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Use of modified cages attached to growing calves to measure the effect of stable flies on dry matter intake and digestibility, and defensive movements

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ABSTRACT

The effects of stable flies on growing calves were examined using fly cages attached to the animals. Dry matter intake, DM digestibility (DMD), and behavioral responses of calves were monitored. Nine Holstein calves were exposed to 3 levels of stable flies (0, 10, 100 flies/animal) 3 times daily for 30 min. The study consisted of a 4-period crossover design; each period included 5-d adaptation, 7-d exposure, and 5-d postexposure. Calves were weighed at the beginning and end of each period. Feed consumption was continuously recorded. Fecal samples taken during and after exposure were used to determine DMD. Three calves were monitored for activity and defensive behavior during exposure. Caged stable flies successfully fed on the calves and invoked defensive behaviors similar to those observed in field studies. Defensive behaviors were proportionate to exposure level, and calves became more proficient at interfering with fly feeding over time. Stable fly exposure increased DMI relative to calf weight and decreased ADG/DMI. Calves initially exposed to 100 flies exhibited more defensive behaviors and lower relative DMI and ADG across all exposure levels relative to calves initially exposed to 10 flies. Stable fly exposure did not affect DMD, number of meals, time eating, or amount eaten per meal. Host defensive behavior, not reduced DMI or DMD, appears to be reducing ADG of calves exposed to stable flies. Results indicate that cages placed on calves may be used to study the effects of stable flies, but host exposure history and behavioral variables must be considered.

Key words: behavior, cattle, digestibility, intake, stable fly

INTRODUCTION

Stable flies are serious pests of cattle worldwide (Moon, 2009). Production losses due to stable fly infestations are estimated to cost US cattle and dairy producers >$2 billion in lost production annually (D. B. Taylor, unpublished data). Infestation levels producing whole-body counts of 50 and 100 flies per animal reduced weight gain in feeder calves by 13.2 and 20%, respectively, relative to calves maintained without stable flies (Campbell et al., 1977). According to those authors, cattle at 50% of the feedlots surveyed in eastern Nebraska had infestation levels of >50 stable flies per animal and 25% had >100 stable flies per animal. Stable flies reduced weight gains of grazing yearling steers by 19% even at low infestation levels, <15 stable flies per animal (Campbell et al., 2001). Among grazing cattle in Kentucky, grazing time, number of bites, and DM mass per bite all decline linearly with stable fly infestation levels (Dougherty et al., 1993).

The mechanisms by which stable flies reduce productivity in cattle remain unclear. Cattle exhibit several behavioral responses to avoid or dislodge stable flies including standing in water, bunching together, stomping the front legs, tail twitching, and head throwing (Campbell et al., 1977;

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Miller, 1995; Mullens et al., 2006). These behaviors have metabolic costs and divert the animals from feeding and drinking. Physiological responses to fly-induced stress ("fly worry"), immunological reactions toward antigens introduced by biting flies, and blood loss may be contributing to lost productivity as well. Results of experiments designed to examine the metabolic and physiological effects of stable flies on cattle have been conflicting. Schwinghammer et al. (1986) observed increases in heart rate, respiration rate, and rectal temperature among other physiological parameters when steers were exposed to 25 and 50 stable flies per day, whereas Estienne et al. (1991) observed no changes in the same parameters. Differences in experimental design might account for the lack of congruence between these studies. In both studies, cages with stable flies were attached to the cattle for 1 h/d exposure. However, Schwinghammer et al. (1986) provided continuous exposure by subsequently releasing the flies into the experimental rooms with the steers, similar to Campbell et al. (1977). The steers in the study by Estienne et al. (1991) were not exposed to stable flies outside of the 1-h cage feeding sessions.

