JOURNAL OF
EMERGING INVESTIGATORS

Article

The effect of calcium on mealworm iron metabolism

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SUMMARY

Calcium is an inhibitor of iron absorption in the mammalian iron metabolism system. The insect iron metabolism system differs from the mammalian iron system. Therefore, inhibition of iron absorption may not occur in insects as in mammals. Supplementation of iron or calcium has been studied in some insect species, as it is a potential solution to nutrient deficiencies. However, simultaneous calcium and iron supplementation has not been studied in insects. We sought to show whether calcium inhibits iron absorption in mealworms, a common edible insect, by supplementing iron and calcium jointly and measuring iron content through spectrophotometry. Based on genetic homologs between the insect and iron mammalian system, we hypothesized that calcium inhibits iron absorption in insects. Our data did not show significant differences in iron content between non-supplemented mealworms, iron-supplemented mealworms, and calcium- and iron-supplemented mealworms. However, patterns of iron content decrease and significantly less weight gain in the jointly iron- and calcium-supplemented mealworms indicate a possibility of absorption inhibition. This offers insight into the variable effects of calcium on insect metabolism. There is a need for further study of insect iron absorption inhibition to quantify calcium supplementation's potential effects on nutrient-rich edible insect development.

INTRODUCTION

Iron-deficiency anemia (IDA) is a worldwide problem. Anemia afflicted approximately 800 million women and children globally as of 2011; iron deficiency caused 42% of those cases in children and 50% of those cases in women (1). IDA is linked to reduced overall growth and health in those affected women and children, as iron is an important micronutrient (a nutrient with low necessary intake) (2, 3). IDA has disproportionately negative impacts and magnitude in low- and middle-income countries (LMIC) (1). The lower productivity and delayed cognitive development linked with anemia pose social and economic costs to LMIC (2). Increasing dietary iron is the primary IDA prevention method (2). However, animal products, the most common iron-rich food sources, are economically inaccessible to many with micronutrient deficiencies (3). As a result, IDA is perpetuated in LMIC. Entomophagy, or the consumption of insects, is a proposed lower-cost alternative iron source (3). Entomophagy is also part of a wider dietary supplementation movement against world hunger (3). Insects, particularly mealworms, are suggested to be more sustainable than traditional livestock (4, 5). Various insects, including mealworms, have sufficient protein content to be a possible protein alternative (6). This potential importance of entomophagy in sustainable anti-hunger and anti-IDA strategies necessitates studies that investigate edible insect iron content optimization (7). Iron absorption inhibitors prevent the absorption of iron for biological use and facilitate iron excretion (8). These inhibitors are a potential barrier to insect iron content optimization (9). Studies investigating the impact of dietary iron absorption inhibitors on insect iron content could provide insight into insect iron content optimization and aid in reducing the worldwide IDA burden. To contribute to this goal, we tested the effect of calcium, a mammalian iron absorption inhibitor, in mealworms.

In this paper, we generalized iron systems as either 'insect' or 'mammalian' due to the dissimilarities between the two (10). In both systems, iron (Fe) is present in two forms: heme (Fe^{2+}), which has higher bioavailability, and non-heme (Fe^{3+}) (11, 12). In the insect system, most iron is contained in proteins distinct from mammalian iron-carrying proteins, resulting in a unique iron absorption process (3). In insects, the primary factors that affect mineral content are feed, life stage, and species (7). Like in mammals, the most straightforward way to increase insect iron content is increasing dietary iron, either short-term (a practice termed "gutloading") or long-term (13). Basic insect iron content and supplementation have been investigated in various species (6, 14–18). However, the effect of edible iron absorption-inhibiting compounds, which are known to influence iron absorption in humans, are not well-studied in insects or considered in the entomophagy industry (7, 10). This could prevent optimization of edible insect nutritional content.

Many insect species are edible and may aid in the entomophagy movement (19). We used yellow mealworms (*Tenebrio molitor*) in this experiment. Mealworms are ideal for a short-term project, as they grow faster and more easily than other edible insects (20). Mealworms are also a prime example of an edible insect and can survive on a diversity of diets, so they can be nutritionally altered easily (19). Mealworms are one of the most commonly produced insects for non-human consumption (e.g., pet feed) (20, 21). Although achieving representation of the entire edible insect industry is not feasible and mealworms are not consistently consumed worldwide, the widespread use and ease of production made *T. molitor* an ideal model (20).

