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Palatability, Consumption, and Physiological Effects of the Green Microalgae *Chlorella* sp. as a Feed Substitute for the Western Honey Bee *Apis mellifera* in a Laboratory Setting

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Abstract

Western honey bees '*Apis mellifera'* are vital pollinators which play a significant role in global food security. Honey bees are faced by numerous environmental pressures including lack of forage which lead to large losses annually of managed honeybee colonies. To offset these pressures, many beekeepers manage colonies with the addition of artificial diets, many of which contain products that do not meet the nutritional requirements needed by honey bees or require large amounts of resources to grow. Recent literature has indicated that algae may be a viable nutritional resource for honey bees, meeting the nutritional requirements needed, requiring less resources, and having a smaller environmental footprint. However, the studies are limited and do not discuss palatability of the diet or provide much evidence for consumption. This study aimed to see if honey bees in laboratory settings were consuming diets containing algae products, how consumption compared when bees were fed modern commercial diets and natural pollen, and determining if physiological impact results matched those in the previous literature. Our findings indicated that honey bees will consume algae diets at rates similar to commercial feeds, and that some physiological results are comparable to those found in the current literature. Additionally, we discuss the implications these results could have for helping guide commercial honey bee diets in a more sustainable manner of raw material production.

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1. Introduction

Western honey bees '*Apis mellifera'* are vital insects in the world, playing an important role as pollinators in most terrestrial environments where they may visit nearly 90% of flowering species in their native range (Hung et al., 2018). Honey bees are also of great importance to global food security, with *A. mellifera* responsible in the United States alone for pollinating more than 100 commercial crop plants and providing tens of billions of dollars in economic benefits (Khalifa et al., 2021). Honey bees are subjected to numerous stressors in their environment including pests, diseases, environmental changes, loss of forage, and chemical exposure from human activity leading to large annual colony losses among beekeepers (Hristov et al., 2020). To offset these stressors, nearly 90% of beekeepers in the US utilize artificial diets in their management practices, usually utilizing ingredients that contain agricultural products such as soy and eggs, however, many of these ingredients use large amounts of resources such as land and water in order to produce their final product and can contribute to environmental issues impacting honey bees (Ricigliano et al., 2022; Long & Krupke, 2016; Grzinic et al., 2023). Additionally, some of these products may be unpalatable or lacking in the nutritional profile required by honey bees to maintain proper individual and colony health (Noordyke & Ellis, 2021). In recent years, algae, particularly microalgae, has shown potential as an alternative, sustainable, and eco-friendly alternative as a diet substitute meeting the nutritional needs for honey bees while negating the environmental impacts imposed by feed substitutes using modern agricultural products and their preceding management techniques. However, experimental parameters, methodologies, and results are varied in the current available literature and many of the experiments have not had follow up research. Additionally, available data on consumption rates, direct evidence of algae intake into the digestive track, and information determining the palatability of algae-based diets are limited in the current literature (Nichols & Ricigliano, 2022). As such, further testing is required to determine the viability of this new field in honey bee nutrition and sustainable resource management. Our experiment set out to determine three questions that arose from gaps in the current literature. First, is there physical evidence that honey bees are consuming and intaking artificial diets containing algae into their digestive systems? Second, are artificial diets containing algae similarly consumed by honey bees when presented alongside pollen and current artificial diets? And finally, do the physiological results reflect what is reported in the current literature?

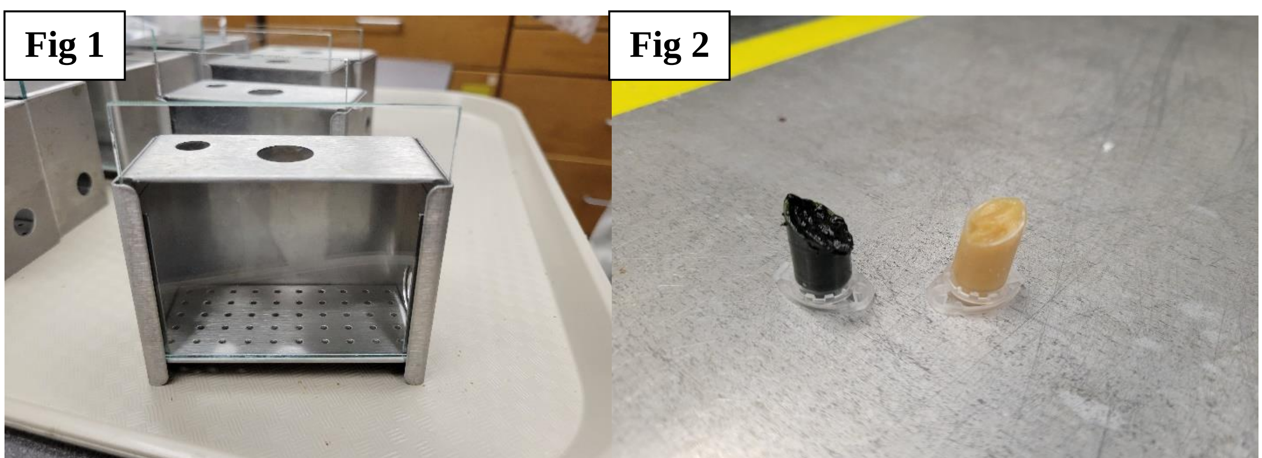
2. Methodology

2.1 Diet Preparation

Diets were prepared by mixing a common commercial substitute diet of 25 g brewer's yeast, 50 g soy flour, 75 g divert sugar, and 15 g canola oil. Additionally, 25 g of commercial bee syrup 'Prosweet' was added to increase palatability of the control and test diets. For experimental diets, soy flour was substituted with an equal amount of either harvested pollen, or commercial *Chlorella* powder. Water was added to the diet to create a creamy consistency and stored in a refrigeration unit at 4.8° C. Upon use, diets were returned to room temperature and additional water added to restore the creamy consistency from any evaporation in storage.