Table 1. Composition of diet

Ingredient	% DM
Bromegrass hay	53.4
Wet corn gluten feed	38.6
Molasses	5.4
Fine ground corn	1.5
Limestone	0.73
NaCl	0.23
Trace mineral premix[1]	0.04
Vitamin A, D, E premix	0.01

[1]Trace mineral premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

Additional studies to quantify the effects of stable flies on DMI, DM digestibility (**DMD**), and defensive movements of cattle under controlled conditions are needed. The goals of the present research were 1) to quantify changes in DMI, DMD, and defensive movements of calves when exposed to stable flies; 2) to determine the effects of successful and unsuccessful feeding attempts by stable flies on the above parameters; 3) to determine whether changes in DMI or DMD or the metabolic costs of defensive movements are responsible for the reductions in ADG observed in previous studies; and 4) to characterize the efficacy of using modified cages strapped to an animal's back as an exposure method to examine the effects of stable flies on cattle.

Figure 1. Stable fly cage with 100 flies.

MATERIALS AND METHODS

Animals and Procedures

Nine Holstein steer calves with an average initial weight of 187 ± 17.8 kg were housed individually in 3-m × 3-m pens with slotted floors, in an environmentally controlled room at 68.7 ± 0.16°C and 56.6 ± 0.74% RH ($\bar{X}$ ± SEM). Animal care procedures followed those reviewed and approved by the University of Nebraska Institutional Animal Care Program (IACUC 05–06–041c). A 3-period crossover experimental design using a diagram-balanced Latin square with a fourth period repeating the third added to allow for testing carryover was used. Each 17-d period consisted of a 5-d adaptation, 7-d exposure, and 5-d postexposure period.

Calves were fed once per day a diet consisting primarily of bromegrass hay and wet corn gluten feed (Table 1) in feed bunks suspended from load cells (Omega, Stamford, CT). Intake was continuously monitored similar to the system described by Cooper et al. (1997). For each day of the 7-d exposure periods, feed remaining in bunks (refusals) were weighed before feeding at 0730 h. Daily intake, time spent eating, intake rate (daily intake/time spent eating), number of meals consumed, and average meal size were calculated during the 7-d exposure. Calves were weighed on d 1 of each period and at the end of period 4; d 1 weights for periods 2 to 4 were used as final weights for periods 1 to 3, respectively. While in the chute, the backs of the calves were shaved and Velcro strips for attaching stable fly cages were glued to the back directly behind the shoulder blade, off center, and just missing the backbone.

