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Effects of Ibuprofen and Caffeine Concentrations on the Common Bed Bug (Cimex lectularius L.) Feeding and Fecundity

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Abstract

Bed bugs are ectoparasites of humans and require a blood meal for their growth and reproduction. Since humans consume Ibuprofen as pain medication and drink coffee (caffeine), bed bugs are likely to acquire these drugs through blood feeding. In this study, we determined the biological effects of Ibuprofen and caffeine on bed bug feeding, fecundity and egg hatch. Five concentrations of Ibuprofen and caffeine were incorporated into reconstituted human blood (RHB). Control treatment had no Ibuprofen or caffeine. Each treatment had six replications. Groups of 20 adult bed bugs (10 males: 10 females)/treatment/replication were pre-weighed, allowed to feed for 45 minutes, and then reweighed. After feeding, bed bugs were transferred into a glass jar equipped with harborage and all the jars were placed in an environmental chamber undisturbed for three 7-day intervals to determine fecundity and nymph emergence. Ibuprofen data showed that the mean mass of the 20 adult bed bugs increased by 0.1074 g (125.65%) in the control but by 0.1336 g (157.30%) at 200 ppm after feeding. After 7 days, 306 and 146 eggs were laid by the 60 female bed bugs fed on 0 and 200 ppm Ibuprofen, respectively. After 2 weeks, 94% eggs hatched with no significant differences amongst treatments. In the caffeine experiments, the bed bug mean mass increases were 0.1219 g (163.90%) in control and 0.0790 g (104.62%) at 50 ppm caffeine. After 7 days, 264 eggs were laid by 60 female bed bugs in control but only 81 eggs were laid at the 50 ppm caffeine. Nymph emergence was >80% for all caffeine concentrations after 2 weeks. These results demonstrated that increasing Ibuprofen concentrations had positive effects on mass gain but negative effects on egg laying capacity of bed bugs. Caffeine concentrations had negative effects on bed bug feeding, fecundity and egg hatch.

Keywords: Cimex lectularius; Bed bugs; Ibuprofen; Caffeine; Feeding; Fecundity

Introduction

The bed bug (Cimex lectularius L., Hemiptera: Cimicidae) infestations have steadily increased and widely reported in North America, Western Europe, and Australia, since the 1990s, and are causing serious concerns to hotel/rental industry, public health professionals and the public at-large. Bed bugs indiscriminately infest buildings regardless of low- or high-income homes (98%), luxury hotels or motels (75%), College dorms (47%) or nursing homes (46%) [1]. The bed bug resurgence was attributed to an increase in international travel and trade, and use of less toxic insecticides [2-4]. The U.S. Department of Commerce reported that there were 51 million international tourists in 2000, a 15% increase over a period of 1995-2000 [4]. The bed bug management practices were affected from cancellation of chlorinated hydrocarbon insecticides, minimum use of organophosphates, carbamates, pyrethroids, use of less toxic insecticide-gel baits, and insect growth regulators [5]. The bed bug resurgence was also reported in other countries including Korea [4], Malaysia and Singapore [6], Italy [7] and China [8]. This recent bed bug resurgence has created a renewed interest to seek new information on bed bug biology, molecular based population genetics, and sustainable management techniques. Research on bed bug resistance to commonly used insecticides was conducted by numerous researchers [3,9-12].

Humans use various products containing Ibuprofen to relieve headache, minor aches, and pains. Humans also routinely consume coffee and various soft drinks containing caffeine. Since bed bugs principally feed on humans in residential environment, they can potentially acquire Ibuprofen and caffeine from blood stream. However, the biological impacts of these drugs on bed bugs have not been studied.

Ibuprofen (iso-butyl-propanoic-phenolic acid; (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid) is a non-steroidal anti-inflammatory drug. It is commonly used to reduce fever and treat pain or inflammation caused by headache, toothache, back pain, arthritis, menstrual cramps, or minor injury. Ibuprofen is the active ingredient in more than 50 brand names and generic medications, such as Advil® and Motrin® that humans consume. It is also used in combination with other drugs for control of other illness, such as flu (Advil® Flu and Body Ache Caplets), cold (Motrin® Children’s Cold) [13,14].

