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# Efficacy of a federally approved flea bait, orally administered to white-footed mice (*Peromyscus leucopus*), against blood feeding *Ixodes scapularis* larvae under simulated field conditions

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ABSTRACT

A promising alternative approach to conventional vector control practices is the use of systemic insecticides/acaricides orally administered to relevant mammalian host species to control blood feeding disease vectors. In the United States, Lyme disease continues to be the most prevalent vector-borne disease with the Centers for Disease Control and Prevention estimating approximately 500,000 Lyme disease infections each year. Previous research has demonstrated the potential usefulness of a low dose fipronil bait in controlling *Ixodes scapularis* larvae feeding on white-footed mice. However, no such acaricide-only product is approved for use in treating white-footed mice to control *I. scapularis*. The purpose of the study was to evaluate the use of a federally approved fipronil flea control bait (Grain Bait) in controlling *I. scapularis* parasitizing white-footed mice (*Peromyscus leucopus*). A simulated field trial was conducted in which Grain Bait was presented to grouped white-footed mice alongside an alternative diet for 168 h. Mice were fitted with capsules and manually parasitized with *I. scapularis* larvae. Replete larvae detaching from each mouse were collected and monitored for molting to nymphs. The inside of each capsule was observed to evaluate tick attachment. Blood was collected from all Treatment group mice via cardiac puncture to determine the fipronil sulfone concentration in plasma (CP) for each animal. Results indicated that Grain Bait would be consumed in the presence of an alternative diet and that bait acceptance was greater for males, relative to females. Treatment with Grain Bait prevented 100% larvae from feeding to repletion at Day 7 post-exposure and prevented 80% of larvae from feeding to repletion and 84% from molting at Day 21 post-exposure, relative to Control groups. Molted nymphs were not recovered from mice that had CP detectable ≥18.4 ng/ml. The results suggest that this federally approved flea product could be utilized for tick control and that other medically important vector-host relationships should be considered.

## 1. Introduction

In recent centuries, vector-borne diseases have been responsible for more human disease and death than all other causes combined (Gubler, 1991), with a global reemergence of old communicable diseases, and emergence of new ones, including Lyme disease, having occurred within the past 50 years (Cabelo and Springer, 1997). Vector control may serve as a promising solution to controlling medically important arthropod species. However, conventional vector control methods such as broadcast acaricide applications present logistical and economic hurdles, environmental concerns, and potential for insecticide resistance (Ginsberg and Stafford, 2005; Piesman and Eisen, 2008; Ginsberg et al., 2017). An alternative approach being investigated is the use of systemic insecticides/acaricides, orally administered to relevant mammalian host species, to control blood feeding vectors. This approach is promising in that it can be applied discriminately, targeting only the host which subsequently reduces insecticide application rates, thus reducing logistical and environmental hindrances. One particularly promising compound is the phenalpyrozol, fipronil.

Fipronil is a broad-spectrum insecticide that disrupts and GABA-gated and glutamate-gated chloride channels of arthropods (Raymond-Delpech et al., 2005). When administered orally to a host, it acts systemically controlling parasitizing vectors during blood feeding. Fipronil-based systemic insecticides have demonstrated high efficacy in controlling mosquitoes and phlebotomine sand flies parasitizing cattle and rodents (Ingenloff et al., 2013; Poché et al., 2013, 2015, 2016, 2017;

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Derbali et al., 2014), ticks parasitizing white-footed mice (*Peromyscus leucopus*) and white-tailed deer (*Odocoileus virginianus*) (Poché et al., 2020a, 2021, 2023), and fleas parasitizing small rodents such as black-tailed prairie dogs (*Cynomys ludovicianus*) (Eads et al., 2019, 2022a; D.M Poché et al., 2017, 2020b) and the great gerbil (*Rhombomys opimus*) (2018). Research also indicates the effectiveness of oral fipronil in controlling fleas and ticks parasitizing *Rattus* spp. rats (Rajonhson et al., 2017; Jacob et al., 2021) and canines (*Canis familiaris*) (dos Santos et al., 2022). Research regarding systemic insecticides for rodent treatment has been ongoing, with a patent being awarded in 2011 (Borchert and Poché, 2011).

One vector-host relationship of concern in the United States (US) is *Oropsylla* spp. fleas and black-tailed prairie dogs and the transmission of plague (*Yersinia pestis*), a bacterium first known to be introduced to the western US in 1900 (Eads et al., 2022b). Black-tailed prairie dogs represent a keystone species of the North American Great Plains (Miarinjara et al., 2022) and plague epizootics within individual colonies can lead to prairie dog mortality of up to 100% (Stapp et al., 2004; Pauli et al., 2006). Although plague no longer results in the millions of human deaths that occurred in Europe in the Middle Ages (United States Centers for Disease Contol and Prevention, 2021), it is still of substantial concern to conservation biologists and is regarded as an emerging disease with changes in land-use increasing the probability of interaction between host species and humans (Perry and Fetherston, 1997). Additionally, zoonotic plague is detrimental to endangered black-footed ferrets (*Mustela nigripes*) (Matchett et al., 2010, 2021), which are dependent upon black-tailed prairie dogs for food (Roelle et al., 2006, Eads and Biggins, 2015). A field trial conducted in 2016 in northern Colorado resulted in the registration of Kaput® Flea Control Bait with Fipronil, a granular flea bait (Grain Bait) containing 0.005% fipronil for use against *Oropsylla* spp. fleas infesting black-tailed prairie dogs (R.M Poché et al., 2017). Additional field trials were conducted in northern Colorado and South Dakota at reduced application rates and these trials indicated that a single application of Grain Bait could control 100% of fleas parasitizing prairie dogs for 3–4 months (Eads et al., 2019, 2022; Poché et al., 2020b). The studies in South Dakota further suggested that fipronil could significantly suppress flea abundance for 12–24 months (Eads et al., 2019, 2020, 2022a). Additionally, Grain Bait was shown to effectively control 100% *Xenopsylla* spp. fleas parasitizing great gerbils up to 80 days in southern Kazakhstan (Poché et al., 2018). Grain Bait is currently approved by the US Environmental Protection Agency (EPA) for use in black-tailed prairie dog colonies to control parasitizing *Oropsylla* spp. fleas and prevent infected flea bites (EPA Reg No. 72500-28). This is one of two systemic insecticides approved for use by the US EPA, the other containing imidacloprid for use against fleas infesting California ground squirrels (*Otospermophilus beecheyi*) (Borchert et al., 2009; Jachowski et al., 2011) (EPA Reg. No. 72500-17). The above results have indicated the effectiveness of Grain Bait against fleas. Considering the effectiveness of fipronil against a variety of arthropod vectors, it is worth investigating the usefulness of Grain Bait in disrupting other medically important vector-host relationships.