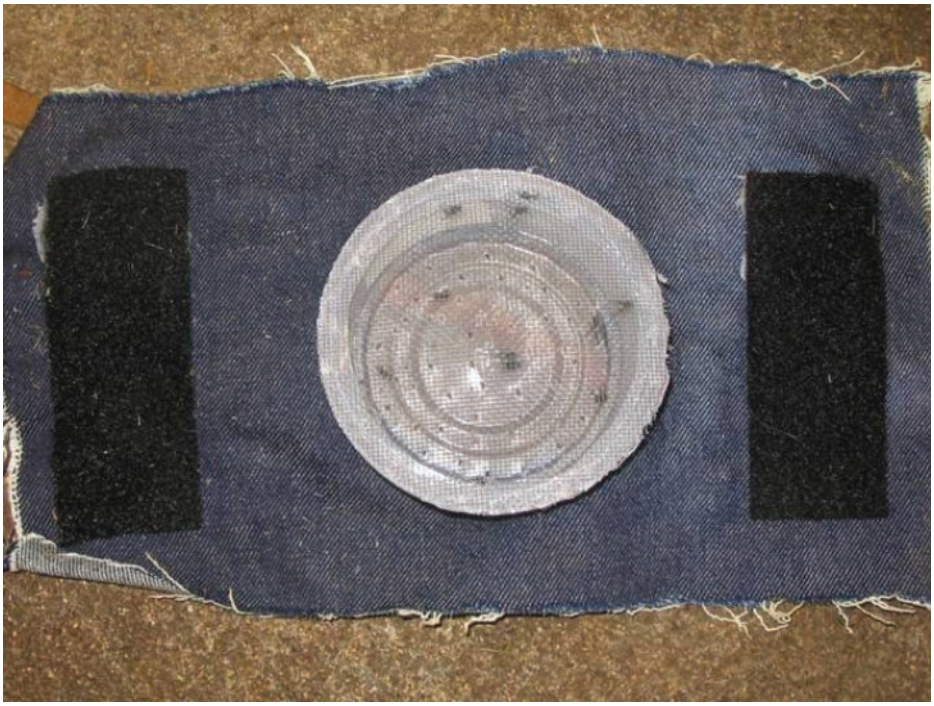

Figure 2. Stable fly cage with denim strap.

Stable fly larvae were reared in a media consisting of 500 g of wheat bran, 200 g of cottonwood sawdust, 115 g of fishmeal, and 1,600 mL of tepid water. Pupae were harvested after 14 d by sifting from the media (Berkebile et al., 2009). Pupae were transferred to paper cups and placed into cages constructed from 3.8-L plastic buckets, with the center portion of the lid cut out and replaced with screen (≈3,000 pupae/cage). Adult flies were provided sugar (10% sucrose solution) for 48 h and then starved for 24 h before being used in this study.

Three- to 5-d-old adult stable flies were counted and transferred to experimental cages each morning. Flies were anesthetized with CO_2 for 30 s. Groups of 10 and 100 flies were placed into 11-cm cages with the center of the lid removed and replaced with a convex mesh screen (Figure 1). Cages with flies were maintained on damp towels to provide moisture until use later the same day.

Cages were attached to the calves with denim strips, 20 × 60 cm with a 10-cm round hole in the middle. Velcro was hot glued to the denim to attach to the Velcro strips on the calves. Fly cages were inserted into the hole in the denim (Figure 2) and held in firm contact with the skin of the calf by stretching tightly and matching Velcro strips (Figure 3).

Calves were exposed to stable flies for 30 min, 3 times per day (0900, 1200, 1500 h) during the 7-d exposure period. After exposure, cages were removed from the calves, and the flies were killed by freezing. Stable flies were dissected immediately and scored for the presence or absence of blood in the gut. Three levels of exposure were used: 0, 10, and 100 stable flies per cage. Three calves were randomly assigned to each of 3 exposure series: A, B, and C. Calves in series A received the 0 fly exposure during period 1, 10 fly exposure during period 2, and 100 fly exposure during periods 3 and 4. Calves in series B received the 10 fly exposure during period 1, 100 fly exposure during period 2, and 0 fly exposure during periods 3 and 4. Calves in series C received the 100 fly exposure during period 1, 0 fly exposure during period 2, and 10 fly exposure during periods 3 and 4.

Three calves, one from each series, were chosen randomly and monitored for behavioral activity during each 30-min stable fly exposure. The number of head movements, tail movements, kicks, and circles walked, as well as time spent standing and eating, was recorded for each monitored calf.

Fecal samples taken on d 5, 6, 7, 12, 13, and 14 of each period at 0900, 1200, and 1500 h were used to determine exposure and postexposure DMD. The 0900-, 1200-, and 1500-h samples were composited on an equal wet-weight basis daily for each animal, dried in a 60°C oven for 48 h, and ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA). Exposure samples, d 5, 6, and 7, were composited on an equal dry-weight basis, as were postexposure samples, d 12, 13, and 14. Feed samples were similarly dried and ground to pass through a 1-mm screen. Feed and exposure and postexposure fecal samples were analyzed for DM, AIA, and ADF. Analysis of ADF was conducted using techniques outlined by Van Soest (1964). Samples were further analyzed for AIA by ashing at 525°C as outlined by Van Soest et al. (1991). Digestibility of diet DM was calculated using AIA as an internal marker in both fecal and feed samples (Van Keulen and Young, 1977).