In mammalian systems, several edible compounds inhibit dietary iron absorption (9). Among iron absorption inhibitors,

https://doi.org/10.59720/24-166

calcium is unique because it inhibits both heme and non-heme iron absorption (22). Calcium intake is insufficient for many people globally (23). Insect calcium content and optimization have been studied in various insect species as an avenue for reducing calcium deficiency (23–26). Since both calcium and iron may be supplemented in insects, knowledge of calcium's effects on insect iron absorption is pertinent. If iron and calcium are supplemented simultaneously in insects, it is unknown if calcium will inhibit iron absorption. Although information on mammalian systems is not directly applicable to insect systems, links to the mammalian calcium-iron absorption inhibition pathway have been found in insects (10, 27). One major hypothesis on calcium's inhibition of mammalian iron absorption is that calcium affects divalent metal transporter 1 (*DMT1*), which participates in both heme and non-heme iron transport (22). *DMT1* is a homolog of *Malvolio* (*Mvl*) in insects, which is similarly thought to be involved in iron uptake and transport (10, 27). This connection implies that calcium may inhibit iron absorption in the insect system (and accordingly the mealworm system) like in the mammalian system. *Mvl* is likely present in *T. molitor*, but it has not yet been shown in other studies or in NCBI's gene database. *Mvl* is present in almost all insect species with sequence data, including another beetle (10, 28). Furthermore, calcium may be more likely to affect insect iron systems than other iron absorption inhibitors due to its wide effect on heme and non-heme mammalian iron (19). Thus, because calcium is a supplement of interest that seems likely to inhibit iron absorption in the insect system, we used calcium as the potential iron absorption inhibitor in this study. Our initial hypothesis was that calcium would inhibit insect iron absorption. However, the insect system's distinct genealogy and the weak scientific literature surrounding mammalian iron-calcium interactions and insect iron metabolism may reasonably indicate that the insect iron system could interact uniquely with calcium.

When studying calcium as an iron absorption inhibitor, several factors influence its observed inhibitory effect, including the calcium compound used, dose administered, and usage length (11, 22, 29). Long-term calcium supplementation does not affect iron content in humans, contrasting the significant inhibitory effect during short-term (one meal) studies (22, 30). These factors are relevant when gauging calcium's inhibitory effect in insects, as their effects and mechanisms may differ in insects. In the insect iron system, the effects of different calcium salts are not well-known; the dose for significant iron absorption inhibition and time to develop resistance to iron absorption inhibition may be unique. We therefore considered the short-term effects of gutloading and controlled for calcium salt and dose.

Based on genetic links in the mammalian and insect iron systems, we hypothesized that calcium would inhibit iron absorption. This would cause calcium-supplemented mealworms to have lower iron content than mealworms without calcium supplementation. Our data showed significant changes in weight gain but not iron content due to calcium supplementation. As such, we could not confirm that calcium supplementation inhibits insect iron absorption. The effects of calcium on insect metabolism should be investigated further.

RESULTS

We tested the ability of calcium to inhibit iron absorption using the mealworm digestive system, a phenomenon seen in mammals that could influence the mineral contents of insects grown for entomophagy. We split mealworms into 3 experimental groups, each with 6 samples of 30 mealworms. We divided groups by dietary supplementation: neither iron nor calcium (NI/NC), iron but no calcium (I/NC), and both iron and calcium (I/C). NI/NC was expected to represent baseline iron content. I/NC was a positive control for iron supplementation. We gutloaded each sample with their respective supplements and then tested each sample's iron content through spectrophotometry.

We compared three measurements across groups to test the correlation between the utilized supplements and iron content. The primary data sets were iron content (converted to mg Fe / 100 g dry matter (DM)) and weight change after gutloading. We also compared the initial sample weights to test for bias due to non-random sample assignment.