Several studies were conducted on the use of Ibuprofen to treat insect bites and stings [15,16] and other illnesses in humans [17,18], but no research was conducted to determine the effect of Ibuprofen on insect biology. There were studies on the impact of Ibuprofen on aquatic life, such as in Daphnia magna Mueller, Litopenaeus spp. Farante and Planorbis carinatus Muller [19-22]. Heckmann et al. [19,20] revealed that the population growth rate in water fleas (Daphnia spp.) was significantly reduced when exposed to Ibuprofen, and the survival was affected at the highest concentration. Alfaro-Montoya [21] studied the impact of Ibuprofen on marine shrimps (Litopenaeus spp.) and found significant differences in treated females with developing ovaries (45 ± 19%) as compared with females in control (12 ± 8%). Pounds et al. [22] detected a reduction in the weight of mollusks (P. carinatus) exposed to Ibuprofen.

Caffeine (1,3,7-trimethylxanthine) is a bitter white crystalline
alkaloid, a central nervous system stimulant and the most widely consumed addictive substance in the world [23]. Worldwide consumption was estimated around 70 to 76 mg/person/day [24]. The caffeine consumption in USA was estimated at 196 to 238 mg daily [23,25]. The common dietary sources of caffeine are coffee, chocolate, tea and some soft drinks. The amount of caffeine in food products varies, depending on the serving size, type of product and preparation method [26-28].

Fernandes et al. [29] reported that caffeine and chlorogenic acid (a phenolic compound from coffee) applied on coffee leaves stimulated the movement of the green scale insect (Coccus viridis, Green), thus reducing their feeding compared to untreated leaves. Caffeine, the phenolic, and the alkaloidic compounds derived from coffee leaves increased egg-laying by the coffee leaf miner, Leucopeta coffeella (Guérin-Ménéville) [30].

The biological effects of Ibuprofen and caffeine when consumed by bed bugs have not been previously studied. Therefore, this research was undertaken to gather data to fulfill this deficiency. We proposed the hypothesis that Ibuprofen and caffeine may negatively affect the bed bug biology. This hypothesis was tested with the following objectives: 1) determine the mass/body weight change in the bed bugs from blood feeding; 2) delineate the bed bug fecundity; and 3) ascertain nymph emergence.

Materials and Methods

Insects

The colony of bed bugs (Harlan strain) used throughout this study was established from live specimens obtained from the Department of Entomology, University of Minnesota, St. Paul, MN on March 2011. The Minnesota bed bug colony is descendants from the colony acquired from the Insect Control Research Center (Baltimore, MD) [31], with specimens collected from a natural infestation in Gainesville, FL sometime before 1983. In our laboratory, the bed bugs were confined in 500 mL Van Water and Rogers (VWR) short wide-mouth glass jar (VWR International, Radnor, PA), containing 9.0 cm circular VWR filter papers (Radnor, PA) with four folds for harborage and substrate for eggs. The glass jars containing bed bugs were covered with organza fabric (JoAnn’s Fabric, Lincoln, NE) for ventilation and containment. All jars were maintained in a Percival Scientific environmental growth chamber (Perry, IA) at 23 ± 2°C, 55 ± 5% relative humidity, and a photoperiod of 12:12 (Light: Dark with light on 10 AM) [32]. The bed bugs were fed weekly with expired reconstituted human blood (RHB) obtained from the Nebraska blood bank (Lincoln, NE).

Treatments

This study included five concentrations of Ibuprofen (40, 80, 120, 160 and 200 ppm) and caffeine (10, 20, 30, 40 and 50 ppm) plus the control as individual treatments (Tables 1 and 2). A stock solution from each of Ibuprofen and caffeine standard was prepared (Ibuprofen: 200 ppm, Fluka PHR1004; caffeine: 50 ppm, Fluka PHR1009; Sigma-Aldrich, St. Louis, MO) and diluted further to attain desired concentrations as specified in Tables 1 and 2. The Ibuprofen concentrations were determined based on an average human body having 5 liters of blood [33]. The Ibuprofen and caffeine were incorporated in the RHB.