Statistical Analyses

Stable fly feeding success was evaluated relative to exposure level, time of exposure, series (order of exposures), and experimental period with a mixed linear model (Proc Mixed, SAS Institute Inc., 2008). Calf, exposure level, experimental period, day of exposure (1–7 within each exposure period), and time of day were considered categorical variables. Exposure level, time, and their interaction were considered fixed effects. Calf and day of exposure were considered random effects. Time was modeled with an autoregressive covariance structure. Alpha = 0.05 was used for all models, and means are presented as $\bar{X} \pm SE$.

Calf movement was analyzed using generalized linear mixed models (Proc GLIMMIX, SAS Institute Inc.,

Figure 3. Stable fly cage on the back of a calf.

2008) with the lognormal distribution. Exposure level, time, period, day, and calf were considered categorical independent variables and the calf × day interaction was considered random. Two analyses were done. In the first, all 3 levels of stable fly exposure, 0, 10, and 100, were included, and the percentage of flies successfully ingesting blood was excluded due to the absence of data for this parameter at the 0 fly exposure level. In the second analysis, the 0 fly exposure level was excluded, and percentage of flies ingesting blood was included as a continuous independent variable.

Dry matter intake relative to weight, number of meals, time eating in minutes, meal length, and mean kilograms per meal were analyzed with mixed linear models (Proc MIXED, SAS Institute Inc., 2008). Fixed effects of series, period, exposure level, day, and carryover were included in the model, with calf considered to be the random effect. Carryover, the effect of the previous exposure level on host responses to current and subsequent exposures, was estimated by including the preceding exposure level as a fixed effect in the model. When carryover was significant, contrasts were used to determine which exposure levels produced carryover. Pairwise comparisons of exposure levels were evaluated with contrast statements. Contrasts were carryover of exposure 0 versus 10, 0 versus 100, and 10 versus 100. Toeplitz, ante-dependence, autoregressive, compound symmetry, and unstructured models were tested, with the final covariance structure selection being based on the lowest Akaike information criterion.

Digestibility was analyzed using mixed linear models (Proc MIXED, SAS Institute Inc., 2008). Exposure level, period, calf, and sample (exposure or postexposure) were categorical independent variables. The effects of exposure, period, and sample were examined, as well as the interaction between sample and exposure level. Calf was considered to be random.

Effects of stable flies on ADG were analyzed using mixed linear models (Proc MIXED, SAS Institute Inc., 2008). Exposure level, series (or-

Table 2. Percentage of stable flies successfully ingesting blood from experimental calves and exposure series for each calf

	Stable flies successfully feeding[1] (% ± SE)					
Calf No.	Period 1	Period 2	Period 3	Period 4	Total	Series[2]
168		60.0 ± 7.40	9.0 ± 4.82	17.6 ± 6.05	28.9 ± 4.51	A
175		88.6 ± 2.70	63.8 ± 5.41	57.9 ± 7.40	70.1 ± 3.56	A
177		72.9 ± 6.55	0.4 ± 0.21	0.0 ± 0.22	24.4 ± 4.85	A
167	66.7 ± 4.80	66.1 ± 8.16			66.4 ± 4.67	B
174	58.6 ± 5.08	31.8 ± 8.46			45.2 ± 5.30	B
178	36.7 ± 5.95	77.0 ± 5.95			56.8 ± 5.21	B
169	5.4 ± 3.38		0.5 ± 0.48	0.0 ± 0.00	2.0 ± 1.16	C
170	44.2 ± 5.47		4.3 ± 2.72	0.0 ± 0.00	16.2 ± 3.22	C
179	31.0 ± 3.99		23.3 ± 6.98	29.0 ± 6.76	27.8 ± 3.47	C
Mean	40.4 ± 2.63	66.1 ± 3.15	16.9 ± 2.63	17.4 ± 2.69	35.2 ± 1.65	

[1]n = 21 exposures per period.