Mean iron content trends were somewhat variable (**Figure 1**). The mean iron content for group NI/NC (M = 3.8471; SD = 2.3491) was similar to that of I/NC (M = 4.2962; SD = 2.5924). Group I/C had a lower mean iron content of 2.5163 mg Fe / 100 g DM (SD = 2.4307). Neither ANOVA nor pairwise tests showed significant differences, which did not indicate that either iron or calcium supplementation impacted iron concentration. Variation was more pronounced for weight change data (**Figure 2**). Group NI/NC had a mean weight change of 0.00920 g/worm (SD = 0.00497); group I/NC had a similar mean weight change of 0.00909 g/worm (SD = 0.00500). Group I/C had a lower mean weight change of 0.00244 g/worm (SD = 0.00248). Mean weight gains were significantly different across the groups (ANOVA p = 0.023816); pair-wise, group I/C was significantly different from group NI/NC (p = 0.03874) and group I/NC (p = 0.04184). Initial weight distribution may have been uneven, as sample distribution wasn't fully randomized (pupated, dead, and underdeveloped mealworms weren't nutritionally equivalent to the sample and were avoided) (6). However, initial weights for NI/NC (M = 2.2883; SD = 0.1477), I/NC (M = 2.2567; SD = 0.2002), and I/C (M = 2.340; SD = 0.2130) were taken and there were no significant differences (ANOVA F = 0.29699, p = 0.747322). This gives stronger credence to the measured difference in weight gain. The differences in weight gain between groups indicate that joint calcium and iron supplementation reduced mealworm weight gain, but iron supplementation alone did not increase weight gain. Additionally, there was a notable trend of gradual increase over samples 1–6 in iron content and weight data (**Figure 3**, **4**).

We linearized the standard curve data, which we created to convert measured absorbance to iron content. We used the data from 0.00, 0.25, 0.50, 0.75, and 1.00 mM solutions of $Fe(NO_3)_3$ in 0.1 M HCl to create a polynomial trend line (n = 5; $-1.91x^2 + 4.47x - 0.0475$; R^2 = 0.995) and a linear trend line (n = 5; $2.56x + 0.192$; R^2 = 0.948). We used the data from 0.00, 0.25, and 0.50 mM solutions to create a linear trend line (n = 3; $3.57x - 0.0207$; R^2 = 0.998).

DISCUSSION

Calcium inhibits iron absorption in mammals and could affect the optimization of insect nutrition for entomophagy. We tested for calcium inhibition of iron absorption by comparing mealworms without supplements (NI/NC), mealworms with iron supplementation (I/NC), and mealworms with iron and calcium supplementation (I/C).

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https://doi.org/10.59720/24-166

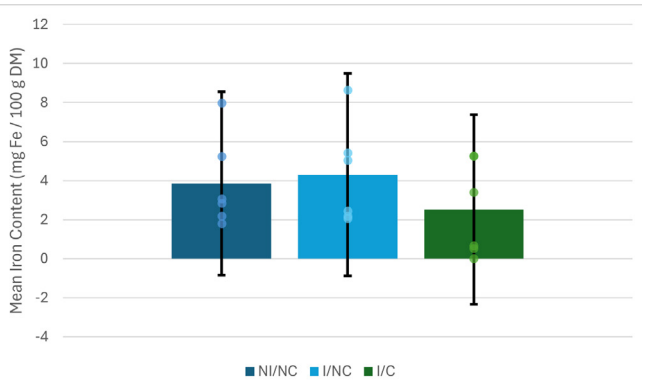

Figure 1. Effect of calcium and iron supplementation on mealworm iron content. Data is presented as mean ± 2SE iron content (n = 6 samples per group) for each group. For each group, 6 samples of 30 mealworms were fed for 48 hours under control conditions (NI/NC), with supplemented iron (I/NC), or with supplemented iron and calcium (I/C). Absorbance of each sample was measured through spectrophotometry and converted to iron content using a standard curve. One-way ANOVA (F = 0.84993, p = 0.447034) and subsequent pairwise tests (NI/NC:I/NC p = 0.94652; NI/NC:I/C p = 0.62602; I/NC:I/C p = 0.44163) were conducted.

