Short communication: Effects of mesh leggings on fly pressure and fly avoidance behaviors of pastured dairy cows

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ABSTRACT

Ectoparasitic stable flies (*Stomoxys calcitrans* [L.]), horn flies (*Haematobia irritans* [L.]), and face flies (*Musca autumnalis* De Geer) negatively affect dry matter intake, milk production, and health of pastured dairy cows. These flies cause fly avoidance behaviors and are a major welfare concern for dairy producers. The objective of this study was to evaluate the effects of mesh Shoofly Leggings (Stone Manufacturing & Supply, Kansas City, MO) on fly avoidance behaviors and numbers of flies attacking pastured dairy cows. In a crossover design, lactating dairy cows ($n = 80$) were randomly assigned to groups with and without leggings (Shoofly Leggings worn on all legs). All cows were managed in one group. Cows were observed for 2-wk periods, and then treatments were reversed in the next 2-wk interval. Counts of stable flies, horn flies, and face flies on all cows were recorded twice daily (once in morning per cow: 0930 to 1230 h; and once in the afternoon per cow: 1330 to 1630 h), 3 times per wk on Tuesday, Wednesday, and Thursday of each week during the study period. The total number of flies per cow was greater on cows with leggings than cows without leggings. The number of horn flies per cow was greater on cows in with leggings (26.4 flies/side) compared with cows without leggings (24.1 flies/side). Stable fly numbers were similar for cows with and without leggings (12.8 flies/leg). A random subset of 20 focal cows per group was observed during 5-min intervals to record frequencies of 4 behaviors: leg stomps, head tosses, skin twitches, and tail swishes. Counts of head tosses (2.6 vs. 3.1), skin twitches (20.9 vs. 19.6), and tail swishes (21.3 vs. 19.3) were similar for cows without leggings versus cows with leggings, respectively. However, foot stomps were 39% lower for cows with leggings compared with cows without leggings, and leg

stomps were 26% higher in the afternoon than in the morning (2.9 vs. 2.4, respectively). A positive correlation was observed between stable and horn flies and all insect avoidance behaviors. Numbers of stable flies were 1.5 times greater in the afternoon than in the morning. The results of this study indicated that flies were associated with cow fly avoidance behaviors regardless of the use of leggings, but leggings effectively reduced foot stomps by 39%, so their use may provide some relief from stable fly injury to pastured dairy cows.

Key words: fly avoidance behavior, stable fly, dairy cow, pasture

Short Communication

Biting and nonbiting filth flies reduce the welfare, health, and economic vitality of dairy cattle. Biting stable flies (*Stomoxys calcitrans* L.) and horn flies (*Haematobia irritans* L.) puncture cattle skin, create a subsurface hematoma, and feed on blood for 2 to 5 min (Lavoipierre, 1965; Eicher et al., 2001). Stable flies attack cattle mainly on their legs, whereas horn flies occur more generally on their hosts' backs, flanks, and bellies (Moon, 2019). Nonbiting face flies feed on facial secretions of cows, which can spread pinkeye (Hall, 1984), but they will also feed at wounds caused by biting flies or other causes of skin trauma.

Stable flies caused an estimated \$360 to \$925 million annual loss to the US dairy industry from 2005 to 2009 through decreased milk production (Taylor et al., 2012). Additional losses are likely to involve reduced DMI and reduced feed conversion efficiency by lactating cows, reduced weight gain by growing replacement heifers, and losses in pasture quality and facility longevity when herds are bunched during hot weather (Campbell, 1976; Wieman et al., 1992). Losses attributable to horn flies and face flies remain to be quantified.

Filth flies are abundant on dairy farms because fly breeding media, such as fresh and aged manure, decaying hay, soiled bedding, and other moist OM, are abundant (Moon, 2019). Previous fly control research

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has focused on methods involving rigorous sanitation, sprays, dust bags, feed additives, pour-ons, insecticide ear tags, and vacuum fly traps. None of those treatments has proven to be effective for all types of flies, or superior for any one of them (Thomas and Skoda, 1995; Campbell et al., 2001; Kienitz et al., 2018).