On a global scale, ticks are recognized as one of the two the main arthropod pathogen vectors of disease agents of humans and animals (mosquitoes being the other) (Colwell et al., 2011) and ticks and wildlife species encompass vector-host relationships of increasing medical and veterinary concern (Dantas et al., 2012). In the US, Lyme disease continues to be the most prevalent vector-borne disease (Rosenberg et al., 2018) with CDC estimating approximately 500,000 Lyme disease infections each year, primarily in the midwestern and northeastern regions (Kugler et al., 2015, 2021). The geographical distribution of the tick species *Ixodes scapularis* (blacklegged ticks) (principal vector) and human Lyme disease instances have expanded and the numbers of reported human cases have steadily increased over the past 30 years (Bacon et al., 2008; Eisen et al., 2016a, 2016b). The white-footed mouse is a frequent host for immature *I. scapularis* and is a primary reservoir for *B. burgdorferi* s.s. in the midwestern and northeastern US, where human Lyme disease incidence are most prevalent (Bunikis and Barbour, 2005; LoGiudice et al., 2003). Larvae may acquire *B. burgdorferi* while taking blood meals during the summer and subsequently emerge as nymphs the following spring. These nymphs are primarily responsible for the transmission of *B. burgdorferi* s.s. to humans, with nymphal infection ranging from 15 to 25% (Lehane et al., 2021). Both fluralaner (Pelletier et al., 2020, 2022) and fipronil (Poché et al., 2020a, 2021) have been evaluated as potential acaricides for administration to *Peromyscus* spp. mice to control immature *I. scapularis*. Oral acaricides offered to *B. burgdorferi* s.s. rodent hosts present a promising means of controlling immature *I. scapularis* parasitizing rodent pathogen reservoirs. Control of ticks parasitizing rodents has potential to disrupt the enzootic transmission cycle, further reducing density of infected host-seeking ticks (Eisen and Stafford, 2020).

Previous research has demonstrated the potential usefulness of a low dose fipronil bait block formulation in controlling *I. scapularis* larvae feeding on white-footed mice (Poché et al., 2020a, 2021). A no-choice test conducted under lab conditions with individually housed white-footed mice indicated that 100% *I. scapularis* larvae could be controlled up to 15 days post-fipronil bait exposure when mice were exposed to fipronil bait for 48 h (Poché et al., 2021). The fipronil bait block was further tested in a choice-test conducted under simulated field conditions with group-housed white-footed mice in which they were exposed to fipronil bait presented in commercial bait stations alongside an EPA approved field rodent challenge diet (United States Environmental Protection Agency, 1991). This study indicated that fipronil bait was palatable in the presence of alternative food sources and that efficacy was obtainable up to 35 days post-exposure dependent upon the concentrations of fipronil sulfone detected in the plasma (Poché et al., 2021).

While the above studies were useful in development of a bait block formulation, with intentions to eventually submit data to the United States Food and Drug Administration (FDA) for product approval, the Grain Bait formulation should be explored as well. Firstly, while the formulation differs, the two products have the same nominal concentration of fipronil (0.005%) suggesting that efficacy could be similar. Secondly, the Grain Bait is already EPA-approved for use in the US. Finally, because it is granular it would be more conducive to alternative application procedures such as spot-treatment in white-footed mouse habitat.

## 2. Materials and methods

The primary objective of the current study was to investigate the efficacy of Grain Bait, presented to white-footed mice, in controlling blood feeding *I. scapularis* larvae. Successful deliverability and efficacy could indicate potential for a multi-use product which could justify the addition of white-footed mice and *I. scapularis* ticks to the current Kaput® Flea Control Bait with Fipronil (EPA Reg. 72,500-28) product label.

### 2.1. White-footed mice and I. scapularis ticks

Test animals were obtained from a previously described outbred white-footed mouse colony (Poché et al., 2020a). Larvae were obtained from the Oklahoma State Tick Rearing Facility (Stillwater, OK, USA) and maintained in a regulated insectary (Poché et al., 2020a).

All white-footed mouse procedures performed during this study, and the test protocol, were approved by the Genesis Institutional Animal Care and Use Committee (IACUC) (February 9, 2022) and followed Animal Welfare Act (AWA) and Genesis IACUC policies (Genesis Laboratories, Inc. Protocol No. 21002).

### 2.2. Fipronil bait

Grain Bait (Scimetrics Limited Corp., Wellington, CO, USA) is an

EPA-approved product utilized in flea control in black-tailed prairie dogs (Kaput® Flea Control Bait with Fipronil, EPA Reg No. 72500-28). The nominal fipronil concentration of 0.005% (50 mg/kg) was confirmed using a validated high-performance liquid-chromatography (HPLC) method (53.8 mg/kg).

### 2.3. Experimental design

#### 2.3.1. Pre-exposure (acclimation)

Acclimation was similar to methodology described by Poché et al. (2021). During acclimation, mice were housed in groups of 5, separated by sex, in metal stock tanks (enclosures) having a surface area ~11,700 $cm^2$. Wood shavings were used to absorb urine and feces and were replaced weekly. Each enclosure was supplied an animal shelter and bedding material (cotton).

Mice were acclimated to test conditions for a minimum of 3 days. A 12 L:12D photoperiod was selected and environmental conditions in the test rooms (temperature: ~20–25 °C; relative humidity: ~30–70%) and health of test mice were monitored daily. Test mice were provided commercial rodent diet (Envigo, Indianapolis, IN) and tap water (via gravity-fed bottle) *ad libitum*. A veterinarian inspected the animals prior to exposure to ensure study suitability.

#### 2.3.2. Exposure - group assignment

Mice were assigned to groups using a random sequence generator. Each group (Treatment, Control) was composed of 20 mice (10 male, 10 female). Treatment group mice were exposed to Grain Bait for 168 h and Control group mice were untreated. The 168 h exposure period was identical to the extended exposure period utilized by Poché et al. (2021). Under field conditions, the ability to keep bait stations in the field for extended periods would increase the probability of treating large proportions of mouse populations. Mice were further assigned to subgroups containing 10 mice (5 male, 5 female) which were differentiated based on the timepoint of tick attachment. This sample size was selected based on the EPA recommendation of 6–10 subjects (10 preferred, 6 acceptable) per group when evaluating pesticides against pests of humans and pets such as fleas and ticks (United States Environmental Protection Agency, 1991).