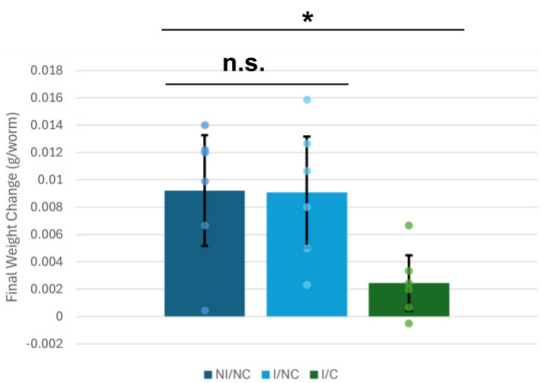

Figure 2. Effect of calcium and iron supplementation on mealworm weight gain. Data presented as mean ± 2SE weight gain (n = 6 samples per group) for each group. For each group, 6 samples of 30 mealworms were fed for 48 hours under control conditions (NI/NC), with supplemented iron (I/NC), or with supplemented iron and calcium (I/C). Weight was measured before and after supplementation to find weight change. One-way ANOVA (F = 4.84464, p = 0.023816) and subsequent pairwise tests (NI/NC:I/NC p = 0.99911; NI/NC:I/C p = 0.03874; I/NC:I/C p = 0.04184) were conducted. *p < 0.05.

I/C mealworms had significantly lower weight gain than NI/NC and I/NC, but no group had significantly different iron content. This did not support our hypothesis of inhibition, as iron absorption inhibition would have caused iron loss. However, imprecise spectrophotometer measurements and variable data between samples caused a high standard deviation in iron content. This made statistical differences less likely when combined with the low sample size for each group (n = 6). However, I/C had visibly lower mean iron content than NI/NC and I/NC (**Figure 1**). This is the expected trend if calcium supplementation inhibits iron absorption.

There was no difference in weight change or iron content between groups NI/NC and I/NC. This indicates that the iron supplementation did not increase iron content, which may be due to the administered iron's form or amount (0.0117 mg powder-form ferrous fumarate). It is possible that mealworms are not biologically receptive to, or will not consume, ferrous fumarate powder. Alternatively, the dosage may have been insufficient to measurably increase iron content, or powder loss may have made the iron supplementation ineffective.

If we consider the trends in the iron content data with the assumption that iron absorption inhibition reduced iron content, the effect of calcium was not in line with earlier assumptions about gut iron and tissue iron. According to the established understanding of insect mineral metabolism, a short-term diet should only affect gut iron (13). As such, the supplemented iron, the primary dietary iron source, should have been the only biological iron affected by calcium. Thus, since supplemented iron did not appear in the iron content data, iron available for absorption inhibition should have been negligible. The decreases in iron content and weight gain after calcium supplementation raise questions about the biological range of iron absorption inhibition in mealworms. These trends may be due to trace amounts of iron in the feed. Although we chose feed without listed iron content, nutritional labels do not list iron to or past a tenth of a milligram.

The gradual increase in iron content and weight gain across the six samples is also relevant to the iron content data. This trend may be due to the sample storage method or time. We gutloaded two newly bought samples of each group each week for three weeks. After each gutloading, we kept the respective samples frozen until the fourth week, when we completed burning. The trend of increase in iron content and weight may be due to time-correlated differences between samples (e.g., age of mealworms from PetSmart and/or time frozen). The containers that stored the mealworms are another possible contributor. We stored samples 1–2 in unused weigh boats from freezing until filtration, while we stored frozen samples 3–6 in the containers that previously held them during gutloading (the containers were first cleaned out). Although we could still reliably measure relative iron content between groups, the chronological trend diminishes the accuracy and precision of the iron content and weight data.

The divergence in statistical significance between weight gain data and iron content data limits the conclusion. General trends in the data were congruent, as NI/NC and I/NC were similar for both data sets, whereas I/C had less iron content and significantly less weight gain. The weight gain data indicate that calcium affected mealworm metabolism. In the context of the weight gain data, the iron content trends indicate that calcium may have affected iron content. However, due to the lack of statistical differences in iron content, the data are not conclusive. To support whether iron absorption inhibition impacted weight gain, further research quantifying the effects of calcium supplementation and iron loss on weight gain is needed.