2.2 Experiment Setup

Experiments were conducted in small, ventilated metal cages with removable glass covers (**Figure 1**). For each experiment, frames of emerging brood were collected from United States Department of Agriculture- Agricultural Research Service (USDA-ARS) colonies and placed for 24 hours in an incubator set to 34° C. Newly emerged bees were then collected and 60 individuals placed into each individual cage. Caged bees were provided with a 1:1 sugar water solution delivered via a 5 ml syringe and placed in an incubator for 24 hours at 30° C with a small tray of water to maintain humidity. After 24 hours, bees were divided into control and experimental groups. Diets were placed in modified 2 ml tubes (**Figure 2**) and placed horizontally into the cages. Cages were then placed randomly back into the incubator at 30° C and rotated every 24 hours to deter environmental effects such as differences in humidity from impacting final consumption and physiological results. Diets were replaced every 48 hours and recorded to determine consumption. Additionally, sugar syrup was checked daily and replaced when levels reached below 2 ml, and dead bees removed and recorded.



Figures 1 & 2: Cages used for the experiment courtesy of USDA (**1**); modified 2 ml tubes loaded with Chlorella and control diets which were used in feeding tests (**2**).

2.3 Palatability Test 1

For the preliminary palatability test, honey bees were loaded into two cages on June 10th, 2023. After 24 hours, the cages were fed a control diet of the commercial feed substitute, and an experimental diet of commercial substitute with commercial *Chlorella* powder replacing the soy powder. Tubes containing the diet were replaced every 48 hours with weights recorded prior to diets being placed in cages and after removal. This was repeated until termination of the test on June 21st, 2023.

2.4 Palatability Test 2

Honey bees were loaded into nine cages on June 23rd, 2023. After 24 hours cages were divided into three treatments. Three cages were fed a control diet of commercial feed substitute, three cages were fed a second control diet of commercial substitute with soy replaced with natural pollen, and three cages were fed with an experimental diet of commercial diet with soy replaced with commercial *Chlorella* powder. Diets were replaced every 48 hours with weights recorded prior to diets being placed in cages and after removal. This was repeated until termination of the test on June 30th, 2023.

2.5 Consumption Test 1

Honey bees from three different genetic lines were loaded into six cages on June 26th, 2023. Multiple genetic lines were chosen to determine if consumption and physiological effects were constant among all lines or to determine if genetic variations affected the impact of algae-based diets. Cages were divided by genetic lines, two cages with Russian line honey bees, two cages with Italian line honey bees, and two cages with Pol line honey bees. The two cages for each were further divided by diet type with the control cage fed with commercial feed substitute, and the experimental cage fed with commercial feed substitute with *Chlorella* powder replacing the soy. Diets were replaced every 48 hours with weights recorded prior to diet being placed in cages and after removal. This was repeated until termination of the test on July 3rd, 2023 and bees removed for dissection for Physiological Test 1.

2.6 Consumption Test 2

Honey bees from no specific line were loaded into 12 cages on August 9th, 2023. The cages were arranged into four different diet treatments of three cages each. One group was fed the commercial diet as a control, and the other groups were fed the commercial diet with the soy replaced with either pollen, *Chlorella*, or spirulina powder. For this test, the diet did not contain any canola oil added to the formulation to determine if the consistency impacted consumption rates. Diets were weighed and replaced every 24 hours until August 16th when the first set of bees were removed from the cages for dissection for Physiological Test 2. The remainder bees were kept in the cages and fed sugar syrup for one more week until removed for dissection for Physiological Test 2 on August 22nd, 2023.

2.7 Physiological Test 1

Twenty-four individuals from each cage of Consumption Test 1 were removed and placed in separate 50 ml tubes divided by line and treatment. Tubes were placed in a styrofoam container of ice to cubes induce a state of torpor. Each line and treatment group of knocked out bees were then placed on a dissection tray of foil sitting on a container of ice cubes to ensure individuals would remain unconscious during termination and the dissection procedure. Knocked out bees first had their heads removed via the use of micro scissors and heads were placed in a 50ml tube sitting in ice to ensure heads did not regain consciousness as nervous and cellular functions were terminated. After removal of heads, midguts and hindguts of four individuals were removed to determine consumption by visual evidence. Midguts and hindguts were removed from the abdomen using entomological forceps to grip the posterior abdominal section and gently pull the gut section out of the opening created via the separation of the posterior abdominal segments. Gut sections were then placed on a petri dish and photographed to provide visual evidence of consumption. All individuals upon removal of heads (and individuals who had guts removed) had abdomens removed via micro scissors and thoraxes were placed in 50ml tubes set in ice separated by treatment and genetic line. Sealed 50ml tubes containing head and thoraxes were then placed into a refrigeration unit set at 4.8° C for 12 hours on July 3rd, 2023. On July 4th, 2023, thoraxes and heads were weighed and data recorded. Heads and thoraxes were placed back in sealed 50ml tubes. On August 9th, 2023, heads and thoraxes were placed on plastic weigh plates were set in a drier set at 60° C for 24 hours. Heads and thoraxes were then weighed to take into account dry weights, and results recorded.

2.8 Physiological Test 2

24 individuals from each cage of Consumption Test 2 were removed and placed in separate 50 ml tubes divided by treatment. Tubes were placed in a styrofoam container of ice to induce a state of torpor. Each line and treatment group of knocked out bees were then placed on a dissection tray of foil sitting on a container of ice to ensure individuals would remain unconscious during termination and the dissection procedure. Knocked out bees first had their heads removed via the use of micro scissors and heads were placed in a 50 ml tube sitting in ice to ensure heads did not regain consciousness as nervous and cellular functions were terminated. All individuals upon removal of heads had abdomens removed via micro scissors and thoraxes were placed in 50ml tubes set in ice separated by treatment. Sealed 50 ml tubes containing head and thoraxes were then placed into a refrigeration unit set at 4.8° C for 12 hours on August 15th, 2023. On August 16th, 2023, heads and thoraxes were placed on plastic weigh plates and were set in a drier set at 60° C for 24 hours. Dry weights of heads and thoraxes were then recorded. This process was repeated on another series of 24 bees from each cage on August 22nd, 2023, and results recorded on August 24th, 2023.

2.9 Data Analysis

Recorded results were placed onto spreadsheets on Microsoft Excel. For consumption data, loss of weight due to water evaporation of diets were determined via the evaporation controls, and total average consumption calculated by subtracting average loss of water weight from the results. Numerical data from palatability, consumption, and physiological recordings were then transferred from Excel to GraphPad Prism Version 10.0.2 (232) for Windows/iOS (GraphPad Software, La Jolla California USA, www.graphpad.com) for graphing and statistical analysis. Student's T-tests and two-way ANOVA tests were performed to determine statistical significance.