#### 2.3.3. Exposure

At initiation of the exposure period, all commercial rodent diet was removed from the enclosures. Each Treatment group enclosure (5 mice) was presented with approximately 100 g Grain Bait in a single commercial bait station (Protecta® LP, Bell Laboratories, Inc., Windsor, WI, USA) and approximately 100 g of EPA field rodent challenge diet (CD) (United States Environmental Protection Agency, 1991) in an open food container. The fipronil bait station and CD were positioned against the wall of the enclosure at opposing sides and were positioned equidistance from the water source and shelter as described by Poché et al. (2021). Each Control group enclosure was presented CD exclusively.

Grain Bait was presented to white-footed mice within the Treatment group for 168 h. The Grain Bait and CD were removed once daily, weighed to the nearest 0.1 g, and immediately returned to the enclosures. Fipronil bait and CD were replenished *ad libitum*. At the conclusion of 168 h, all fipronil bait and challenge diet were removed and replaced with commercial rodent diet. At the conclusion of exposure, all bedding was removed and replaced with clean bedding to ensure that no fipronil bait was present in the enclosures. During post-exposure, mice remained in group enclosures, were provided commercial rodent diet and tap water *ad libitum,* and were observed daily for general health. Test mice remained in group enclosures until tick attachment.

#### 2.3.4. Tick attachment

Tick attachment was performed at Day 7 and Day 21 post-exposure. Treatment and Control subgroups parasitized with ticks and Day 7 and Day 21 were identified as T7 and C7, and T21 and C21, respectively. At the initiation of the tick attachment period, the appropriate Treatment and Control subgroup mice were transferred from the animal study room to the insectary. Mice were then housed in individual wire cages each suspended above a moat of water used to collect detached larvae. Forty (40) larval ticks were applied within a small capsule attached to each mouse. The tick attachment procedures are explicitly described in Poché et al. (2020a).

#### 2.3.5. Post-tick attachment

The post-tick attachment procedures are explicitly described in Poché et al. (2020a). Three methods of observing and recovering ticks were used during the tick feeding period (post-tick attachment): (1) searching the water in moats for detached non-engorged and replete larvae, (2) using microscopy to observe attached ticks within the capsules, and (3) monitoring molting of detached, replete larvae.

1. *Moat Observations and Tick Recovery* – Twice daily, the water in the moats under each cage was searched for non-engorged or replete ticks in the same manner previously described (Poché et al., 2020a, 2021).
2. *Microscope Tick Observations* –The inside of each capsule was carefully scanned for attached non-engorging larvae (brown, often desiccated) and engorging larvae (bloated and white, grey, red or pink in color) in the same manner previously described (Poché et al., 2020a, 2021). The presence of red feces were also a clear indicator of the presence of engorging/actively feeding larvae.
3. *Monitoring of Detached Replete Larvae* –Replete larvae within the Control and Treatment subgroups were retained separately in glass test tubes and were monitored over an ~8-week observation period to determine molting success utilizing methodology previously described (Poché et al., 2021).

#### 2.3.6. Blood sample collection

At the conclusion of Day 4 post-tick attachment, blood samples were taken from all Treatment group mice to provide indication of individual bait consumption (Poché et al., 2021). Modifications were made to the procedure based on recommendations by Poché et al. (2021). To maximize blood collection, blood draws were performed utilizing live mice. To do so, mice were anesthetized using an isoflurane vaporizer set to maximum isoflurane output (5%) and an oxygen flow rate of 2L/min. Once mice reached the anesthetic plane, they were transferred from the induction chamber to a nosecone, the oxygen flow rate was reduced to 0.5 L/min, but the isoflurane remained at 5%. While mice were under heavy anesthesia, ~100 μl of blood was collected directly from each animal via cardiac puncture using methodology similar to that described by Williams et al. (2020). After blood collection, mice were immediately euthanized via cervical dislocation while still under heavy anesthesia (Leary et al., 2020). Blood was additionally collected from 4 Control group mice to obtain a baseline.

Blood samples were placed into microtainers containing a serum separator (BD Microtainer®, Gurgaon, Haryana, India), were centrifuged for 10 min at 6100 revolutions per minute (rpm) and stored at −20 °C. Plasma was then delivered to the Center for Environmental Medicine (CEM) Analytical Laboratory at Colorado State University (Fort Collins, CO, USA) for analysis.

### 2.4. Data analyses

#### 2.4.1. White-footed mouse body weights and fipronil granular bait consumption

Body weights of all mice were recorded prior to Grain Bait exposure and at the conclusion of the post-tick attachment period. Differences in body weight between test groups (treatment *vs.* control) and within each test group (initial weight *vs.* final weight) were estimated. Grain Bait consumption was estimated daily (to the nearest 0.1 g) for each test group. Total Grain Bait consumption in each group was then used to

estimate the average total fipronil consumed by each mouse each day and total fipronil consumption in mg/kg per individual mouse.

#### 2.4.2. Tick observations and recovery

All larvae collected from moats, observed via microscopy, and monitored for molting were explicitly defined based on developmental status:

##### 2.4.2.1. Moats

Non-engorged/flat = No discernable blood meal, collected from moats.

Replete/Engorged = Darkly colored, bloated larvae collected from moats.

##### 2.4.2.2. Microscopy (observable within capsules only)

Non-engorging = Attached flat larvae having no discernable blood meal, or expired (often desiccated), previously engorging larvae having succumb to fipronil toxicity.

Engorging = Attached, actively feeding, and bloated (often surrounded by red feces)

##### 2.4.2.3. Post repletion

Replete/Engorged = Darkly colored, bloated larvae within test tube.

Molted = Emerged nymph.

Representative images of larva observed via microscopy are presented in Fig. 1.

Noticeable differences and non-differences in (1) initial and final bodyweights, (2) daily Grain Bait consumption, (3) the numbers of non-engorged and replete larvae collected from moats per test group; (4) attached non-engorging and engorging larvae within capsules per test group; and (5) larvae within capsules successfully detaching per test group, were further analyzed to estimate statistical differences using a Wilcoxon Signed-Rank test where $P \leq 0.05$ was considered significant (Wilcoxon, 1945).