In relation to existing literature, the conclusion becomes more complex. Although there is no other literature on the inhibition of iron absorption in insects, the iron data may agree with the iron content decrease seen in mammalian iron absorption inhibition (22). However, the statistically significant weight data do not directly support iron absorption inhibition, as iron and calcium's effects on weight are contentious across mammalian and insect literature.

Calcium's effects on body fat metabolism and consequent weight gain are debated. Various studies describe a positive,

https://doi.org/10.59720/24-166

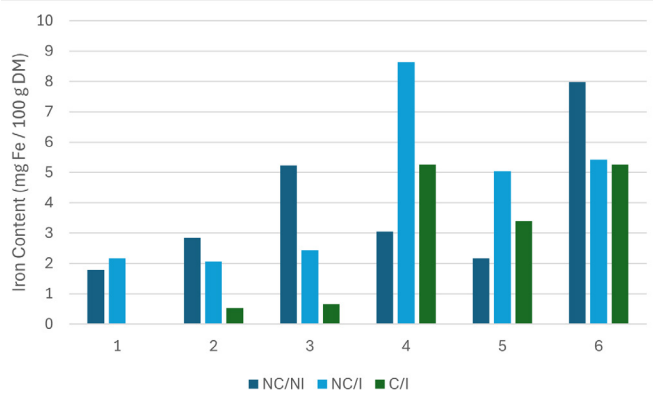

Figure 3. Iron content after supplementation across samples 1–6. Iron content (*n* = 6 samples per group) for each sample in each group. Sample numbers reflect the chronological order of experimentation on each sample. For each group, 6 samples of 30 mealworms were fed for 48 hours under control conditions (NI/NC), with supplemented iron (I/NC), or with supplemented iron and calcium (I/C). Absorbance of each sample was measured by spectrophotometry and converted to iron content using a standard curve.

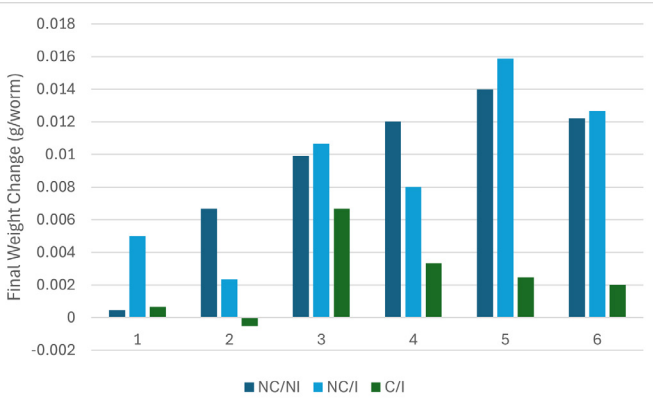

Figure 4. Weight change after supplementation across samples 1–6. Weight change (*n* = 6 samples per group) for each sample in each group. Sample numbers reflect the chronological order of experimentation on each sample. For each group, 6 samples of 30 mealworms were fed for 48 hours under control conditions (NI/NC), with supplemented iron (I/NC), or with supplemented iron and calcium (I/C). Weight was measured before and after supplementation to find weight change.

little, or no correlation between calcium and lipolysis; calcium's form (e.g., calcium salt vs dairy) impacts that relationship (31–34). In insects, a study found positive relationships between calcium and fat metabolism (35). Furthermore, literature on whether calcium inhibits zinc absorption is inconsistent. Zinc affects weight, making calcium's effect on zinc another possible confounding variable, although no relevant studies tested mealworms (36–43). It is also unknown how iron loss definitively affects weight gain. In mammals, literature is sparse and shows both increases and decreases in weight gain due to iron supplementation (44, 45). Literature on insects showed that iron's impact on weight gain varies by species, geographic population of a species, initial iron content, and iron dose (15, 18). Due to the variable literature on potential factors in weight gain, it is not possible to assert whether calcium's effect on weight gain in our study was through fat metabolism, inhibition of zinc absorption, and/or inhibition of iron absorption. The data thus do not support the initial hypothesis that iron absorption inhibition occurs in mealworms, but they are not fully conclusive and leave room for further inquiry.