3. Results

3.1 Palatability Test 1

After eight days of feeding, diet with soy replaced by *Chlorella* was consumed at around the same rate as control diet. Average 48-hour consumption rate of *Chlorella* diet was 0.4637 g and commercial diet was had an average consumption rate of 0.3957 g (**Figure 3**). However, differences were non-significant with a *P* value > 0.05 (0.6777). Mortality rate was negligible with a total mortality <1% from both the test cage and control.

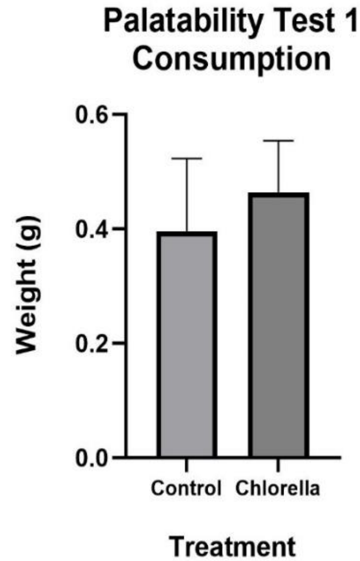


Fig. 3 P= 0.6777

Figure 3: Consumption results in first palatability test.

3.2 Palatability Test 2

After seven days of feeding there was a significant difference in mean consumption rates between the control commercial diet (0.6018 g) and the diet with pollen (0.8214 g) with a *P* value < 0.05 (0.0330) (**Figure 4**). There was no significant difference between the consumption rates of the control commercial diet (0.6018 g) and diet with *Chlorella* (0.5968 g) with a *P* value > 0.05 (0.9577) (**Figure 5**). There was also no significant difference between the diet with pollen (0.8214 g) and the diet with *Chlorella* (0.5968 g) with a *P* value > 0.05 (0.0515) (**Figure 6**). A two-way ANOVA test showed no significance between the control commercial diet and the diet with *Chlorella* with a *P* value > 0.05 (0.9916) and significance between the control diet and diet with pollen with a *P* value < 0.05 (0.0002). The ANOVA also showed significance between the diet with pollen and the diet with Chlorella with a *P* value < 0.05 (0.0001).

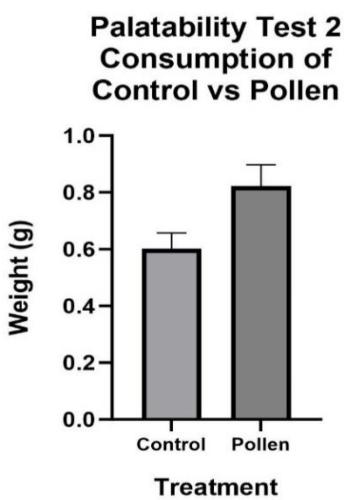


Fig. 4 P= 0.0330*

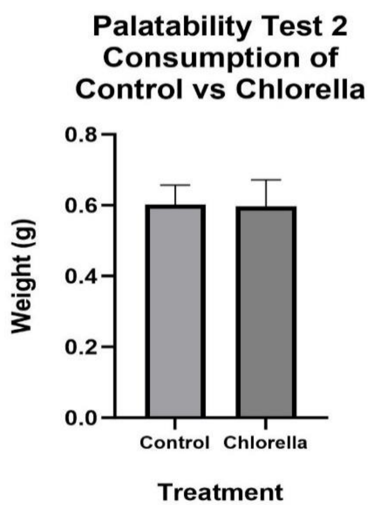


Fig. 5 P= 0.9577

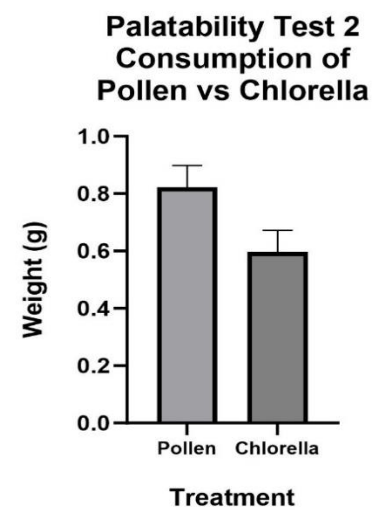
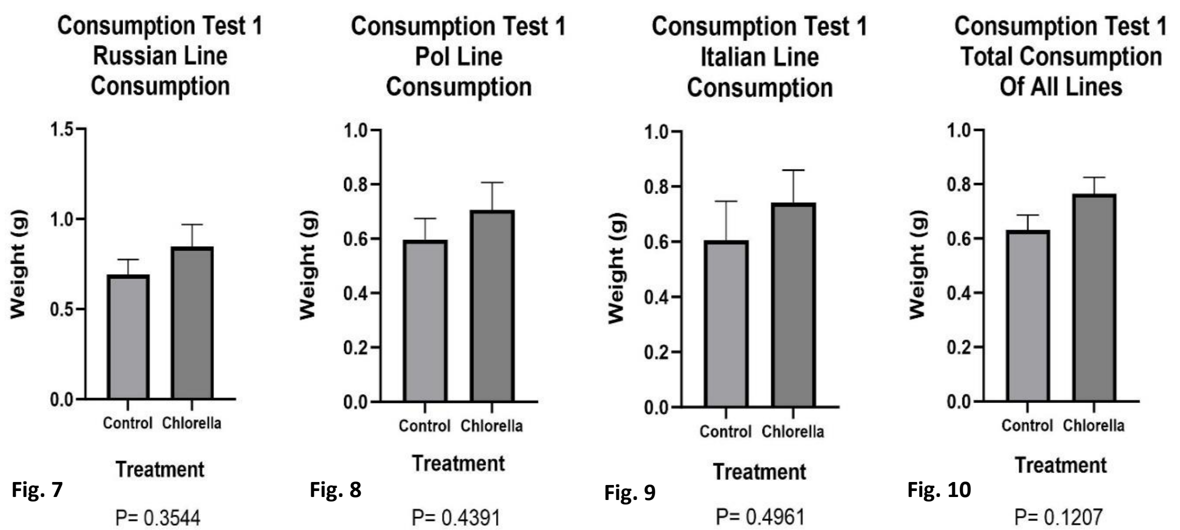


Fig. 6 P= 0.0515

Figure 4-6: Consumption results of the second palatability test between the three different diets.