#### 2.4.3. Fipronil plasma concentration

The CP (ng/ml) was estimated for each individual mouse euthanized ($n = 24$). The limit of quantification (LOQ) was 0.04 ng fipronil/ml plasma, which was markedly lower than the LOQ utilized by Poché et al. (2021) (1.25 ng/ml). Comparisons were made between the CP recorded for T7, relative to T21 (Wilcoxon Signed-Rank test: $P \leq 0.05$).

#### 2.4.4. Mortality estimates

The efficacy of Grain Bait in preventing attached larvae from feeding to repletion was calculated using Abbott's formula (Abbott, 1925), accounting for the Control groups.

$$Efficacy\ (\%) = 100 * \left(\frac{C - T}{C}\right)$$

Where:

$C$= Number of attached larvae feeding to repletion per mouse in Control
$T$ = Number of attached larvae feeding to repletion per mouse in Treatment

The efficacy of the fipronil bait in preventing replete larvae from molting was calculated using Abbott's formula but the variables were redefined:

$C$= Number of replete larvae molting to nymphs in Control
$T$ = Number of replete larvae molting to nymphs in Treatment

All statistical and data analyses were performed using JMP Statistical Software (Version 15) (Cary, NC, USA) and Microsoft Excel.

## 3. Results

### 3.1. Mouse body weights

A summary of bodyweights can be found in Table 1. Within each subgroup, the average final weight exceeded the average initial weight, with the exception of the Treatment group males parasitized at Day 7 exposure (T7) (Initial: 22.9 g, Final: 22.1 g). All mice proceeding to the

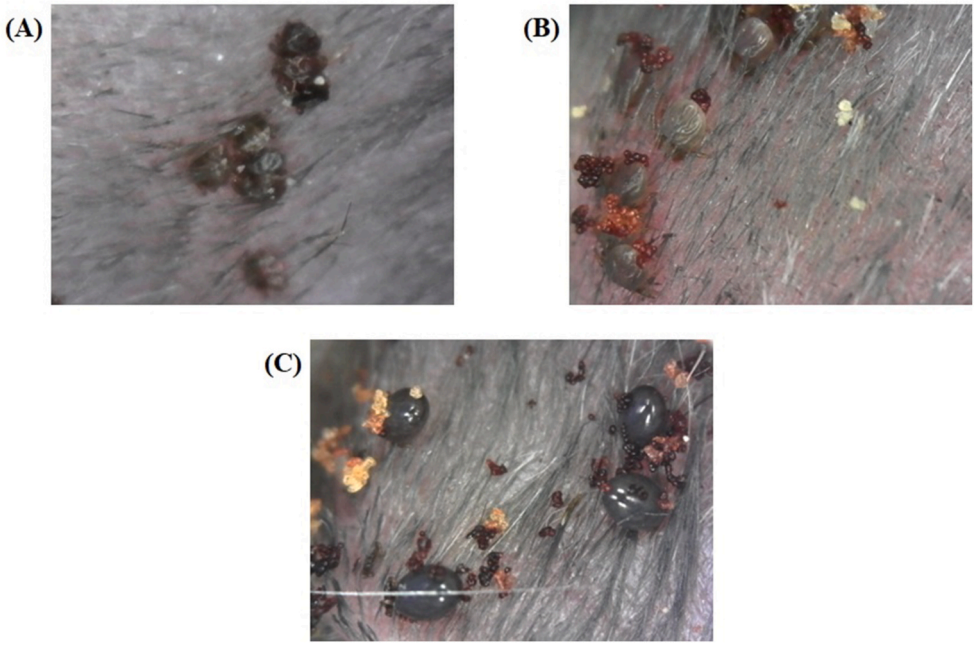


**Fig. 1. Capsule observations via microscopy.** (A) Non-engorged (deceased) larvae, (B) engorging larvae, (C) fully engorged (replete) larvae nearing detachment.

**Table 1**
Initial and final bodyweights (g) for white-footed mice within each test group (Mean ± SD).

| Test Group | Tick Attachment (Post-exposure) | Sex | Bodyweights | |
|---|---|---|---|---|
| | | | Initial | Final |
| Treatment | Day 7 (T7) | Male | 22.9 ± 6.5 | 22.1 ± 4.4 |
| | | Female | 19.4 ± 1.1 | 21.1 ± 1.5 |
| | Day 21 (T21) | Male | 20.3 ± 1.7 | 20.5 ± 1.9 |
| | | Female | 19.2 ± 3.8 | 20.7 ± 2.7 |
| Control | Day 7 (C7) | Male | 18.8 ± 0.6 | 19.2 ± 0.5 |
| | | Female | 19.0 ± 1.7 | 19.8 ± 1.4 |
| | Day 21 (C21) | Male | 22.3 ± 1.9 | 22.4 ± 2.7 |
| | | Female | 20.1 ± 5.1 | 21.9 ± 2.6 |

exposure and post-exposure periods had initial weights within the EPA recommended allowances (15–40 g) (United States Environmental Protection Agency, 1991). No significant differences were detected between Treatment and Control when comparing initial weights ($Z = 0.379$, $p = 0.7048$) or final weights ($Z = 0.419$, $p = 0.6750$). Final body weights did not differ significantly from initial weights within the Treatment ($Z = 1.380$, $p = 0.1675$) or Control groups ($Z = 1.488$, $p = 0.1367$).

### 3.2. Feed consumption

All mice appeared normal and healthy throughout the study and no signs of toxicity to Grain Bait were observed. Results indicate that Grain Bait will be consumed in the presence of an alternative feed (Table 2). On average, male mice within the treatment group consumed more Grain Bait relative to female mice. Grain Bait made up 55.6% (T7) and 43.0% (T21) of the total diet consumed by male mice and 19.9% (T7) and 33.7% (T21) of the total diet consumed by female mice.

A summary of the average fipronil consumed (mg/kg) per mouse is presented in Table 3. Male mice within the Treatment group consumed an average of 17.4 (T7) and 15.7 (T21) mg/kg/mouse fipronil. Female mice within the Treatment group consumed an average of 8.5 (T7) and 14.1 (T21) mg/kg/mouse fipronil.

The amount of Grain Bait eaten by Treatment group males was significantly greater relative to females ($Z = 2.139$, $p = 0.0325$). No significant difference was detected when comparing Grain Bait and CD consumption among males within the Treatment group. Females within the Treatment group consumed significantly more CD relative to Grain Bait ($Z = -2.920$, $p = 0.0035$).