Experimental design

The experimental setup was a complete randomized design with feeding groups consisted of one randomly selected experimental unit (EU) of each treatment. The EUs consisted of 20 adult bed bugs (10 M:10 F) kept in 125 ml VWR straight wide-mouth glass jars (Radnor, PA) and sealed with the organza fabric for the duration of the study. The bed bugs were provided with one 9.0 cm VWR filter paper with four folds for harborage. Each treatment (of Ibuprofen, caffeine and control) was replicated six times and was conducted at different time intervals.

Feeding experiment

The bed bug feeding setups used for the treatments were modeled after the Chin-Heady et al. [35]. Six 50 mm petri dishes were filled with the appropriate concentration of treated blood (e.g., for the Ibuprofen, each 50 mm petri dish was filled with one of the concentrations (0, 40, 80, 120, 160 and 200 ppm) (Figure 1a). The petri dishes were labeled, sealed (with Nescofilm, LabShop, UK) and placed on a 316.7 cm² Sybron Thermolyne 1000 hotplate/stirrer (VWR, Radnor, PA) set at 36 ± 2°C. One 125 ml beaker with 50 ml water was placed in the center of the hot plate to monitor the temperature (Figure 1b). After the blood was warmed up, the appropriate EU (0, 40, 80, 120, 160 or 200 ppm, one replication each) was placed upside down on the correspondingly labeled 50 mm petri dish to feed for 45 minutes (Figure 1c). After the 45 minutes, the petri dishes with blood were agitated to resuspend any settled blood cells. This process was repeated with each replication of EUs (0, 40, 80, 120, 160 or 200 ppm) until the bed bugs were fully fed. The 45-minute feeding regime was based on Johnson [36] who reported that bed bugs feed to repletion between 20 and 30 minutes if undisturbed.

Mass gain/loss

The groups of 20 adult bed bugs in ach experimental unit (EU) were pre-weighed on an Ohaus GA110 analytical balance (Parsippany, NJ) and the mass recorded. The bed bugs were allowed to feed for 45 minutes, removed from the EU and reweighed. The difference between the two masses is due to the amount of blood consumed. The EUs were then placed in the environmental chamber (at conditions described earlier) undisturbed for one week.

Fecundity and nymph emergence

After one week in the environmental chamber, the adult bed bugs
were removed from the EUs and the numbers of eggs laid on the filter paper in each EU were recorded. The EUs including the filter papers with eggs were returned to the environmental chamber undisturbed for two 7-day intervals to allow the bed bug nymphs to emerge. Emerged nymphs were counted twice, at 1 week and 2 weeks after eggs were laid. The percent nymph emergence was calculated by comparing the number of eggs laid versus the number of nymphs emerged.

**Statistical analysis**

To determine the effect of Ibuprofen and caffeine on the bed bug feeding and fecundity, the data were analyzed using analysis of variance (ANOVA), SAS Enterprise Guide 4.3 [37]. The significant differences were further tested with the pairwise comparisons of the treatment levels using the generalized linear model (GLM) procedure with multiple comparisons corrected by the Tukey’s test.

**Results**

**Mass Gain/Loss and egg production**

A significant effect (p<0.05) of the Ibuprofen concentrations was observed on bed bugs mass percent increase (F=2.57, df=5, 30, P=0.047), and eggs production (F=5.38, df=5, 30, P=0.0012) (Table 1). The regression R² and coefficient of variation were 0.2997 and 13.023 for the mass percent increase, and 0.4732 and 27.039 for bed bugs egg production, respectively. There was a significant increase in blood consumed by the bed bugs with 200 ppm Ibuprofen when compared to lower Ibuprofen concentrations and the control. Addition of Ibuprofen to the bed bug’s diet also resulted in a significant reduction in egg production at 160 and 200 ppm concentrations (Table 3).

Significant differences (p<0.05) were observed in percent mass decrease (F=5.147, df=5, 30, P=0.0007) and less egg production (F=7.02, df=5, 30, P=0.0001) when bed bugs were fed on caffeine incorporated blood meals (Tables 2 and 4). Regression R² and coefficient of variation were 0.4682 and 18.42 for the mass percent increase and 0.5461 and 31.09 for egg production, respectively. The analysis of variance determined that the addition of caffeine to the bed bugs’ diet had a significant negative impact on the bed bugs feeding and fecundity.