3.3 Consumption Test 1

The consumption test over three different lines showed that showed no significant differences in 48 hour consumption rates between the control commercial diet and diet with *Chlorella*. This was reflected as well when cumulative consumption averages of all lines were compared. Average consumption rate for *Chlorella* diet was 0.8468 g for Russian line bees, 0.7060 g for Pol line bees, 0.7425 g for Italian line bees, and a cumulative consumption rate of 0.7561 g. Average consumption rate for control diet was 0.6918 g for Russian line bees, 0.5969 g for Pol line bees, 0.6046 g for Italian line bees, and a cumulative consumption rate of 0.6311 g. There were no significant differences in consumption rates between diets for all tested groups with a P value > 0.05 for Russian line bees (0.3544) (**Figure 7**), Pol line bees (0.4391) (**Figure 8**), Italian line bees (0.4961) (**Figure 9**), and cumulative line consumption (0.1207) (**Figure 10**). A two-way ANOVA showed no significant differences between consumption by all lines of either diet.



Figures 7-10: Results from consumption test among different lines of honeybees.

3.4 Consumption Test 2

The consumption test looking over different diet types revealed a significant preference for diet with pollen compared to the other diets. Bees consumed the control diet and diet with *Chlorella* at roughly equal amounts when averaged, and there was no consumption of diet with spirulina powder. Average consumption rates over 24 hours was 0.8954 g for diet with pollen, 0.4567 g for diet with *Chlorella*, and 0.4447 g for the control diet. There was a significant difference in consumption between bees fed diet with pollen and bees fed either diet with *Chlorella* or control diet, with both having P value < 0.05 (<0.0001) (**Figures 11 & 12**). There was no significant difference between bees fed diet with *Chlorella* and bees fed control diet, with a P value > 0.05 (0.7310) (**Figure 13**). A two-way ANOVA showed the same significant and non-significant results between the consumption of the different diets.

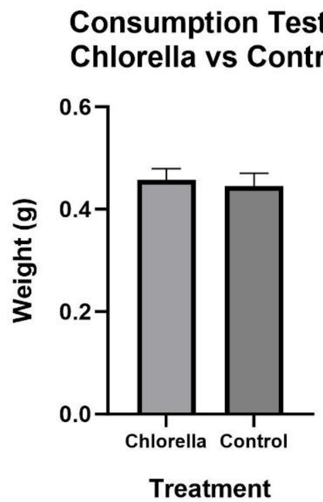


Fig. 11

P= 0.7310

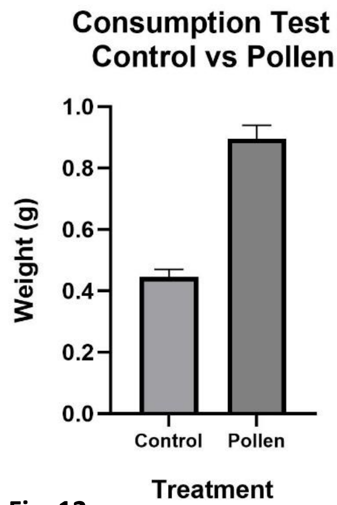


Fig. 12

P= <0.0001*

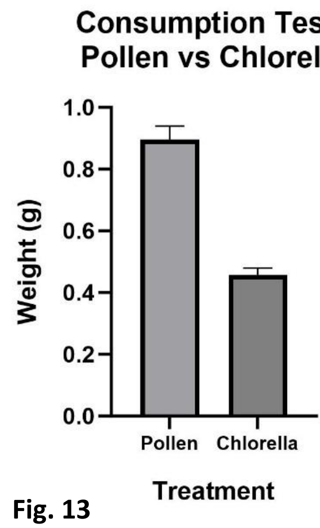


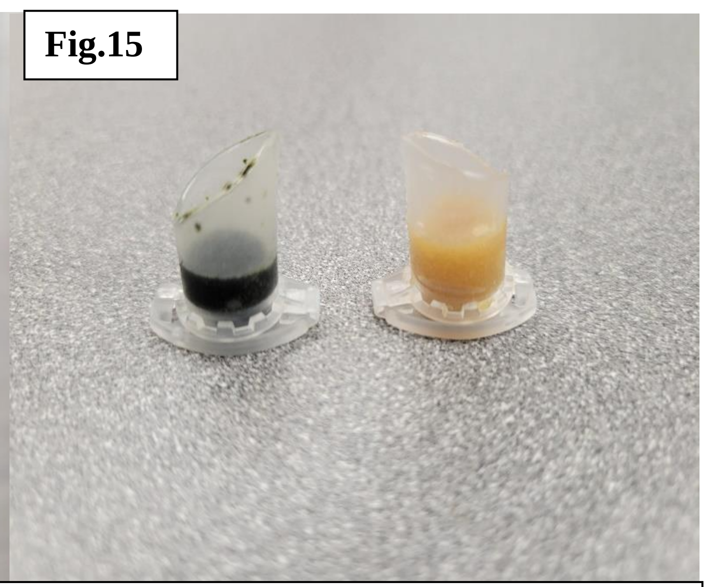
Fig. 13

P= <0.0001*

Figures 11-13: Consumption results between different diets among the second consumption test.

3.5 Physiological Test 1

Besides visual observations of feeding behavior (**Figures 14 & 15**), dissection of the hindguts revealed that commercial diets with soy replaced with *Chlorella* were consumed by all the tested lines of honey bees (**Figures 16-18**). Physiological results varied depending on the line tested and measured variable, with some results showing statistically significant differences between both diet formulations, and some results showing very little difference between diets with no statistical significance. Average wet head weights for bees fed control diet were 0.01273 g among Russian line bees, 0.013 g among Italian line bees, 0.01262 g among Pol line bees, and



Figures 14 & 15: Visual confirmation of honeybees consuming diet both directly (**14**) and after tubes were removed (**15**).