**Table 2**
Comparison of consumption of Grain Bait and challenge diet (CD) within the Treatment and Control groups of white-footed mice.

| Test Group | Tick Attachment (Post-exposure) | Sex | Grain Bait/CD | Consumption (g) |
|---|---|---|---|---|
| Treatment (7-day Grain Bait Exposure) | Day 7 (T7) | M | Grain Bait | 39.9 |
| | | | CD | 31.8 |
| | | F | Grain Bait | 16.5 |
| | | | CD | 66.5 |
| | Day 21 (T21) | M | Grain Bait | 31.9 |
| | | | CD | 42.2 |
| | | F | Grain Bait | 27 |
| | | | CD | 53.2 |
| Control (Untreated) | Day 7 (C7) | M | CD | 112.6 |
| | | F | CD | 79.6 |
| | Day 21 (C21) | M | CD | 118.9 |
| | | F | CD | 101.1 |

### 3.3. Tick observations and recovery

In total, 1600 *I. scapularis* larvae were introduced onto 40 test group mice (Control = 800, Treatment = 800).

#### 3.3.1. Collection moats

In the Treatment groups and Control groups, totals of 190 (23.8% of total larvae introduced) and 153 (19.1% of total larvae introduced) non-engorged larvae were respectively collected from within moats (Tables 4 and 5). Most non-engorged larvae were collected during the early stages of post-tick attachment, with 142 (92.8%) within the Control groups being collected at or before Day 1 post-tick attachment and 153 (80.5%) within the Treatment groups collected at or before Day 1. Non-engorged larvae were collected from the collection moat of every single test mouse. From Day 2 to Day 4, the number of non-engorged larvae collected within the Treatment groups ($n = 37$) was noticeably greater relative to the Control groups ($n = 11$).

Within the Treatment subgroups a total of 19 detached replete larvae (2.4% of total larvae introduced) were collected over the course of all post-tick attachment periods (Tables 4 and 5) with 0 ticks being collected from the moats of mice infested at Day 7 post-exposure. Within the Treatment subgroups, the proportion of mice from which replete larvae were collected was 0% (Day 7) and 40% (Day 21). Within the Control subgroups, a total of 331 detached replete larvae (41.4% of total larvae introduced) were collected over the course of all post-tick attachment periods (Tables 4 and 5). A total of 236 replete larvae were collected at Day 7 and 95 at Day 21. All mice within all Control subgroups had replete larvae collected from them and Day 3 and Day 4 post-attachment.

The combined Control subgroup mice had a significantly greater number of replete ticks collected, relative to combined Treatment mice for combined sexes ($Z = -5.342$, $p < 0.0001$), female mice ($Z = -3.447$, $p < 0.0006$), and male mice ($Z = -3.888$, $p < 0.0001$). C7 had a significantly greater number of replete ticks collected, relative to T7 ($Z = -4.001$, $p < 0.0001$), and C21 had a significantly greater number of replete ticks collected, relative to T21 ($Z = -3.262$, $p < 0.0011$). No significant differences were detected when making similar comparisons for non-engorged ticks collected from the moats.

#### 3.3.2. Capsule observations

A summary of capsule observations is presented in Table 6. Cumulatively, within the Control subgroups, the number of non-engorging larvae decreased from 26 at Day 2–4 at Day 4 post-exposure. The number of engorging larvae decreased from 276 at Day 2–68 at Day 4, which was reflected in the number of replete larvae that were collected in moats. Contrarily, within the Treatment subgroups, the number of observable non-engorging larvae increased from 227 at Day 2–239 at Day 4, and the number of engorging larvae deceased from 35 at Day 2–11 at Day 4. Representative images of larvae observed attached to Treatment and Control mice at Day 2 and Day 4 are presented in Fig. 2.

The combined Control subgroup mice had a significantly greater number of engorging larvae successfully detach, relative to combined Treatment mice for combined sexes ($Z = -5.314$, $p < 0.0001$), female mice ($Z = -3.647$, $p < 0.0003$), and male mice ($Z = -3.724$, $p < 0.0002$). The combined Treatment subgroup mice had a significantly greater number of larvae that were non-engorging, relative to combined Control mice for combined sexes ($Z = 5.337$, $p < 0.0001$), female mice ($Z = 3.450$, $p < 0.0006$), and male mice ($Z = 3.931$, $p < 0.0001$). C7 and C21 respectively, had significantly greater numbers of engorged larvae detached relative to T7 ($Z = -4.005$, $p < 0.0001$) and T21 ($Z = -3.392$, $p = 0.0007$). T7 and T21 respectively, had significantly greater numbers

**Table 3**
Estimated individual Grain Bait and fipronil total and daily consumption by Treatment group white-footed mice ($n = 20$).

| Tick Attachment (Days Post-Exposure) | Sex | Bodyweight (g) | Total Consumption | | | Daily Consumption | | |
|---|---|---|---|---|---|---|---|---|
| | | | Grain Bait (g) | Fipronil (mg) | mg/kg | Grain Bait (g) | Fipronil (mg) | mg/kg |
| Day 7 (T7) | Male | 22.9 ± 6.5 | 8.0 | 0.40 | 17.4 | 1.1 | 0.06 | 2.5 |
| | Female | 19.4 ± 1.1 | 3.3 | 0.17 | 8.5 | 0.5 | 0.02 | 1.2 |
| Day 21 (T21) | Male | 20.3 ± 1.7 | 6.4 | 0.32 | 15.7 | 0.9 | 0.05 | 2.2 |
| | Female | 19.2 ± 3.8 | 5.4 | 0.27 | 14.1 | 0.8 | 0.04 | 2.0 |

**Table 4**
Summary of flat and replete *I. scapularis* larvae collected from moats per day post-tick attachment.

| Group ID | Tick Attachment (Post-exposure) | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Flat | Replete | Flat | Replete | Flat | Replete | Flat | Replete | Flat | Replete | Flat | Replete |
| Treatment ($n = 20$) | Day 7 (T7) | 68 | 0 | 40 | 0 | 4 | 0 | 1 | 0 | 2 | 0 | 115 | 0 |
| | Day 21 (T21) | 28 | 0 | 17 | 0 | 17 | 0 | 11 | 5 | 2 | 14 | 75 | 19 |
| Control ($n = 20$) | Day 7 (C7) | 58 | 0 | 34 | 0 | 2 | 0 | 0 | 85 | 0 | 151 | 94 | 236 |
| | Day 21 C21) | 24 | 0 | 26 | 0 | 6 | 0 | 3 | 31 | 0 | 64 | 59 | 95 |
| Total Treatment | | 96 | 0 | 57 | 0 | 21 | 0 | 12 | 5 | 4 | 14 | 190 | 19 |
| Total Control | | 82 | 0 | 60 | 0 | 8 | 0 | 3 | 116 | 0 | 215 | 153 | 331 |

Flat = non-engorged, Replete = Fully engorged.