Further observation revealed that the maximum mass increase was at the highest concentration of Ibuprofen (200 ppm) in the blood, 157.30

### Table 1:

Average weight of the 20 bed bugs (10 M: 10 F) before and after feeding, plus mass and percent increase for each Ibuprofen concentration added to their blood

| Treatment* Ibuprofen (ppm) | Bed Bugs Mean Mass (g) (x SEM) | Mean % Increase (x SEM) |
|----------------------------|--------------------------------|------------------------|
| N** Before Feeding | After Feeding | Increase |
| 0 | 0.0856 ± 0.0019 | 0.1930 ± 0.0043 | 0.1074 ± 0.0035 | 125.65 ± 4.59 a |
| 40 | 0.0831 ± 0.0022 | 0.2014 ± 0.0049 | 0.1183 ± 0.0038 | 142.67 ± 5.03 a |
| 80 | 0.0850 ± 0.0008 | 0.1939 ± 0.0064 | 0.1090 ± 0.0066 | 128.42 ± 8.12 ac |
| 120 | 0.0824 ± 0.0012 | 0.1992 ± 0.0036 | 0.1068 ± 0.0029 | 141.86 ± 3.16 a |
| 160 | 0.0849 ± 0.0023 | 0.2185 ± 0.0123 | 0.1096 ± 0.0111 | 131.88 ± 12.91 ae |
| 200 | 0.0849 ± 0.0016 | 0.2185 ± 0.0062 | 0.1336 ± 0.0054 | 157.30 ± 5.80 bd |

*Each treatment had six replications
**Total numbers of bed bugs per treatment

### Table 2:

Average weight of the 20 bed bugs (10 M: 10 F) before and after feeding, plus mass and percent increase for each caffeine concentration added to their blood

| Treatment* Caffeine (ppm) | Bed Bugs Mean Mass (g) (x SEM) | Mean % Increase (x SEM) |
|----------------------------|--------------------------------|------------------------|
| N** Before Feeding | After Feeding | Increase |
| 0 | 0.0745 ± 0.0015 | 0.1964 ± 0.0060 | 0.1219 ± 0.0058 | 163.9001 ± 8.81 a |
| 10 | 0.0823 ± 0.0028 | 0.2056 ± 0.0039 | 0.1233 ± 0.0043 | 151.2402 ± 8.11 ac |
| 20 | 0.0779 ± 0.0019 | 0.1919 ± 0.0042 | 0.1139 ± 0.0049 | 147.0597 ± 9.06 ae |
| 30 | 0.0822 ± 0.0032 | 0.1882 ± 0.0073 | 0.1060 ± 0.0091 | 131.2207 ± 14.58 a |
| 40 | 0.0779 ± 0.0035 | 0.1665 ± 0.0076 | 0.0885 ± 0.0044 | 113.7580 ± 3.43 bd |
| 50 | 0.0747 ± 0.0020 | 0.1538 ± 0.0148 | 0.0790 ± 0.0135 | 104.6169 ± 15.95 bd |

*Each treatment had six replications
**Total numbers of bed bugs per treatment

### Table 3:

The number (and average per replication) of eggs laid by 60 female bed bugs in one week after consuming Ibuprofen in reconstituted human blood, plus the percent bed bug nymph emerged at week one and two after eggs were laid. Different letters represent significant differences (p<0.05) between the various test concentrations.

| Treatment* Ibuprofen (ppm) | Egg Production | Mean % Nymphal Emergence (x SEM) |
|----------------------------|----------------|---------------------------------|
| N** Total | X ± SEM | Week 1 | Week 2 |
| 0 | 60 | 306 | 51.0 ± 3.74 a | 28.76 ± 7.86 | 97.06 ± 0.96a |
| 40 | 60 | 300 | 50.0 ± 3.65 ac | 30.33 ± 4.92 | 98.33 ± 0.95a |
| 80 | 60 | 274 | 45.67 ± 2.42 ac | 33.94 ± 3.01 | 99.27 ± 0.47a |
| 120 | 60 | 268 | 44.67 ± 6.18 ac | 33.58 ± 6.95 | 94.78 ± 2.17 a |
| 160 | 60 | 196 | 32.67 ± 5.88 b | 60.71 ± 7.92 | 99.49 ± 0.79 a |
| 200 | 60 | 146 | 24.34 ± 0.80 bd | 59.59 ± 12.72 | 97.26 ± 1.92 a |