Several factors limit the conclusion's scope. First, calculated iron contents were imprecise and likely inaccurate due to malfunction of spectrophotometers. For comparison between groups, they are adequate; however, they cannot be directly compared to iron measurements in other studies. Additionally, we did not measure the calcium content of our samples. We cannot identify the initial iron and calcium contents at which the results occurred. This is an impactful stipulation that limits scope, as the effects of mineral supplementation vary based on parental mineral content and mineral intake before supplementation, which were both uncontrolled in this study, as well as mineral intake during experimentation (15, 22, 30). It is also not possible to delineate the conclusion's scope regarding biological iron types. The spectrophotometry procedures were only designed to measure Fe^{3+}. It is plausible, however, that the test accounted for some or most biological iron through chemical reactions that converted Fe^{2+} to Fe^{3+} and through the denaturation of iron-binding proteins (46, 47). In mammals, calcium is unique in its absorption inhibition of all iron types (heme/ferrous Fe^{2+} and non-heme/ferric Fe^{3+}) (10, 12). Our conclusion cannot be specifically applied to either iron form due to the method complexities. In addition, we used 48-hour gutloading periods; the effects of long-term calcium supplementation in insects may be distinct. Due to all the above limitations, the conclusion indicates a possibility of iron absorption inhibition in mealworms but cannot confirm inhibition or inhibition's biochemical conditions.

The results of this study should not be extrapolated to species other than *T. molitor*. Previous insect experiments show that supplementation's effects on mineral content and secondary effects such as weight vary between species and even between different geographic populations of the same species (15, 25). It is possible that the iron and weight trends in mealworms will not be seen in other species. If similar trends are seen in other species or populations, they will likely be mathematically distinct.

The possibility of calcium inhibiting iron absorption in insects provides information on the possible biochemical and genetic structure of mealworm iron metabolism. The weight data offer insight into the disputed biological relationship between calcium and weight by introducing the potential factor of iron absorption inhibition and expanding the literature's breadth of species. The possibility that calcium supplementation could reduce iron content or weight gain is impactful for mealworm producers determining a nutrition program. This conclusion has wider impacts in the context of entomophagy's importance in improving nutrition in LMIC.

The correlation of calcium supplementation with decreased weight gain and possible decrease in iron content in mealworms raises the possibility of iron absorption inhibition in insects. Further study into the nature of iron absorption inhibition in insects is necessary. Experiments testing the effects of a range of calcium doses on weight and iron content should be performed over a range of initial iron contents and weight statuses. This may require a series of experiments to aid in identifying iron absorption inhibition's

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biochemical mechanisms and extent in insects. Experiments should be done with high accuracy in data collection to aid the entomophagy industry in the optimization of insect growth and mineral content. This accuracy should be obtained through larger sample sizes, more sensitive spectrophotometry technology, and controls on possible confounding variables such as zinc. The study also raised questions about the extent of mealworm iron absorption inhibition regarding iron forms and storage. It is necessary to identify such trends in a variety of species, particularly those utilized for entomophagy. The literature on iron supplementation should also be expanded to more species, including mealworms, as existing mathematical extrapolations appeared possibly ineffective at increasing mealworm iron content (25).

MATERIALS AND METHODS

Due to the mealworms' commercial source, they had unknown initial mineral contents (13). Group NI/NC was a negative control that received no supplementation to account for unknown mineral concentrations. The remaining groups received iron, but group two received no calcium (I/NC), while group three received calcium (I/C). Iron gutloading was expected to increase gut iron content. Tissue iron content consists of pre-existing iron that a 48-hour diet should not affect (13). I/NC was expected to act as a positive control with increased gut iron content compared to NI/NC (the baseline for tissue iron content). Group names are denoted by supplementation: NI (no iron), NC (no calcium), I (iron), and C (calcium).

We based our parameters on a similar study that used a sample size of 20 mealworms and replicated each group 6 times (48). Each group therefore had six replicate samples. However, to meet the 2.5 g sample weight recommended for iron content testing, we adjusted our sample size to 30 as a result of the weight per mealworm (about 0.08 - 0.10 g) and death and pupation (1-2 mealworms per 48 hours) seen in pre-testing (46). Each group had 180 mealworms; we tested 540 mealworms in total.