**Table 5**
Summary of the total number and mean non-engorged and replete *I. scapularis* larvae collected from moats over the course of post-tick attachment.

| Mouse Test Group | Days Post-Grain Bait Exposure | Total Larvae Introduced onto Mice | Larvae Collected from Moats | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total Non-engorged Larvae Recovered | Mean ± SD Non-engorged Larvae per Mouse | Proportion of Mice with Non-engorged Larvae (%) | Total Replete Larvae Recovered | Mean ± SD Replete Larvae per Mouse | Proportion of Mice with Replete Larvae (%) |
| Treatment | Day 7 (T7) | 400 | 115 | 11.5 ± 5.1 | 100 | 0 | 0 | 0 |
| | Day 21 (T21) | 400 | 75 | 7.5 ± 3.9 | 100 | 19 | 1.9 ± 3.3 | 40 |
| Control | Day 7 (C7) | 400 | 94 | 9.4 ± 3.9 | 100 | 236 | 23.6 ± 9.5 | 100 |
| | Day 21 (C21) | 400 | 59 | 5.9 ± 2.4 | 100 | 95 | 9.5 ± 4.9 | 100 |
| Treatment Total | | 800 | 190 | 9.5 ± 4.9 | 100 | 19 | 1.0 ± 2.5 | 20 |
| Control Total | | 800 | 153 | 7.7 ± 3.6 | 100 | 331 | 16.6 ± 10.3 | 100 |

**Table 6**
Mean number of attached *I. scapularis* larvae (±SD) per mouse observable within each capsule within each test group.

| Test Group | Days Post-Grain Bait Exposure | Observable Attached Ticks, Post-Tick Attachment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 2 | | | | Day 4 | | | |
| | | Non-Engorging | | Engorging | | Non-Engorging | | Engorging | |
| | | Total | Mean ± SD | Total | Mean ± SD | Total | Mean ± SD | Total | Mean ± SD |
| Treatment | Day 7 (T7) | 130 | 13.0 ± 5.6 | 4 | 0.4 ± 0.7 | 134 | 13.4 ± 5.3 | 0 | 0 |
| | Day 21 (T21) | 97 | 9.7 ± 5.1 | 31 | 3.1 ± 2.3 | 105 | 10.5 ± 5.9 | 11 | 1.1 ± 1.9 |
| Control | Day 7 (C7) | 11 | 1.1 ± 0.7 | 148 | 14.8 ± 4.4 | 4 | 0.4 ± 0.7 | 15 | 1.5 ± 0.7 |
| | Day 21 (C21) | 15 | 1.5 ± 1.5 | 128 | 12.8 ± 3.1 | 0 | 0 | 53 | 5.3 ± 1.5 |
| Treatment Total | | 227 | 11.4 ± 5.5 | 35 | 1.8 ± 2.2 | 239 | 12.0 ± 5.6 | 11 | 0.6 ± 1.4 |
| Control Total | | 26 | 1.3 ± 1.2 | 276 | 13.8 ± 3.8 | 4 | 0.2 ± 0.5 | 68 | 3.4 ± 2.3 |

of imbedded non-engorging larvae relative to C7 ($Z = 3.828$, $p < 0.0001$) and C21 ($Z = 3.686$, $p = 0.0002$).

### 3.3.3. Nymphal development (molting)

A summary of molting success is presented in Table 7. Zero (0) replete larvae were collected from moats of T7 mice and thus molting success was not calculable. Of the 19 replete larvae collected within T21, 12 (63.2%) molted by the end of the post-repletion period. Of the 234 larvae collected within C7, 155 (66.2%) molted. Of the 95 replete larvae collected within C21, 75 (78.9%) molted. Within the Treatment groups, the proportions of mice which had larvae successfully molt were 0% (T7) and 30% (T21). Within the Control groups, the proportions of mice which had larvae successfully molt were 100% (C7) and 90% (C21).

### 3.4. Fipronil plasma concentration

A list of all plasma samples analyzed for CP is presented in Table 8. Fipronil sulfone was the only metabolite detectable > LOQ of 0.04 ng/ml. All mice within the Treatment groups had CP at levels detectable > LOQ. The mean CP per mouse was 160.4 ng/ml (T7) and 25.6 ng/ml (T21). No CP was detected in Control samples. Males within T7 (224.7 ng/ml) and T21 (42.9 ng/ml) had markedly higher respective CP values than did females within T7 (96.0 ng/ml) and T21 (8.3 ng/ml). This supports the consumption results as well as the nymphal observations, as 3 female mice within T21 yielded molted nymphs, relative to 0 male mice. Nymphs were not recovered from mice with CP ≥ 18.4 ng/ml. The lowest was a female in T21 (2.5 ng/ml) which yielded 7 molted nymphs.

Not surprisingly, CP was significantly higher for mice in T7 relative to T21 ($Z = -3.288$, $p = 0.0010$). Male mice had noticeably higher CP,