*Each treatment had six replications
**Total numbers of bed bug females per treatment
The bed bug nymph emerged at week one and two after eggs were laid. Different letters represent significant differences (p<0.05) between the various test concentrations.

The number (and average per replication) of eggs laid by 60 female bed bugs in one week after consuming caffeine in reconstituted human blood, plus the percent egg production and mean % nymph emergence:

Table 4: The number (and average per replication) of eggs laid by 60 female bed bugs in one week after consuming caffeine in reconstituted human blood, plus the percent egg production and mean % nymph emergence.

| Treatment* Caffeine (ppm) | Egg Production | Mean % Nymph Emergence |
|--------------------------|----------------|------------------------|
|                          | N** Total | x ± SEM | Week 1 | x ± SEM | Week 2 |
| 0                        | 60 264   | 44.00 ± 2.44 a     | 11.74 ± 5.06 | 94.70 ± 1.82a |
| 10                       | 60 170   | 28.34 ± 4.51 b     | 10.59 ± 5.27 | 91.76 ± 4.62a |
| 20                       | 60 176   | 29.34 ± 4.36 b     | 14.77 ± 6.94 | 97.16 ± 1.62a |
| 30                       | 60 164   | 27.34 ± 3.23 b     | 10.37 ± 5.20 | 93.29 ± 4.67a |
| 40                       | 60 127   | 21.17 ± 2.38 c     | 11.61 ± 10.78 | 92.13 ± 2.94a |
| 50                       | 60 81    | 13.50 ± 7.01 d     | 8.64 ± 6.81  | 81.48 ± 10.07a |

Each treatment had six replications
**Total numbers of bed bug females per treatment

Table 4: The number (and average per replication) of eggs laid by 60 female bed bugs in one week after consuming caffeine in reconstituted human blood, plus the percent egg production and mean % nymph emergence.

The number of eggs produced by the 60 female bed bugs in the control for the caffeine treatment was 264, with an average of 44.00 ± 2.45 (x ± SEM) per replication (Table 4). There was a decrease in the number of eggs produce by the bed bugs fed with the increasing concentration of caffeine in the RHB. The least number of eggs were produced by the 60 female bed bugs fed with the highest concentration of caffeine (50 ppm) in the RHB reflecting a total of 81 eggs, 13.5 ± 2.86 per replication (Table 4). The difference between the number of eggs laid by the bed bugs in the control vs. the highest caffeine concentration (50 ppm) was 64%. There were significant differences (p<0.05) in the number of eggs produced by the control vs. all concentrations of caffeine in the RHB (Table 4).

For the caffeine consumption study, of the 264 eggs laid by the bed bugs in the control treatment 11.7 and 94.7% hatched after week one and two, respectively (Table 4). The Ibuprofen may have affected the egg’s development, which resulted in the 52% reduction in eggs laid at the 200 ppm Ibuprofen concentration, but it did not affect nymph emergence. After two weeks, there were no significant differences in nymph emergence among the treatments. There was a 97.26% hatch rate for the eggs (Table 3) in the Ibuprofen treatments.

Caffeine effect on fecundity and nymph emergence

The number of eggs produced by the 60 female bed bugs in the control vs. all other caffeine concentrations (<30 ppm) did not affect bed bugs feeding (Table 2), had only 81% nymph emergence (Table 4). Although low caffeine concentrations (<30 ppm) did not affect bed bugs feeding (Table 2), there was a significant reduction in the number of eggs laid (Table 4). For two weeks, there were no significant differences in nymph emergence among the treatments (Table 4).

