circular plots at 50 m intervals along each transect (Neff 1968, Doenier et al. 1997), and collected all pellet groups to remove old pellets (fall survey) and measured deposition of new pellets (spring survey). In the spring survey, we also collected pellet groups containing bait marker within 1 m of transects. In addition, the area within 30 m of the feeding site was surveyed for marked pellet groups. Bait marker detected in the spring survey was used to evaluate animal movement among the feeding sites. We did not observe a decrease in deer use during the transition from alfalfa hay to corn marked with metallic

flakes at our feeding sites. Although corn was not previously present in Sandhill Wildlife Research Area, deer quickly acclimated to the corn marked with metallic flake-soy lecithin combination and palatability of corn bait seemed unaffected by this marking system.

We readily detected marked pellet groups by visual inspection during the spring pellet surveys. We recovered all marked pellet groups within 400 m of each feeding site; 63% of the marked groups were recovered within 50 m of the feeding site. We recovered 1 pellet group containing 2 distinct bait markers indicating movement of an individual between 2 feeding sites, likely within 48 hour. This movement was confirmed by camera surveillance which recorded a radio-collared male deer visiting 2 distinct feeding sites. Recovery of marked pellet groups was correlated with the annual deer-use ($r = 0.99$, $df = 2$, $P = 0.004$) and spring pellet density ($r = 0.90$, $df = 2$, $P = 0.09$) at feeding sites (Figure 1). The most active feeding site yielded the highest recovery of marked pellet groups, while sites with less deer-use resulted in substantially lower recovery of marked pellet groups.

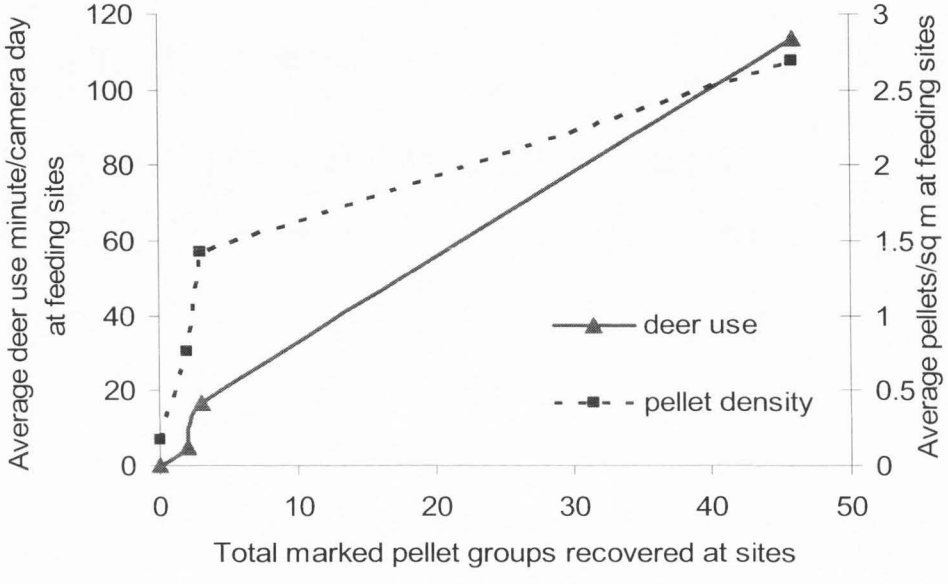

Figure 1. Average white-tailed deer use (deer-use minutes/camera day), estimated pellet density (pellets/m^2), and number of marked pellet groups recovered at 4 feeding sites in Sandhill Wildlife Area, Babcock, WI during fall-spring 2004-2005.

DISCUSSION

Our experimental feeding trials with domestic sheep showed that the metallic flake marker and soy lecithin adhesive was the most effective, reliable, and cost-effective marking combination. Microtaggants or REE may be feasible for small-scale or captive studies where specific research objectives justify the higher costs associated with these markers. However, lower detection or sophisticated analysis required for Microtaggants and REE markers make them impractical for large-scale field

studies. Plastic chips, while inexpensive, did not readily adhere to shelled corn and were difficult to detect in fecal material. In contrast, metallic flakes were comparatively inexpensive, persistent, and easily detected in fecal material of sheep and free-ranging white-tailed deer. With penned sheep, soy lecithin adhesive appeared to be more effective than molasses. However, our limited feeding trials could not exclude reduced palatability for the soy lecithin adhesive. As an alternative, we suggest that bait also be coated with molasses to disguise soy lecithin flavoring.

Our field study using the metallic flake marker in free-ranging deer confirmed the efficacy of this technique for studying deer behavior and movement. Metallic flake particles were easily detected with the unaided eye and provided efficient field identification. Further, white-tailed deer quickly acclimated to corn marked with metallic flake-soy lecithin and molasses. The association between the deposition of marked pellet groups and deer use of feeding sites demonstrated that this system might also be used to provide an index of deer activity and bait consumption.

Minor problems encountered with metallic flakes include minor contamination on most surfaces and clothing; therefore, slight contamination with different metallic flakes may occur during bait preparation, pellet collection, or inspection of retrieved pellet samples. Although metallic flakes are available in 9 colors, several of these colors are mixtures of 2 flake colors, posing limitations on the potential number of distinct markers. Metallic flakes are ephemeral markers allowing short-term temporal measurements. However, bait markers with longer retention time may be preferable for long-term marking studies. Bait marking with metallic flakes would be appropriate for large-scale studies tracking short-term animal movement, feeding behavior of animals, or delivery of vaccines or chemical substances to animals. Successful implementation of a bait marking system requires careful consideration of research design criteria including digestive transit time, animal-use patterns, proportion of bait marker in animal diets, and data collection effort.

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