Stable, horn, and face flies are typically most active from May to October in the Midwest United States, and are active year round in warmer climates. Stable fly feeding may occur at any time during daylight (Mitzmain, 1913), with the most activity occurring between 1000 h and 1600 h (Hoffman, 1968). Muscid flies rest in sunny sites when the air temperature is below 20°C and in shady sites when temperatures are above 30°C. In the northern United States, fly populations are restricted to a distinct breeding season because of the cold winters, and muscid fly populations typically peak in abundance of flies in later summer or early autumn (Moon, 2019).

Attacking blood-feeding flies elicit 5 fly avoidance behaviors from large mammals, including cattle and horses: foot stomps, head throws, skin twitches, tail swishes, and ear flicks (Dougherty et al., 1995; Mullens et al., 2006; Muzari et al., 2010; Mottet et al., 2018). Excessive foot stomping by horses can lead to hoof damage, dermatitis, laminitis, and stunted growth of the keratinous hoof tissue (Krinsky, 1976). Connections between hoof stomps and hoof health in cattle are less well understood. Recently, fly annoyance-reducing leggings became available for horses, and Mottet et al. (2018) reported that mesh leggings on horses reduced foot stomps and head throws. Those findings suggested that leggings might also be useful for pastured dairy cows. Thus, the objective of this study was to evaluate the effects of fly protectant leggings on fly abundance and fly avoidance behaviors of pastured dairy cows.

Our study was conducted at the 250-cow grazing dairy at the University of Minnesota West Central Research and Outreach Center in Morris, Minnesota, from June to September 2017. The University of Minnesota Institutional Animal Care and Use Committee (#1607-33960A) approved all procedures involving animal care and management.

Cows were housed on pasture 22 h per d and were milked twice daily at 0600 and 1700 h in a swing-9 parabone milking parlor. The pastures consisted of multispecies perennial grasses and legumes, including smooth brome grass (*Bromus inermis* L.), orchard-grass (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* L.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and kura clover (*Trifolium ambiguum* L.). A total of 80 cows used in the study were rotationally grazed at a stocking rate of 3 cows per ha. Cows rotated to a new paddock every 2 d based on for-

age availability. Forage height of new paddocks ranged from 17 to 33 cm and forage refusal height ranged from 7 to 9 cm. In addition to pasture, each cow was supplemented with 2.72 kg of organic corn daily with ad libitum access to free-choice minerals and water.

The 80 lactating Holstein and crossbred dairy cows (Heins et al., 2010) were randomly assigned to 2 treatments groups: leggings (Shoofly Leggings worn on all legs) or control (no leggings). Cows were balanced across treatments by parity, breed, and calving date. Shoofly Leggings are a flexible, plastic mesh, loose-fitting, and chemical-free legging that is marketed to equestrians to improve horse comfort (Stone Manufacturing & Supply, Kansas City, MO). The leggings were orange. Following a crossover design, cows were exposed to their assigned treatments for a 2-wk period, and then switched to the alternative treatment during the next 2 wk, and that design was repeated in June, July, August, and September.

One trained observer identified and counted flies on all 80 cattle in the study. Stable flies on front and back legs were recorded as the mean number of stable flies per leg (on front and back legs; only stable flies that had direct contact with the cow), horn flies per animal's side, and face flies per animal's face. Counts of flies were randomly recorded from cows regardless of treatment and were made once in the morning (0930 to 1230 h) and again in the afternoon (1330 to 1630 h) on Tuesday, Wednesday, and Thursday of each week. Counts were recorded once all cows returned to the pasture from the milking parlor, and counts included those flies on the leggings.