Fig. 16

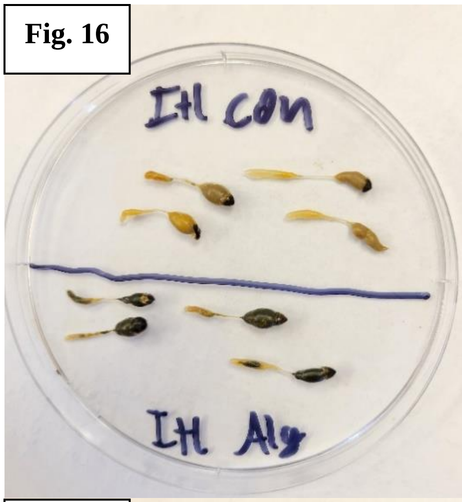


Fig. 17

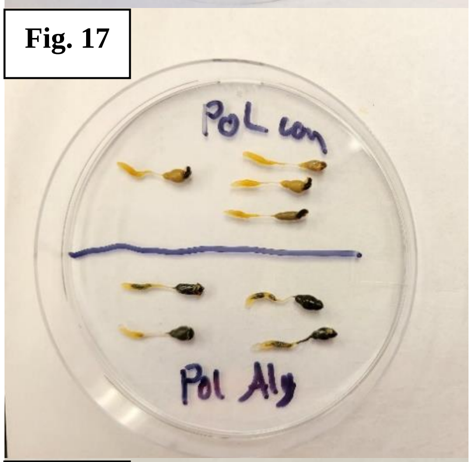
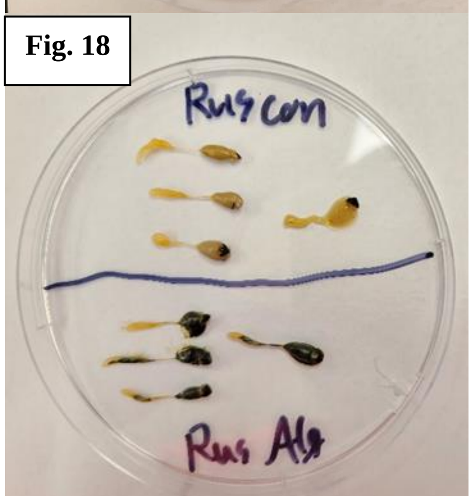
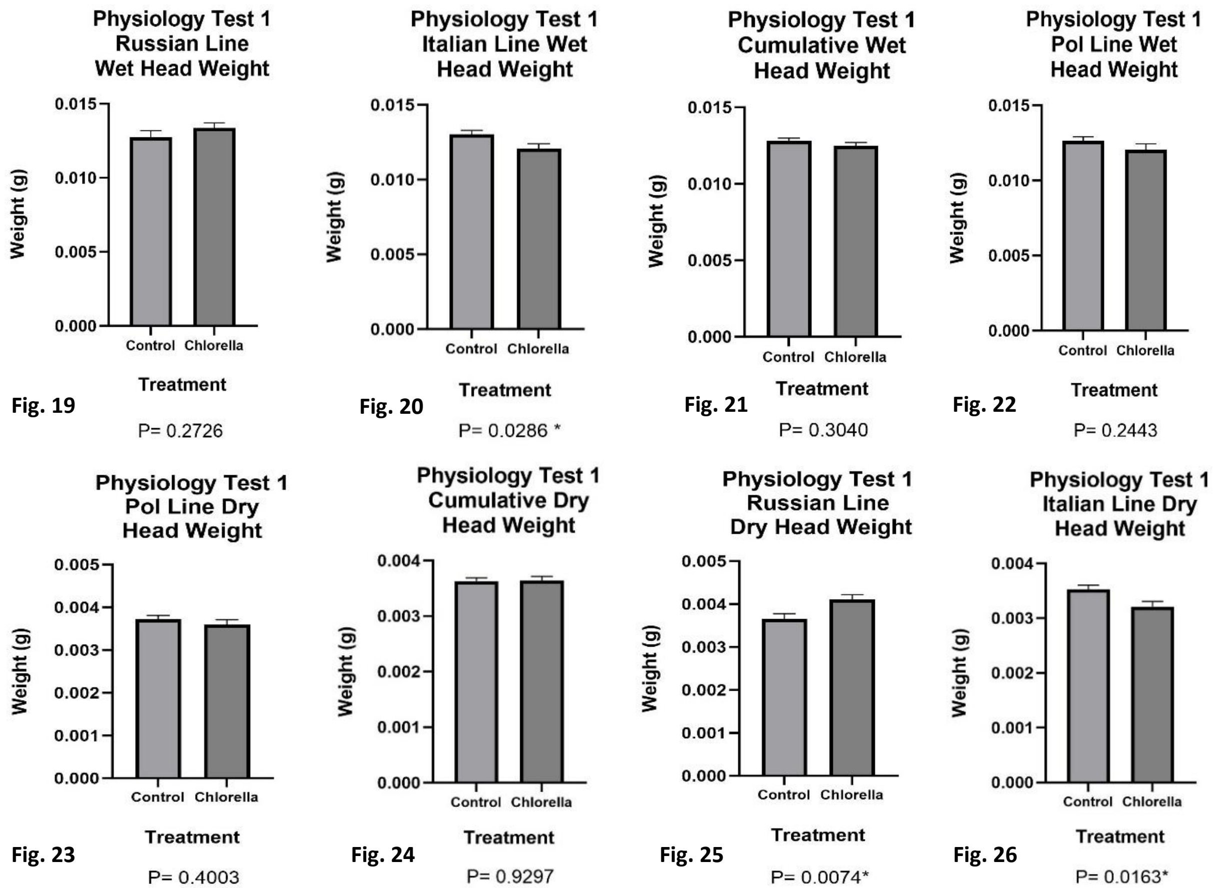


Fig. 18



Figures 16-18: Visual confirmation of algae consumption in hindguts and midguts from all lines tested in the first physiology test. This includes the Italian line (**16**), Pol line (**17**), and Russian line (**18**).