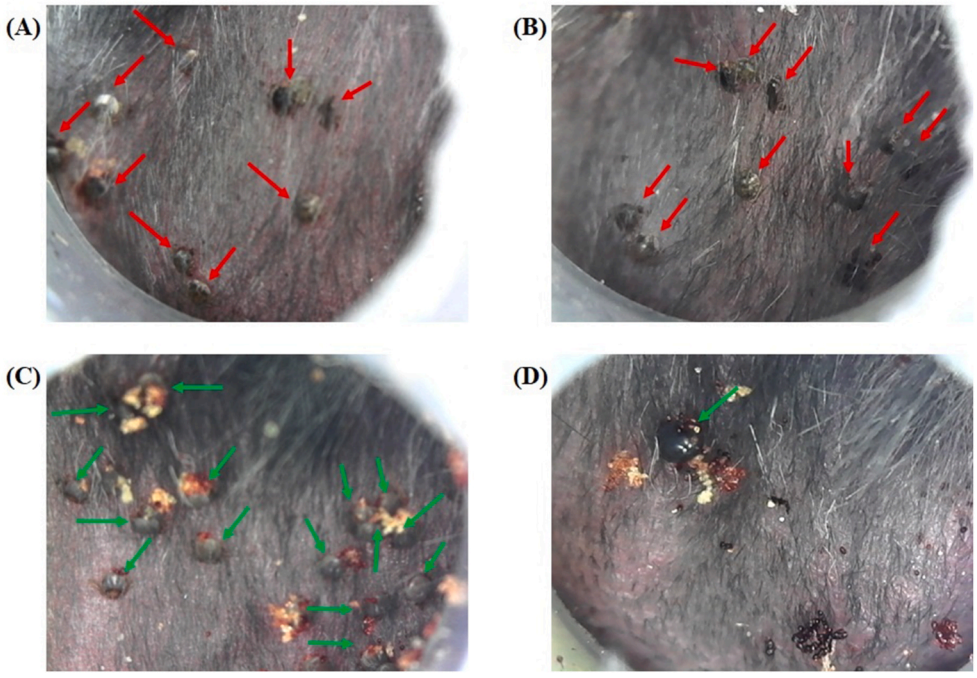


**Fig. 2. Representative images of Day 2 and Day 4 capsules observations.** Non-engorging larvae attached to Treatment mouse at (A) Day 2 and (B) Day 4. Engorging larvae attached and actively feeding on Control mouse at (C) Day 2 and (D) Day 4. At Day 4, the majority of larvae fed to repletion and detached from the Control mice, while the majority died in situ on the Treatment mice. Green arrows indicate live larvae and red arrows indicate dead larvae. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 7**
Summary of replete *I. scapularis* larvae successfully molting for each test group.

| Test Group ID | Days Post-Grain Bait Exposure | Total Mice | *Mean* Larvae Placed in Each Capsule Day 0 | Total Replete Larvae Placed in Desiccator | Mean Replete Larvae Placed in Desiccator | Total Replete Larvae Molting | Mean Replete Larvae Molting |
|---|---|---|---|---|---|---|---|
| Treatment | Day 7 (T7) | 10 | 40 | 0 | 0 | 0 | 0 |
| | Day 21 (T21) | 10 | 40 | 19 | 1.9 ± 3.3 | 12 | 1.2 ± 2.3 |
| | Cumulative | 20 | 40 | 19 | 1.0 ± 2.5 | 12 | 0.6 ± 1.7 |
| Control | Day 7 (C7) | 10 | 40 | 234 | 23.4 ± 9.4 | 155 | 15.5 ± 8.0 |
| | Day 21 (C21) | 10 | 40 | 95 | 9.5 ± 4.9 | 75 | 7.5 ± 3.8 |
| | Cumulative | 20 | 40 | 329 | 16.5 ± 10.2 | 230 | 11.5 ± 7.4 |

**Table 8**
Fipronil sulfone concentrations in white-footed mice utilized in tick attachments. ND = None detected.

| Test Group ID | Sex | Subgroup ID | Days Post-Grain Bait Exposure | | Fipronil Sulfone ng/ml | *n* Replete Larvae | *n* Molted Nymphs |
|---|---|---|---|---|---|---|---|
| | | | Tick Introduction | Plasma Collection | | | |
| Treatment | M | T7 | 7 | 11 | 124.9 | 0 | 0 |
| Treatment | M | T7 | 7 | 11 | 225.3 | 0 | 0 |
| Treatment | M | T7 | 7 | 11 | 390.5 | 0 | 0 |
| Treatment | M | T7 | 7 | 11 | 272 | 0 | 0 |
| Treatment | M | T7 | 7 | 11 | 110.9 | 0 | 0 |
| Treatment | F | T7 | 7 | 11 | 36.2 | 0 | 0 |
| Treatment | F | T7 | 7 | 11 | 87.2 | 0 | 0 |
| Treatment | F | T7 | 7 | 11 | 192.3 | 0 | 0 |
| Treatment | F | T7 | 7 | 11 | 49.6 | 0 | 0 |
| Treatment | F | T7 | 7 | 11 | 114.7 | 0 | 0 |
| Treatment | M | T21 | 21 | 25 | 65.8 | 1 | 0 |
| Treatment | M | T21 | 21 | 25 | 63.2 | 0 | 0 |
| Treatment | M | T21 | 21 | 25 | 52.7 | 0 | 0 |
| Treatment | M | T21 | 21 | 25 | 7.8 | 0 | 0 |
| Treatment | M | T21 | 21 | 25 | 25.2 | 0 | 0 |
| Treatment | F | T21 | 21 | 25 | 8.6 | 4 | 2 |
| Treatment | F | T21 | 21 | 25 | 18.4 | 0 | 0 |
| Treatment | F | T21 | 21 | 25 | 2.5 | 10 | 7 |
| Treatment | F | T21 | 21 | 25 | 6.6 | 4 | 3 |
| Treatment | F | T21 | 21 | 25 | 5.4 | 0 | 0 |
| Control | M | C7 | 21 | 11 | *ND | 21 | 20 |
| Control | F | C7 | 21 | 11 | *ND | 27 | 23 |
| Control | M | C21 | 21 | 25 | *ND | 15 | 13 |
| Control | F | C21 | 21 | 25 | *ND | 17 | 10 |

relative to females, with the difference being nearly significant ($Z = 1.928$, $p = 0.0539$).

### 3.5. Efficacy estimates

The mean number of replete ticks collected per mouse was 0 (T7) and 1.9 (T21) within the Treatment subgroups and 23.6 (7) and 9.5 (C21) within the Control subgroups. The efficacy of fipronil bait in preventing *I. scapularis* larvae from feeding to repletion was estimated to be 100% (T7) and 80% (T21).

The mean number of molted nymphs recorded per mouse at the conclusion of the post-repletion period was 0 (T7), 1.2 (T21), 15.5 (C7), and 7.5 (C21). No replete larvae were collected within T7 and thus 100% of ticks on mice were prevented from molting. In T21, the efficacy of Grain Bait in preventing all ticks on the mice from eventually molting was 84%.

## 4. Discussion

These results expand upon the use of fipronil formulated baits in controlling *I. scapularis* parasitizing white-footed mice and suggest that a federally approved fipronil flea bait has potential to control *I. scapularis* parasitizing white-footed mice. One hundred percent (100%) efficacy was obtained at Day 7 post-exposure and 80–84% efficacy obtained at Day 21 post-exposure. These metrics exceed the recommendations previously described by EPA for fleas and ticks (United States Environmental Protection Agency, 1998) and the results are supported by the results of the previous simulated field study conducted by Poché et al. (2021).