A second trained observer counted fly avoidance behaviors displayed by a subset of 20 focal cows in each group, at the same time as the observer that counted flies. Each cow was observed once per time period (morning and afternoon) for a total of 2 observations per day. Focal cows were identified randomly by parity and breed, and the same cows were used for the entire study. Observers distinguished horn flies from face flies and stable flies. Horn flies were counted individually or in groups of 10 on one side of each cow. Face flies were counted on the cows' faces viewed head on. Stable flies were counted on the visible faces of front and rear legs separately. Fly counts were made from a distance of 2 to 4 m (Denning et al., 2014; Kienitz et al., 2018). For each animal, the observer first counted stable flies, horn flies, and face flies as above, then counted behavioral events in 5-min intervals or durations per cow, and then repeated fly counts at the end of the interval. Events were head tosses, skin twitches, tail swishes, front leg stomps, and back leg kicks. A head toss was recorded if the animal's head was thrust back toward its body, far enough that the nose crossed an imaginary plane

Table 1. Least squares means and 95% CI for counts of 3 species of filth flies on pastured dairy cows without versus with leggings and in the morning versus the afternoon¹

Item	LSM (95% CI)			
	Horn fly (flies/side)	Stable fly (flies/leg)	Face fly (flies/face)	Total flies
Treatment				
Control	24.1 (21.3–27.2)	12.8 (11.6–14.1)	0.25 (0.21–0.29)	37.7 (34.0–41.7)
Leggings	26.4 (23.4–29.8)	12.8 (11.6–14.1)	0.27 (0.23–0.31)	40.1 (36.2–44.4)
<i>P</i> -value of difference	0.003	0.96	0.33	0.02
Time of day				
Morning	25.5 (22.6–28.8)	10.6 (9.6–11.7)	0.26 (0.23–0.31)	36.8 (33.2–40.8)
Afternoon	24.9 (22.1–28.1)	15.4 (14.0–16.9)	0.25 (0.22–0.29)	41.0 (37.0–45.4)
<i>P</i> -value of difference	0.48	0.001	0.67	0.0001

¹Time of day and treatment interaction had $P > 0.33$ for all fly species count outcome analyses.

across the front of the animal's chest (Mullens et al., 2006). A skin twitch occurred if an isolated twitch took place in a localized area or as a continuous shiver over the whole flank for several seconds (Dougherty et al., 1995). A tail swish occurred if the tail was moved from its resting position to one side of the animal's body; a separate swish occurred if the tail crossed to the opposite side of the body (Dougherty et al., 1994). A front leg stomp was defined as a raising of either front leg (while not walking) followed by a forceful thrust back to the ground. A hind leg kick occurred if either hind leg was thrust upward toward the animal's belly (Dougherty et al., 1995). Leg stomps and leg kicks were combined together for analysis.

Mean, high, and low temperature and total precipitation for summer months of 2017 during the study were recorded at the West Central Research and Outreach Center, Morris, Minnesota, dairy weather station. The average high temperature at the West Central Research and Outreach Center dairy ranged from 21.1°C in September to 27.7°C in July. The average low temperature ranged from 9.3°C in September to 15.9°C in July. Precipitation ranged from 23.4 mm/mo in July to 231.6 mm/mo in August across the study.

Period mean fly counts and behavioral frequencies (four 2-wk periods) were analyzed with PROC GLIMMIX of SAS (version 9.4, SAS Institute Inc., Cary, NC). Poisson models were evaluated for each behavior and fly count outcome with fixed effects of treatment, time of day, treatment and time of day interaction, period, and a random effect of cow. The compound symmetry covariance structure was used to specify covariance between errors of cows, and models were corrected for overdispersion. For the analysis of behaviors, the fly counts recorded before and after each observation for each species were averaged. The sum of the 3 fly species counts was used as a covariate in the behavior models. The random effect of observational group was added to the behavior models. To obtain the incidence

rate ratios for the effects of fly species on behaviors, individual models were fitted for each fly species as a covariate. The results of the analyses are reported as back-transformed means from a log-transformation and 95% confidence intervals with significance declared at $P < 0.05$.