0.01279 g cumulatively among all lines. Average wet head weights for bees fed diet with *Chlorella* was 0.01336 g among Russian line bees, 0.012 g among Italian line bees, 0.01205 g among Pol line bees, and 0.01249 g cumulatively among all lines. There was no significant differences in consumption in three of the four groups with a P value > 0.05 among Russian line bees (0.2725) (**Figure 19**), Pol line bees (0.2443) (**Figure 20**), and cumulatively along all lines (0.3040) (**Figure 21**). There was a significant difference with a P value < 0.05 among Italian line bees (0.0286) with wet head weights of bees fed control diet being higher than wet head weight of bees fed *Chlorella* diet (**Figure 22**). Additionally, a two-way ANOVA showed no significant differences between genetic lines in consumption of the control diets, but a significant difference between consumption of diet with *Chlorella* among Russian bees from Italian and Pol line bees with a P value < 0.05 (0.0262). Average dry head weights for bees fed control diet were 0.0036 g among Russian line bees, 0.0035 g among Italian line bees, 0.003721 g among Pol line bees, and 0.003632 g cumulatively among all lines. Average dry head weight among bees fed diet with *Chlorella* was 0.0041 g among Russian line bees, 0.0032 g among Italian line bees, 0.01225 g among Pol line bees, and 0.003632 g cumulatively among all lines. There were no significant differences with a P value > 0.05 between the different diet groups among the Pol line bees (0.4003) (**Figure 23**), and cumulatively among all lines P value > 0.05 (0.9297) (**Figure 24**). There was a significant difference between the different diet groups with a P value < 0.05 among the Russian line bees (0.007) (**Figure 25**) and Italian line bees (0.0163) (**Figure 26**). Dry head weights among Russian line bees were higher in bees fed diet with *Chlorella*, while among Italian line bees dry head weights were higher in bees fed the control diet. A two-way ANOVA showed so significant differences in dry head weight between the different lines fed control diet, but a significant difference between all lines fed diet with *Chlorella* with a P value < 0.05. Average wet thorax weight for bees fed control diet was 0.04049 g among Russian line bees, 0.040 g among Italian line bees, 0.03708 g among Pol line bees, and 0.03938 g cumulatively among all lines. Average wet thorax weight



Figures 19-26: Wet (**19-22**) and dry (**23-26**) head weight results for all lines and cumulatively.

for bees fed diet with *Chlorella* was 0.0403 g among Russian line bees, 0.039 g among Italian line bees, 0.03648 g among Pol line bees, and 0.03864 g cumulatively among all lines. There was no significant difference in wet thorax weight between the different diets in all lines, with a P value > 0.05 among Russian line bees (0.8031) (**Figure 27**), Italian line bees (0.1542) (**Figure 28**), Pol line bees (0.4482) (**Figure 29**), and cumulatively among all lines (0.1816) (**Figure 30**). A two-way ANOVA showed a significant difference of wet thorax weights among bees fed control diet with a P value < 0.05 between Russian and Pol lines (0.0003), and Pol and Italian lines (0.0002), but not between the Russian and Italian lines (0.9947). This was the same among bees of different lines fed diet with *Chlorella*, with a P value < 0.05 between Russian and Pol lines (0.0001), and Pol and Italian lines (0.0059), but not between Russian and Italian lines (0.3516). Average dry thorax weight for bees fed control diet was 0.01283 g among Russian line bees, 0.0128 g among Italian line bees, 0.01252 g among Italian line bees, and 0.01271 g cumulatively among all lines. Average dry thorax weight for bees fed diet with *Chlorella* was 0.01305 g among Russian line bees, 0.0123 g among Italian line bees, 0.01225 g among Pol line bees, and 0.01255 g among all line cumulatively. There were no significant differences in dry thorax weight between diet groups, with a P value > 0.05 among Russian line bees (0.3651) (**Figure 31**), Italian line bees (0.2107) (**Figure 32**), Pol line bees (0.2107) (**Figure 33**), and cumulatively among all lines (0.3708) (**Figure 34**). A two-way ANOVA showed no significant