Each sample was kept in 34 oz plastic containers with dimensions of 22.5 x 14 x 6.5 cm. This height and relative area (cm^2) per mealworm is sufficient based on previous experimentation (6). Each container received 0.03 g carrot per mealworm to provide water (6, 48). Calcium-supplemented samples received 90 g calcium per kg feed; iron-supplemented samples received 51 mg iron per kg feed (25). We used calcium carbonate (NOW Supplements; SKU 1245) as the calcium supplement, and ferrous fumarate (BulkSupplements.com; SKU FERFU100) as the iron supplement (15, 26). There were no previous studies on gutloading iron in mealworms, so our choice of iron supplement was based on precedent of powder supplements in mealworms and the use of ferrous fumarate powder with other insects (15, 25, 26). The iron dosage was based on a study's statistical regression that used data from supplementing other minerals (25). Before the mealworms' addition to the container, supplement(s) were mixed into a potato flake substrate (25). Potato flakes (Onuva Potato Flakes 1lb; GTIN 783191017056) were used, as potato-based substrate has been found to decrease mortality and delay mealworms' development compared to other substrates (49, 50). This is ideal, as *T. molitor* must be in the same life stage for comparison (6, 46). We used a minimum of 0.8 g substrate per mealworm (6). Substrate filled about ¼ inch of the container's height to provide sufficient separation between mealworms and the container's bottom; this height is sufficient based on pre-testing that showed long-term survival. This minimum height and weight necessitated 75 g substrate per sample. Once containers were arranged, "live regular mealworms" purchased from PetSmart (Cat# 5161313) were divided into samples of 30, excluding non-nutritionally equivalent pupae and dead or disproportionately small larvae (6). Each sample was weighed, put into the containers, and left for 48 hours in a lab classroom protected from direct sunlight. 48 hours was used because it is the optimal mealworm gutloading time (13, 25, 26). After samples were set up, all unused mealworms were disposed of in soapy water; pupated and dead sample mealworms were similarly disposed of after gutloading.

After gutloading, samples were weighed. Samples were then frozen to decrease cruelty when burning samples and to preserve samples for spectrophotometry (25). Due to equipment availability, we used spectrophotometry to measure iron content. Spectrophotometry procedures were based on lab procedures from Purdue University (46). The iron testing process began by burning the samples with a Bunsen Burner (Flinn Scientific; AP8285), porcelain crucibles, and pestles. After samples were reduced to ash, their dry weight was recorded; they were then tested for absorbance using spectrophotometry. For each sample, the ash was placed in a beaker; 10 mL 2 M HCl (Flinn Scientific; CAS 7647-01-0) was stirred in for one minute. 10 mL distilled water was then stirred in. Filtrate was collected and 2.5 mL 0.1 M KSCN (Flinn Scientific; Cat# P0178) was stirred into the solution. The solution was then transferred into a test tube. The test tube's absorbance at 460 nm was measured with a spectrophotometer (Flinn Scientific; Cat# AP7026) and recorded (51). Steps from container set-up to absorbance testing were repeated separately for each sample.

A standard curve was developed and linearized to convert the absorbance measurements to iron concentrations (**Figure 4**, **Table 1**). Solutions of 0.00, 0.25, 0.50, 0.75, and 1.00 mM/L $Fe(NO_3)_3$ were prepared in 0.1 M HCl (Flinn Scientific; Cat# H0014) using 0.001 M $Fe(NO_3)_3$ (Flinn Scientific; Cat# F0008) and water. The 0.00 mM solution was prepared using 0.1 M HCl instead of water. Each solution totaled 20 mL and was prepared by stirring in 2.5 mL 0.1 M KSCN and then tested for absorption using spectrophotometry (**Figure 5**). Due to low sample iron contents and polynomial curving, only the 0.00, 0.25, and 0.50 solutions were used in the standard curve (**Figure 4**).

Data were compared with ANOVA and pair-wise tests (52). Within each group, each of the six respective samples was considered a data point used to calculate the group means. Tests were done with the Social Science Statistics website. We considered a *p*-value below 0.05 statistically significant. Before comparing iron content, we used dimensional analysis to calculate each sample's iron content in mg Fe/100 g DM.