Table 1 shows the least squares means and 95% confidence intervals for fly counts by treatment group and time of day. The total number of flies per cow was greater on cows with leggings than cows without leggings ($P = 0.024$). The number of horn flies per cow was greater ($P = 0.003$) on cows in with leggings (26.4 flies/side) compared with cows without leggings (24.1 flies; 21.3 to 27.2 CI).

For the period of the day (morning or afternoon), the total number of flies per cow was greater in the afternoon than in the morning (41.0 flies vs. 36.8 flies, respectively; $P = 0.01$). The difference in total fly numbers was due to greater numbers of stable flies on cows during the afternoon observation (15.4 flies/leg vs. 10.6 flies/leg; $P = 0.01$). The numbers of flies on cows in the current study were similar to Sjostrom et al. (2016) and Kienitz et al. (2018), who reported 30 horn flies/side and 7 stable flies/leg. No time by treatment interaction ($P > 0.10$) was observed.

Table 2 shows the least squares means and 95% confidence intervals for fly avoidance behaviors by treatment group and time of day. Frequencies of fly aversion behaviors differed between treatment groups and times of day, and increased with counts of the individual fly species. Cows with leggings stomped 39% less frequently (2.0 stomps with leggings vs. 3.3 leg stomps without leggings) than cows without leggings ($P < 0.01$), and cows across both treatments in the afternoon stomped 26% more frequently than cows in the morning (2.9 stomps in the afternoon vs. 2.3 stomps in the morning; $P = 0.04$). Eicher et al. (2001) found fly counts to be greatest in the afternoon compared with the morning. The number of head throws, tail swishes, and skin

Table 2. Least squares means and 95% CI for frequencies of fly avoidance behaviors (number per 5-min interval) by pastured dairy cows without versus with leggings and in the morning versus afternoon¹

Item	LSM (95% CI)			
	Head throws	Leg stomps	Tail swishes	Skin twitches
Treatment				
Control	2.6 (2.2–3.1)	3.3 (2.5–4.3)	19.3 (15.9–23.5)	19.6 (16.6–23.1)
Leggings	3.1 (2.6–3.6)	2.0 (1.5–2.7)	21.3 (17.6–25.9)	20.9 (17.7–24.5)
<i>P</i> -value	0.06	<0.0001	0.14	0.33
Time of day				
Morning	2.7 (2.3–3.2)	2.3 (1.8–3.1)	19.7 (16.2–24.0)	20.2 (17.2–23.7)
Afternoon	3.0 (2.5–3.5)	2.9 (2.2–3.8)	20.9 (17.2–25.4)	20.3 (17.2–23.8)
<i>P</i> -value	0.28	0.04	0.36	0.93

¹Time of day and treatment interaction had $P > 0.13$ for all behavior outcome analyses.

twitches were not different between treatment groups or times of day ($P > 0.10$). The number of horn flies on a cow increased ($P < 0.01$) the number of head throws, foot stomps, tail swishes, and skin twitches. For every 1-unit increase in horn flies on a cow, the rate ratio for head throws would be expected to increase by a factor of 1.02 (95% CI: 1.02 to 1.03), foot stomps by a factor of 1.01 (95% CI: 1.00 to 1.02), tail swishes by a factor of 1.01 (95% CI: 1.01 to 1.02), and skin twitches by a factor of 1.01 (95% CI: 1.01 to 1.02). The number of face flies on a cow had a positive effect on the number of head throws ($P = 0.049$). For every 1-unit increase in face flies on a cow, the rate ratio for head throws would be expected to increase by a factor of 1.05 (95% CI: 1.00 to 1.09). The number of stable flies on a cow increased the number of all behaviors ($P < 0.001$). For every 1-unit increase in stable flies on a cow, the rate

ratio for head throws would be expected to increase by a factor of 1.03 (95% CI: 1.02 to 1.04), foot stomps by a factor of 1.03 (95% CI: 1.02 to 1.04), tail swishes by a factor of 1.02 (95% CI: 1.02 to 1.03), and skin twitches by a factor of 1.03 (95% CI: 1.02 to 1.03).