[2]Exposure levels (number of flies) for periods 1 to 4: Series A, 0–10–100–100; Series B, 10–100–0–0; Series C, 100–0–10–10.

der of exposures), and period were considered categorical independent variables. Period weights were considered to be repeated measures with an autoregressive covariance structure. Feed efficiency (ADG/DMI) was normalized with a log-transformation and analyzed similarly.

RESULTS AND DISCUSSION

Stable Fly Feeding

The mean percentage of stable flies successfully ingesting blood during this study was 35.2 (Table 2). Fly feeding success varied among host calves. Fewer than 2% were able to ingest blood when placed on calf 169, whereas more than 70% ingested blood when placed on calf 175 ($P < 0.01$). A higher percentage of the flies successfully ingested blood with 10 flies per cage (36.7 ± 2.38) than with 100 flies per cage (33.7 ± 2.30, $P = 0.02$), and flies tended to be more successful during the 1200 h exposure (37.6 ± 2.89) than during the 900 h (34.4 ± 2.85, $P = 0.09$) and 1500 h (33.6 ± 2.86, $P = 0.03$) exposures. The order in which the exposures were given, series, also tended to have an effect on stable fly feeding success ($P = 0.06$). Only 15.3 ± 1.79% of the stable flies on calves in series C (100–0–10–10) successfully ingested blood, whereas 41.1 ± 2.91% and 56.1 ± 3.01% ingested blood on calves in series A (0–10–100–100) and B (10–100–0–0), respectively. Blood feeding success increased from 40.4% during the first experimental period to a peak of 66.1% ($P < 0.01$) during the second period and then dropped during the third ($P < 0.01$) and fourth ($P < 0.01$) periods.

Calves exhibited several defensive behaviors when experimental cages were affixed, including head throwing, tail switching, circling or spinning, and kicking. The frequencies of the 4 defensive behaviors were correlated (r = 0.56–0.87), so they were combined into a single parameter by summation for further analyses. Defensive movements did not vary with respect to time of day ($P = 0.08$) but did increase in response to the number of flies from 2.5 ± 0.27 per 30 min when no stable flies were in the cages to 35.2 ± 2.92 ($P < 0.01$) and 66.7 ± 4.78 ($P < 0.01$) with 10 and 100 stable fly exposure levels, respectively (Figure 4). The number of defensive movements differed among the 3 calves monitored (Figure 5, $P < 0.01$). Calf 169 (series C) averaged 65 defensive movements per 30 min across the 3 exposure levels, whereas calves 177 (series A) and 174 (series B) responded with an average of only 21 and 23 defensive movements per 30 min, respectively. An interaction between calf and exposure level was observed as well ($P < 0.01$). The number of defensive movements increased from an average of 11.9 during the first experimental period to 15.1 ($P = 0.02$) and 20.2 ($P < 0.01$)