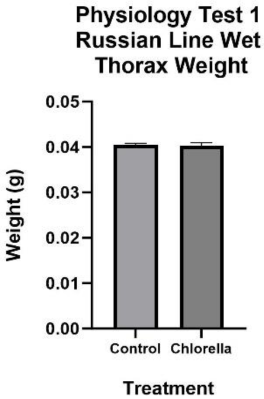


Fig. 27
P= 0.8031

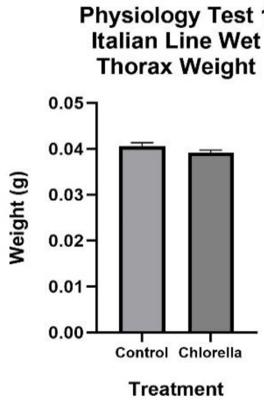


Fig. 28
P= 0.1542

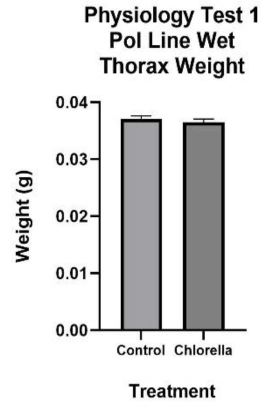


Fig. 29
P= 0.4482

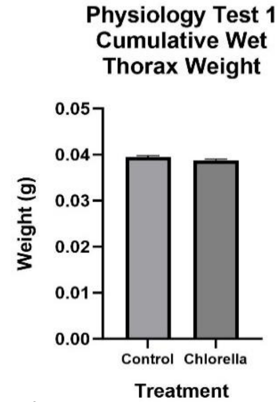


Fig. 30
P= 0.1816

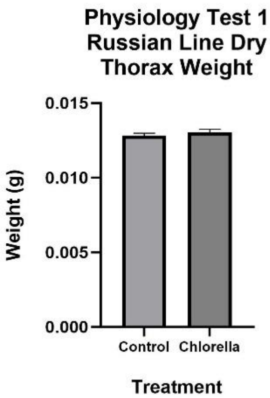


Fig. 31
P= 0.3651

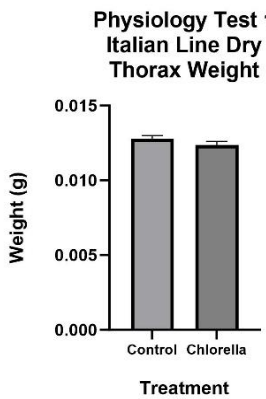


Fig. 32
P= 0.2107

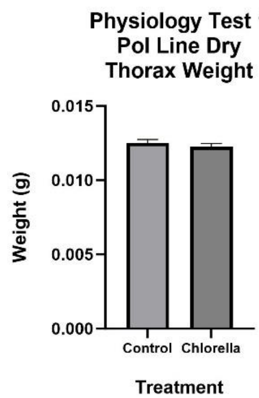


Fig. 33
P= 0.3995

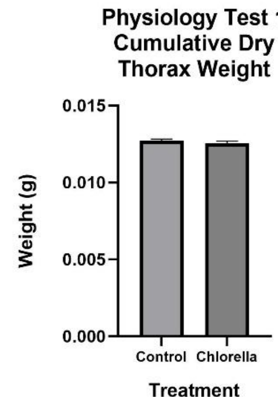


Fig. 34
P= 0.3708

Figures 27-34: Wet (**27-30**) and dry (**31-34**) thorax weights of all lines and cumulatively.

difference in dry thorax weight between all lines fed the control diet, and only a single significant difference between lines fed diet with *Chlorella*, with a *P* value < 0.05 only between Russian and Pol lines (0.0235).

3.6 Physiological Test 2

Average dry head weights on August 17th, 2023, was 0.003443 g among bees fed control diet, 0.004028 g among bees fed diet with pollen, and 0.003613 g among bees fed diet with *Chlorella*. There was no significance between bees fed control diet and diet with pollen with a *P* value > 0.05 (0.1460) (**Figure 35**) and between bees fed diet with pollen and diet with *Chlorella* (0.3016) (**Figure 36**). However, there was significance between bees fed control diet and bees fed *Chlorella*, with a *P* value < 0.05 (0.0255) (**Figure 37**). A two-way ANOVA showed no significant difference in dry head weight between all diet groups. Average dry thorax weights on August 17th, 2023, was 0.01287 g among bees fed control diet, 0.01306 among bees fed diet with pollen, and 0.01291 among bees fed diet with *Chlorella*. There were no significant differences between diets for all lines, with a *P* value > 0.05 among bees fed control diet and diet with pollen (0.2128) (**Figure 38**), among bees fed diet with pollen and diet with *Chlorella* (0.3807) (**Figure**

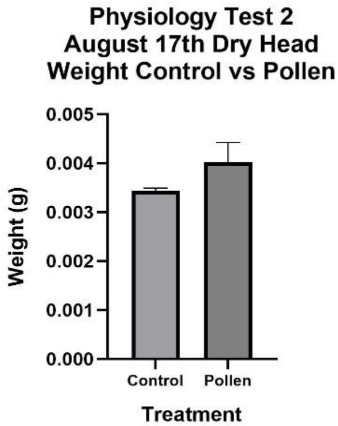


Fig. 35

P= 0.1460

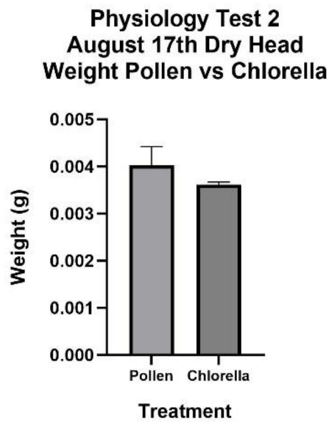


Fig. 36

P= 0.3016

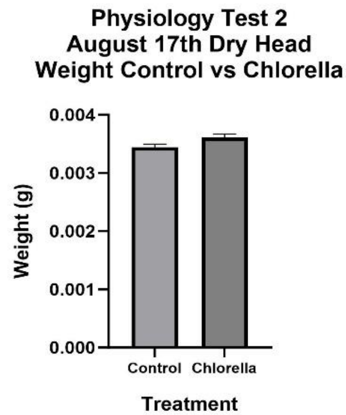


Fig. 37

P= 0.0254*

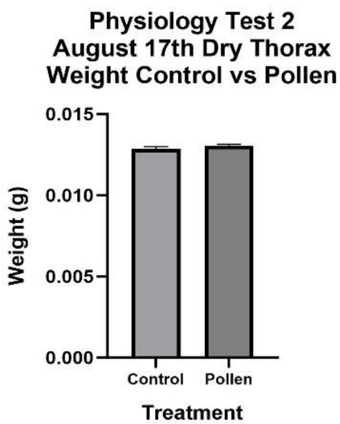


Fig. 38

P= 0.2128

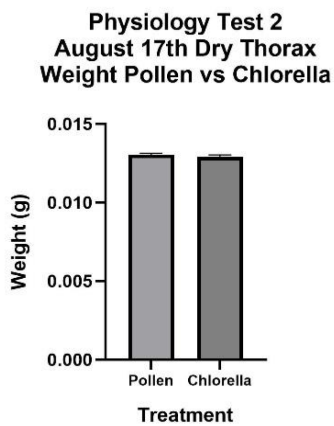


Fig. 39

P= 0.3087

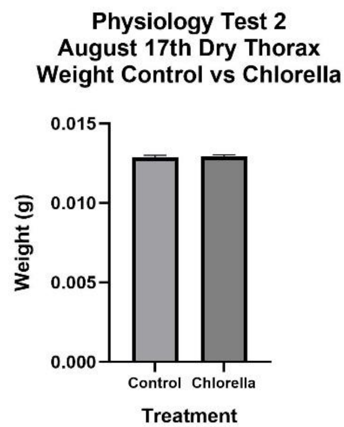


Fig. 40

P= 0.8080

Figures 35-40: Dry weight results of different diets from bees dissected on August 17th. This includes dry head weights (**35-37**) and dry thorax weights (**38-40**).