Figure 1 shows the relationship between foot stomp behaviors and stable flies by treatment. Cows that did not have leggings were most affected by stable flies, whereas the cows with leggings were not as affected by stable flies.

The total number of flies on a cow increased head throws, foot stomps, tail switches, and skin twitches of cows ($P < 0.01$). If the total flies on a cow increased by 1, the rate ratio for head throws would be expected to increase by a factor of 1.02 (95% CI: 1.01 to 1.02), foot stomps by a factor of 1.01 (95% CI: 1.01 to 1.02), tail swishes by a factor of 1.01 (95% CI: 1.00 to 1.02), and

Figure 1. Relationship between foot stomp and leg kick behaviors and stable flies (solid line = cows without leggings; dotted line = cows with leggings). Error bars represent the 95% CI of each treatment mean. Effects were based on estimates for the afternoon and one 2-wk period because of the greatest number of flies present.

skin twitches by a factor of 1.01 (95% CI: 1.01 to 1.02). The results of this study are consistent with previous studies of cows in pasture-based systems and open field environments (Dougherty et al., 1993; Mullens et al., 2006; Kendall et al., 2007), which reported that biting flies, such as stable and horn flies, were predictors of avoidance behaviors such as ear flicks, head throws, foot stomps, skin twitches, tail swishes, and wound licking. Furthermore, Sjostrom et al. (2016) reported that horn fly counts were highest when cows were most active, during grazing, from 0600 to 0800 h and 1600 to 1800 h, which led to decreased rumination and milk production. Similar to Sjostrom et al. (2016), the current study found no difference between morning and afternoon horn fly counts. Many factors may contribute to high afternoon activity of flies, including the physiological state of the host, such as increased temperature (Dougherty et al., 1995) and ambient temperature.

Kendall et al. (2007) reported that the body temperature of pastured dairy cows peaks in the afternoon during the summer, which may help explain why stable fly numbers were higher in the afternoon compared with the morning in the current study. More than likely, the warmer weather increased fly activity. To support this idea, previous studies (Schmidtman et al., 1981; Harvey and Launchbaugh, 1982; Dougherty et al., 1993) found that fly annoyance reduces grazing time, which cows compensate for by grazing early in the morning or late in the evening when temperatures are lower. For the current study, the average ambient temperature ranged from 17.0°C during September 2017 to 23.1°C during July 2017. The average morning temperature ranged from 12.5 to 25.2°C and the average afternoon temperature ranged from 16.6 to 28.3°C. The ambient temperatures of the study were similar to Dougherty et al. (1993), which found cows performed energy-consuming muscle movement behaviors to displace stable flies and modified their grazing habits to avoid all flies regardless of weather.

Interestingly, horn fly numbers were greater on cows with leggings in the current study. Horn flies do occur on legs of cows, and perhaps, horn flies on cows with leggings simply moved up onto the cow's body, which made for greater horn fly side counts. Alternatively, maybe the horn flies preferred cows with leggings since they had less foot stomps. No peer-reviewed studies exist that examine the relationship between cow cleanliness and fly counts in pastured dairy cows. A study conducted by Franks et al. (1964) found that horn flies preferred colors compared with white, which may have contributed to the greater number of flies recorded on cows with leggings because the leggings were orange in color. In a similar study (Mottet et al., 2018) that

examined Shoofly Leggings for equine, more flies were reported on legs without leggings; however, that study had a smaller sample size (6 horses) and a drastically different environment. However, the authors concluded that leggings were effective in reducing foot stomps, which is consistent with the findings of the current study.

The current study suggests that flies cause fly avoidance behaviors in cows regardless of the use of leggings. However, leggings effectively reduced foot stomps and may offer some relief from flies in pastured dairy cows. The findings of this study also suggest that fly activity is greater in the afternoon compared with the morning, so pastured dairy cows are more likely to have greater frequencies of fly avoidance behaviors in the afternoon.

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