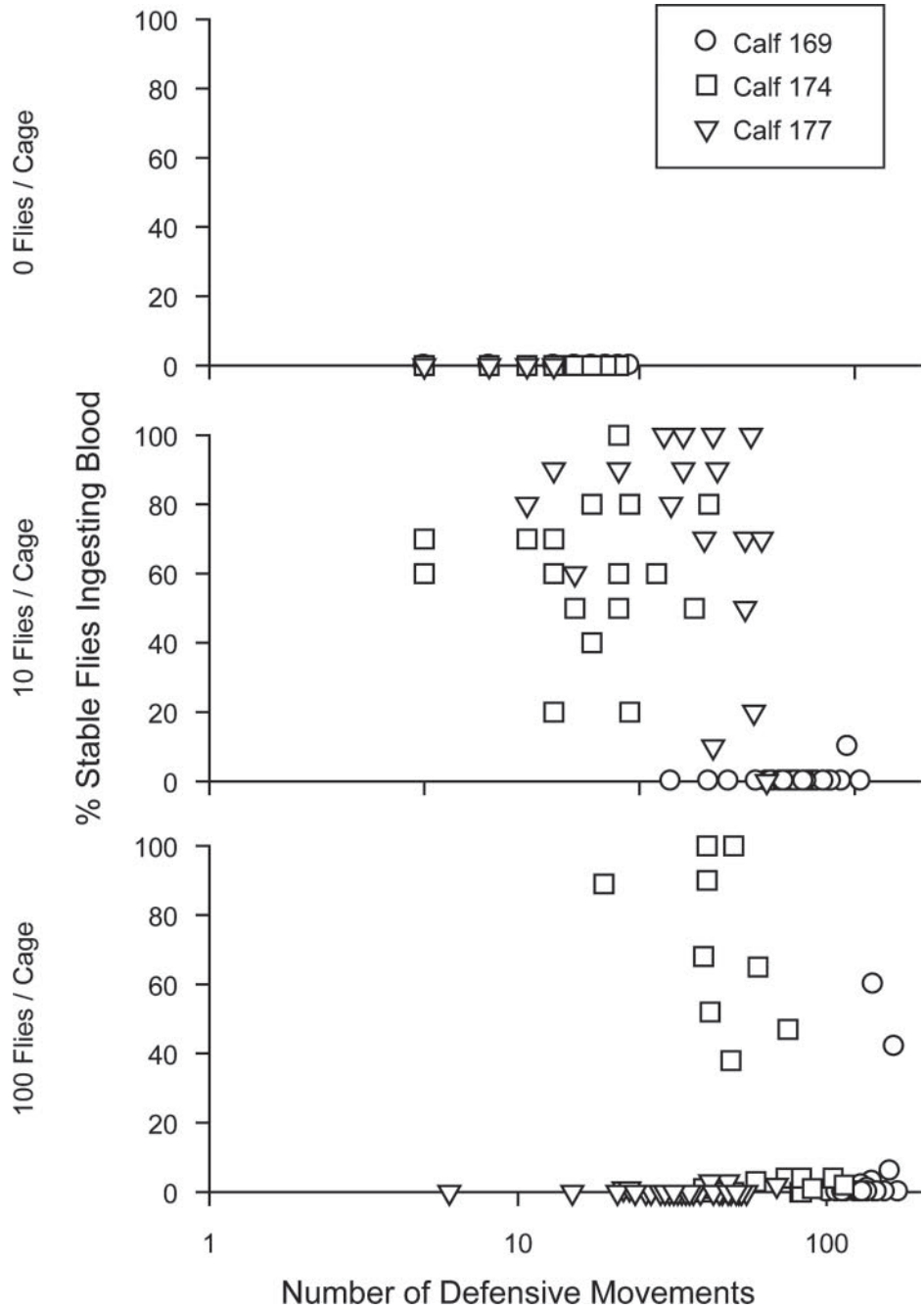

Figure 4. Stable fly feeding success relative to number of host defensive movements per 30-min observation period. Defensive movements included head and tail movements, kicks, and walking in circles.