In addition to controlling larvae up to 21-days post-exposure, the mice exposed to fipronil bait for 168 h in the current study showed no observable signs of fipronil toxicity. The ability to keep the bait in the field for extended periods would logically reduce the labor required when positioning bait stations and increase the probability of treatment reaching a sizable proportion of the rodent population. The CP values obtained from mice during this simulated field study suggest potential for elevated bait acceptance among mouse populations under field conditions. Lowering the LOQ for CP detection from 1.25 ng/ml (Poche et al., 2021) to 0.04 ng/ml greatly improved our ability to detect CP. Contrary to Poché et al. (2021), 100% of mice within the Treatment group in the current study consumed enough fipronil to maintain CP above LOQ up to 7-days and 21-days post-exposure. Results further suggested 100% of parasitizing *I. scapularis* larvae could be controlled if mice had CP $\geq$ 18,4 ng/ml. Additionally, 100% control of *I. scapularis* larvae was obtained for a female mouse with CP 5.4 ng/ml, suggesting control could be obtained at reduced CP levels. A field trial would be useful in confirming these findings.

The consumption, efficacy and CP reported in the current study suggest that Grain Bait is palatable in the presence of an alternative food source, but that modifications to the delivery system might want to be considered. Unlike the results of Poché et al. (2021), in the current study females ate considerably less Grain Bait than did males. We noted that females had a greater tendency to nest in the bait stations and urinate on Grain Bait, which we suspect may have acted as a repellent. This was not as much of an issue with the paraffin-based block (Poché et al., 2021) as the blocks were able to be suspended in the bait stations using pins. Grain Bait as it currently stands is EPA approved for use in controlling fleas on black-tailed prairie dogs. The label indicates that Grain Bait is to be applied outside of active prairie dog burrows. A similar spot-treatment method might be beneficial in *P. leucopus* habitat, especially in large, wooded areas. However, white-footed mouse density in the wild averages roughly 4–12 mice per ~4000 $m^2$ (Aguilar, 2011), suggesting that the density around bait stations in the field would be much lower relative to the density in the current study. Thus, we suspect the issue of crowding and urination within bait stations would be minimized under natural conditions. Future field studies should consider evaluating multiple application procedures under field conditions.

If this federally registered flea product does become approved for use in controlling *I. scapularis* parasitizing white footed mice, managers will need to consider the best options for 1) maximizing the probability for successful tick reduction, and 2) creating a treatment scheme that is logistically and economically feasible. It is important to consider the optimum time of year to apply the bait under field conditions. Targeted larval control should be performed during the summer months, as tick larvae hatch out and feed during this time (Eisen et al., 2016a, 2016b). Previously, researchers have deployed bait stations containing topical tick control formulations in the summer and spring to control larvae and nymphs, respectively (Schulz et al., 2017; Williams et al., 2018; Jordan and Schultz, 2019). While larval control is the obvious priority considering their association with white-footed mice, nymphs play a critical role in the transmission cycle of *B. burgdorferi* s.s. Thus, if logistically and financially feasible, it could be useful to apply Grain Bait during peaks in nymph abundance in order to reduce pathogen transmission to alternative hosts that could serve as *B. burgdorferi* s.s reservoirs (Fish, 1993; Mather, 1993; Eisen et al., 2016a, 2016b). Poché et al. (2021) noted that preliminary data suggested efficacy of 0.005% fipronil against nymphs, and Poché et al. (2023) indicated 0.0025% to be highly efficacious against adult females. Thus, fipronil treatment can control all blood feeding *I. scapularis* life stages. Field trials would be useful in determining the overall effectiveness of spring + summer application and summer only application and would subsequently aid in determining the best treatment strategies.

Male mice consumed fipronil at an average rate of 2.5 mg/kg/day (T7) and 2.2 mg/kg/day (T21). Female mice consumed fipronil at an average rate of 1.2 mg/kg/day (T7) and 2.0 mg/kg/day (T21). This rate of consumption is lower than the 4.7 (male) and 4.8 (female) mg/kg/day reported for the paraffin block formulation (Poché et al., 2021). Similar to Poché et al. (2021) no symptoms of fipronil toxicity were observed, suggesting that Grain Bait may be safely administered to the target species under field conditions. This is further suggested by the fact that significant weight loss was not observed within the Treatment group mice. Considering the oral $LD_{50}$ of fipronil in mice is approximately 95 mg/kg [34], it is not surprising that adverse effects were not observed. The low rate of consumption and lack of adverse effects observed suggest that Grain Bait could be positioned in the field for extended durations. However, additional research explicitly evaluating chronic exposure of white-footed mice to Grain Bait would be useful.

Risk of fipronil toxicity to non-target animals should be minimal. A low dose (0.005%) and low application rate, in addition to the use of a species-specific bait station, should aid in reducing the risk to animal species. Prior research has concluded that fipronil represents reduced risk to non-targets, relative alternative insecticidal compounds, because its effectiveness allows for it to be applied at 100 to 200x less than other insecticides such as malathion and carbaryl (Norelius and Lockwood, 1999). If Grain Bait does become federally approved, managers should carefully determine the ideal application rates prior to bait deployment. For certain vertebrate species such as rabbits that might be more susceptible to fipronil (Gupta and Anadón, 2018), the use of bait stations will reduce risk of exposure. However, it may be advantageous for some wildlife species such as chipmunks (*Tamias* spp.), which may also serve as *B. burgdorferi* s.s reservoirs (McLean et al., 1993), to have access to the bait as well. Field studies would be useful in evaluating any potential impact of Grain Bait treatment on non-targets.

### 4.1. Conclusions

A fipronil grain bait (Kaput ® Flea Control Bait with Fipronil), presented to white-footed mice for 168 h, controlled larval *I. scapularis* at Day 7 and Day 21 post-exposure, significantly reducing the number of larvae feeding to repletion, detaching, and molting to nymphs. The study expands upon the results of Poché et al. (2021) and indicates that

multiple fipronil formulations may be useful in controlling tick vectors. These results suggest that this fipronil product may be palatable to multiple mammalian species, and it may be advantageous to continue to explore targeting other vector-host relationships of veterinary or medical importance.

## Declaration of competing interest

The study described in this manuscript titled, "Efficacy of a federally approved flea bait, orally administered to white-footed mice (*Peromyscus leucopus*), against blood feeding *Ixodes scapularis* larvae under simulated field conditions" is original and is not under consideration by any other journal. All authors approved the final manuscript and its submission. The authors declare that they have no conflict of interest.

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