Concentration (mM):	0.00	0.25	0.5	0.75	1.00
Absorbance (Au):	0	0.831	1.786	2.251	2.49
Transmittance:	100.00%	14.80%	1.60%	0.60%	0.30%

Table 1. Standard curve data for estimating conversion of experimental absorbance to iron content. Solutions of 0.00, 0.25, 0.50, 0.75, and 1.00 mM $Fe(NO_3)_3$ in 0.1 M HCl were tested in a spectrophotometer; absorbance and transmittance were recorded for each concentration.

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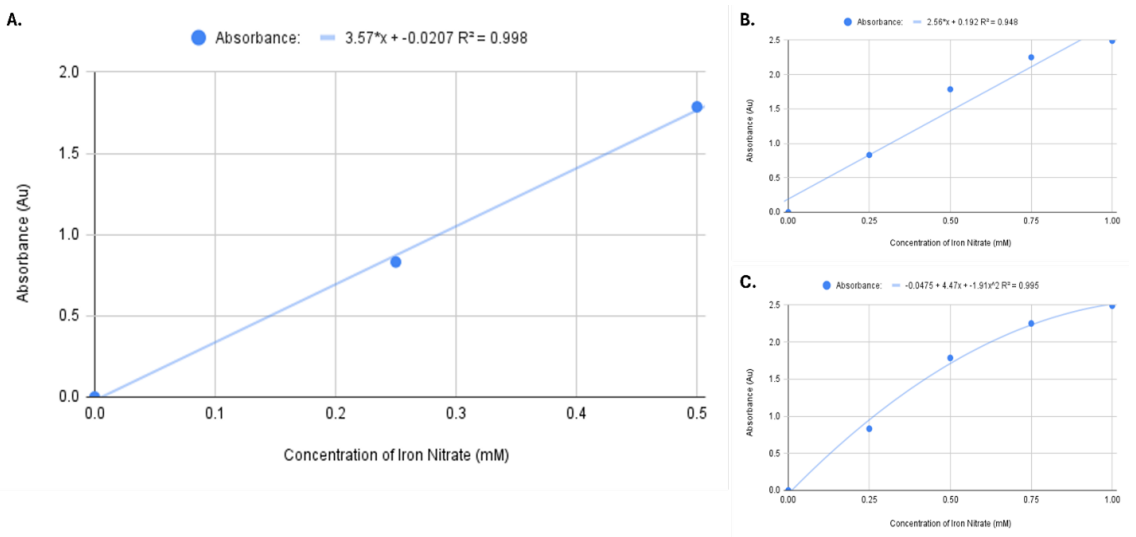

Figure 5. Linearized data of standard curve for iron spectrophotometry. Absorption data for solutions of $Fe(NO_3)_3$ in 0.1 M HCl. Iron nitrate solutions of 0.00, 0.25, 0.50, 0.75, and 1.00 mM were created and tested for absorbance using spectrophotometry. **A)** Data for solutions of 0.00, 0.25, and 0.50 mM shown with a linear trend line. **B)** Data for solutions of 0.00, 0.25, 0.50, 0.75, and 1.00 mM shown with a linear trend line. **C)** Data for solutions of 0.00, 0.25, 0.50, 0.75, and 1.00 mM shown with a polynomial trend line.

These units are a standard iron content measurement utilized for ease of comparison to previous mealworm iron content studies (3, 7, 16). We statistically compared the groups' calculated mean Fe/DM contents.

A Scientific Review Committee approved the study before experimentation.

ACKNOWLEDGMENTS

Thanks to Adam Duong, Aubrie Hollis, and an anonymous friend for their help in counting and distributing mealworms during experimentation. Thanks to Alexander "Xander" Bouldin for his help revising and editing. Thanks to Samantha Supernaw and Margaret Parker-Supernaw for their support and for funding purchase of supplies and obtaining mealworms.Thanks to Ms. Jessica Matthews for her lab space and materials, time, and unending support.

Received: June 19, 2024
Accepted: September 9, 2024
Published: August 1, 2025

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