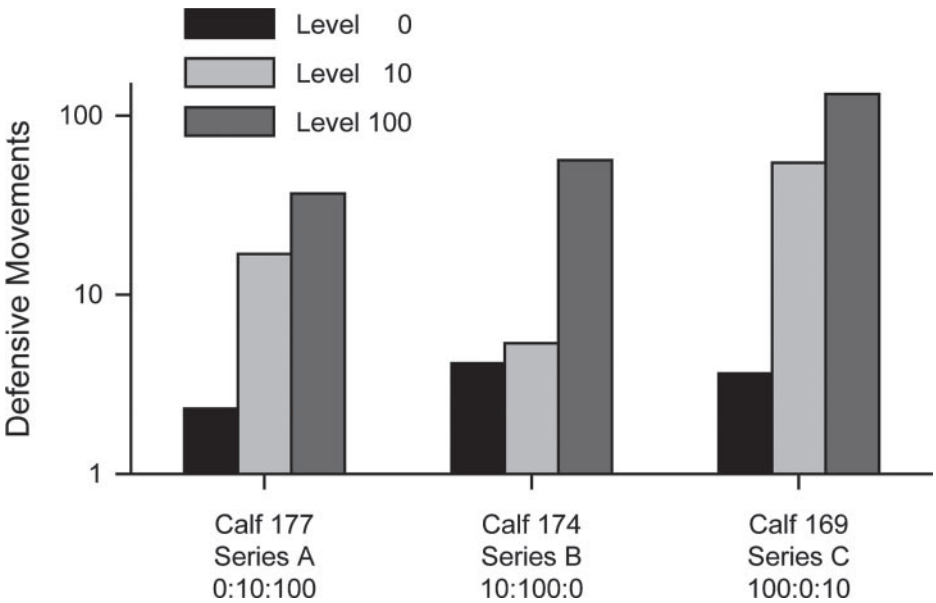

Figure 5. Least squares means for number of defensive movements by observed calves relative to stable fly exposure levels and order of exposure levels (series).

during the second and third periods and then remained unchanged, 17.8 ($P = 0.23$), in the fourth. The percentage of the stable flies successfully ingesting blood was negatively correlated with the log of the number of defensive movements (r = −0.54, $P < 0.01$).

The number of stable flies successfully ingesting blood was lower than expected throughout the experiment and reached very low levels during periods 3 and 4. The calves appear to have learned behaviors to interrupt fly feeding. Calves initially exposed to the highest level of flies, series C, had the lowest percentage of successful fly feeding and the most defensive movements at all exposure levels. Calves receiving the 10 fly exposure before the 100 fly exposure, series A and B, exhibited 66% fewer defensive movements and permitted 3-fold more stable flies to successfully ingest blood relative to those not preconditioned with lower exposure levels. The ability of cattle to adapt to stable fly infestations and develop defensive behaviors to reduce feeding has been observed in field studies as well (Catangui et al., 1993). The increase in stable fly feeding success observed during period 2 may be the result of series C calves, those exhibiting the highest level of defensive movements and lowest fly feeding success, being in the 0 fly exposure level during that period and, therefore, not being included in the average.

Under field conditions, stable flies prefer to feed on the lower front legs of cattle (Berry et al., 1983). Developing a cage that could be maintained on the lower leg was not feasible. However, stable flies were able to feed and evoke defensive behaviors in calves when caged on the back. These defensive behaviors were similar to those reported in field studies (Mullens et al., 2006). These results indicate that stable flies in cages fixed to an animal's back may be useful for examining the effects of stable flies on cattle.

Feed Efficiency

Dry matter intake did not differ among the 7 d of the exposure period ($P = 0.65$), and no carryover effect was observed ($P = 0.07$). Those variables were, therefore, not included in the final analysis. Dry matter intake relative to weight increased during the course of the study from 0.025 kg in the first period to 0.035 kg in the fourth period (Table 3, $P < 0.01$). Calves not challenged with stable flies ingested a lesser percentage of their BW than did those exposed to 100 stable flies (Table 3, $P = 0.03$). Relative DMI tended toward varying with the sequence of the exposures as well (Table 3, $P = 0.06$). Calves initially exposed to 100 stable flies, series C, tended to ingest a lower percentage of their BW than did those initially exposed to 0, series A, and 10, series

Table 3. Least squares means for relative DMI, ADG, and ADG/DMI relative to experimental period, order of exposures (series), and level of exposure

	Period				Series			Level		
Item	1	2	3	4	A	B	C	0	10	100
DMI[1] (kg)	0.025^{A}	0.031^{B}	0.032^{B}	0.035^{C}	0.033^{A}	0.033^{A}	0.026^{A}	0.029^{A}	0.030^{AB}	0.033^{B}
ADG (kg)	1.38^{A}	0.92^{B}	1.32^{A}	1.31^{A}	1.35^{A}	1.19^{AB}	1.15^{B}	1.29^{A}	1.19^{A}	1.21^{A}
ADG/DMI[2]	0.23^{A}	0.16^{C}	0.18^{B}	0.16^{BC}	0.18^{A}	0.16^{B}	0.20^{A}	0.20^{A}	0.18^{B}	0.17^{B}

[A–C]Numbers followed by the same superscript do not differ ($P < 0.05$).