Discussion

This study revealed that Ibuprofen had positive effect on blood consumption but negative effect of on egg production. However, the percent nymph emergence was not significantly affected in various Ibuprofen concentrations. The bed bugs fed on caffeine incorporated RHB had negative effects on both mass gain and egg production. When fed with increasing levels of Ibuprofen, there was a significant decrease in the number of eggs laid by the bed bugs. A caution should be exercised because ibuprofen can have negative side effects in humans, such as increased risk of gastrointestinal bleeding [38,39], and should not be considered as a management strategy for bed bugs.

When fed with increasing concentration of Ibuprofen, although the bed bugs fed to repletion, the number of eggs laid decreased by 47.7% between the control and the highest concentration of Ibuprofen.
in the blood. One reason for the reduction in egg produced may be that Ibuprofen affected the egg development in the bed bugs. Alfaro-Montoya [21] has shown that Ibuprofen negatively affected marine shrimp ovaries. Tesh and Guzman [40] fed three mosquito species various concentrations of ivermectin in human blood and recorded mortality and effect on fertility. These authors reported that dying mosquitoes displayed signs of acute toxicity, such as incoordination, movement difficulty and lethargy. They also observed a noticeable decrease in both the number and viability of the mosquitoes’ eggs. In another study conducted by Sheele et al. [41] to observe the effect of ivermectin on bed bugs, volunteers consumed 200 mg/kg orally and then had bed bugs feed on them, 50 and 63% (n=24) mortality was recorded, compared to 4 and 8% (n=24) in the control group, after 2- and 20-days, respectively.

The result from the caffeine study showed that with increasing caffeine concentrations in RHB, there were negative effects on bed bugs feeding and fecundity, but not on nymph emergence. There was a positive relation between the numbers of egg produced by the bed bugs vs. percent mass increase due to feeding. The more blood the bed bug consumed resulted in more egg production. A negative correlation was observed between increasing caffeine concentration in the blood vs. the number of eggs produced by and the amount of blood consumed by the bed bugs. When fed with increasing concentration of caffeine, bed bugs consumed significantly less (36%) blood and produced significantly fewer eggs, 64% between the control and the highest concentration (50 ppm) of caffeine in the blood. Wright et al. [42] found that honey bees rewarded with naturally occurring caffeine from Coffea and Citrus species, were three times as likely to remember the floral scent as was honey bees rewarded with sucrose alone. It also improves the honey bees’ long-term memory for flowers.

The correlation between the number of eggs produced and the amount of blood consumed was explained by Johnson [36] who reported three interrelated variables, the weight of unfed female, a mass of blood meal and the number of eggs produced. Johnson [36] further reported a positive correlation between the weights of unfed females and a mass of blood meal with the number of eggs produced.Usinger [43] also, observed a correlation between food intake and egg production and longevity, although longevity was not studied in this research, a correlation between food intake and egg production was observed. Similar observations were recorded in other insect species; the checker-spot butterfly (Euptychius editha) had increased egg production and egg mass with increased consumption of carbohydrates and nectar [44]. In previous studies, [45,46] consumption of caffeine was shown to cause mortality in insects. Although not tested in this study, further research is needed to confirm mortality in bed bugs.

In conclusion, Ibuprofen and caffeine negatively affected bed bugs fecundity, although nymph emergence was unaffected. When fed with increasing levels of Ibuprofen, there was an increase in blood consumed, but when fed with increasing levels of caffeine, there was a decrease in blood consumed by the bed bugs. There was a greater than 81% emergence of nymphs from the eggs laid by the bed bugs exposed to Ibuprofen and caffeine. At all caffeine concentrations, the eggs laid were significantly less than the control. A positive correlation was observed between the number of eggs produced and the amount of blood consumed. This was in agreement with other similar studies, which reported a positive association between a mass of blood meal with the number of eggs produced in previous bed bug studies as well as several other insects.

The method developed in this research may be used to study the effects of other compounds, humans consume on bed bugs or even other insects that feed on humans, such as mosquitoes and ticks. Future studies could address the effects of other compounds, such as nicotine or other medication, taken on a regular basis, on bed bugs feeding and fecundity. Some of these studies could be extended to test survival and effects on the nymphs, which were hatched from eggs developed during the exposure to the test compounds.

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