[1]Relative DMI = DMI/initial BW.

[2]Back transformed to original units.

B, stable flies (Table 3). Number of meals, time eating, meal length, and amount eaten per meal did not vary relative to exposure level, series, or period. None of the intake parameters varied relative to day of the exposure period. Dry matter digestibility did not vary among exposure levels ($P = 0.38$), experimental periods ($P = 0.13$), or between samples taken during and after exposures ($P = 0.09$). The interaction between exposure level and when the sample was taken relative to exposure was insignificant as well ($P = 0.62$).

The ANTE(1) covariance structure best fit the autocorrelation of the ADG data resulting from repeated measures on calves. Average daily gain was lower during period 2 than during the other 3 periods (Table 3, $P < 0.01$). For the 4 experimental periods combined, ADG decreased relative to the order of exposure levels (Table 3, $P = 0.05$). Average daily gain was highest for series A (0–10–100–100) and decreased for series B (10–100–0–0) and C (100–0–10–10). Average daily gain did not vary relative to exposure level ($P = 0.35$).

Feed efficiency (ADG/DMI) was lower during experimental periods 2 and 4 than during periods 1 and 3 ($P = 0.02$). For overall exposure levels, series C calves were most efficient and series B calves were least efficient ($P = 0.01$). Efficiency decreased with increasing levels of flies (Table 3, $P = 0.03$).

Three sources of variation were observed during this study. Several parameters varied in relation to experimental period, with period 2 being the most divergent. The percentage of flies successfully ingesting blood was highest for period 2, and feed efficiency and ADG were lowest. In general, number of defensive movements and intake by the calves increased from the first to the last experimental period. Exposure level was a primary source of variation. The number of flies successfully ingesting blood, defensive movements, and relative DMI increased with exposure level, whereas feed efficiency decreased. The final important variable was order of exposure levels, series. Calves initially exposed to 100 flies, series C, exhibited more defensive movements resulting in a lower percentage of the flies successfully ingesting blood, lower DMI, but higher feed efficiency. In contrast, those exposed to control cages with no flies first, series A, exhibited the fewest defensive movements and highest ADG.

The interactions between stable flies and cattle productivity are clearly complex. Calves respond to stable flies with defensive movements that can reduce the feeding success of the flies. The level of defensive movements appears to be related to the calves' previous experiences with stable flies, lower in animals gradually exposed to biting flies, and higher in those initially exposed to higher numbers of flies. Effective defensive movements are learned, and the ability of the animals to impede biting improves with experience. Under our experimental conditions, stable fly exposure appears to increase relative DMI but not affect DMD. When DMI and ADG were analyzed together, a relationship was observed that indicated stable flies affected the conversion of food energy to weight gain. Energy loss due to the defensive movements may account for the reduced conversion efficiency.

IMPLICATIONS

Host defensive responses to stable fly exposure appear to have a greater role in reducing ADG and productivity of cattle than do reduced DMI or DMD. The behavioral responses vary depending on the previous experiences of the animal with this pest. This variation may be responsible for some of the inconsistencies observed in previous attempts to evaluate the effects of stable flies on cattle production. The effects of the defensive movements on NE_m requirements need to be quantified to permit modeling of ADG relative to stable fly population levels. Caged stable flies attached to experimental animals can be used effectively for short-term experiments to study behavioral responses and adaptations of cattle to stable flies.

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