39), and among bees control diet and diet containing *Chlorella* (0.8080) (**Figure 40**). Additionally, a two-way ANOVA showed no significant differences in dry thorax weight between all diet groups. Average dry head weights on August 24th, 2023, was 0.003492 g among bees fed control diet, 0.003331 g among bees fed diet with pollen, and 0.003107 g among bees fed diet with *Chlorella*. There was no significance with a *P* value > 0.05 among bees fed control diet and diet with pollen (0.6820) (**Figure 41**), and among bees fed control diet and diet containing *Chlorella* (0.3280) (**Figure 42**). However, there was a significant difference among bees fed diet with *Chlorella* and bees fed diet with pollen, with a *P* value < 0.05 (0.0013) (**Figure 43**). A two-way ANOVA showed no significant difference in dry head weight between all diet groups. Average dry thorax weights on August 24th, 2023, was 0.01223 g among bees fed control diet, 0.01256 g among bees fed diet with pollen, and 0.01218 g among bees fed diet with *Chlorella*. There were no significant differences among bees fed control diet and bees fed diet with *Chlorella*, with a *P* value > 0.05 (0.7626) (**Figure 44**). However, there was a significant difference among bees fed control diet and bees fed diet with pollen with a *P* value < 0.05 (0.0108) (**Figure 45**), and among bees fed diet with pollen and bees fed diet with *Chlorella*

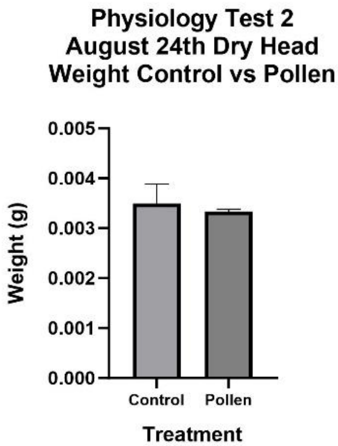


Fig. 41 P= 0.6820

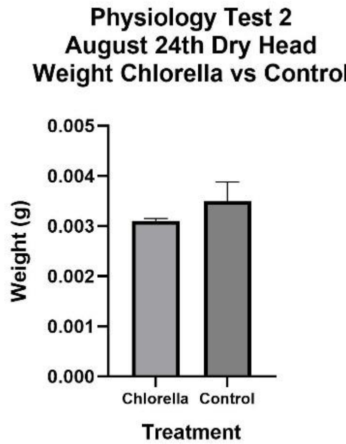


Fig. 42 P= 0.3280

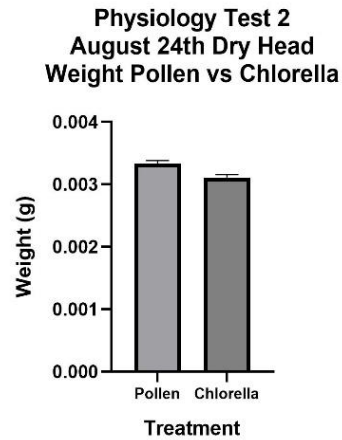


Fig. 43 P= 0.0013*

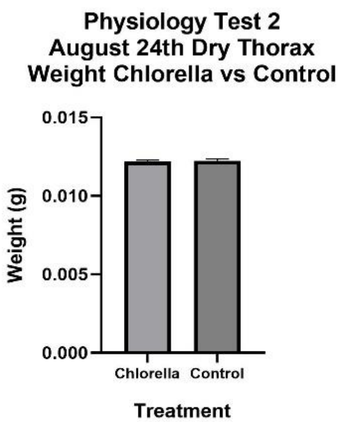


Fig. 44 P= 0.7626

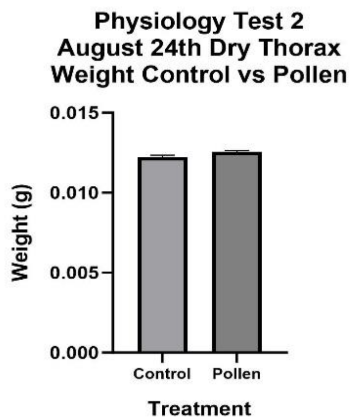


Fig. 45 P= 0.0180*

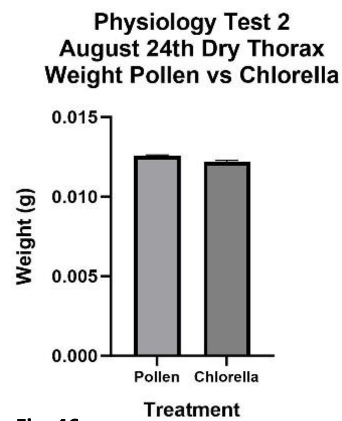


Fig. 46 P= 0.0035*

Figures 41-46: Dry weight results from bees dissected on August 24th. This includes dry head weights (**41-43**), and dry thorax weights (**44-46**).

(0.0035) (**Figure 46**). A two-way ANOVA showed a significant difference in dry thorax weight with a P value < 0.05 between bees fed diet with pollen and bees fed diet with *Chlorella*.

4. Discussion

The experiments showed that when presented diet containing algae, honey bees will consume the diet in laboratory settings. This was confirmed via visual observation of diet consumption both while in the cage and after the feeding period, and evident in dissected hind guts. While the results show that honey bees prefer pollen over algae-based diets, the results also revealed that honey bees were consuming algae-based diets at a level equal to or slightly above its soy-based counterpart, albeit non significantly. However, since algae biomass can be produced on a much quicker and larger scale, can be harvested daily regardless of season, and has a much smaller water footprint per kg of biomass than soy, the results show potential of algae biomass acting as a sustainable replacement for soy-based honeybee diet substitutes (Bošnjaković & Sinaga, 2020:

Ullah et al., 2014; Tzachor, 2019; Martins et al., 2018; Tozzini et al., 2021). Upon completion of the experiments, four articles were found that showed consumption data and were compared to the results of the experiments (Ricigliano et al., 2021; Ricigliano & Simone-Finstrom, 2020; Ricigliano et al., 2022; Jang et al., 2022). The results of this experiment showed similar results where pollen was consumed at a higher rate than the algae-based diet (Ricigliano et al., 2021; Ricigliano et al., 2022), and where algae-based diets were consumed at a rate similar or slightly above that of commercially available plant-based protein supplement diet (Ricigliano & Simone-Finstrom, 2020). Of note is that for the initial test, an evaporation control was not added, nor evaporation rate taken into effect while calculating the results.

The physiology tests revealed mixed results both by line, and over time when it came to head and thorax weights. Results among the Russian line honey bees in the first physiology test showed a significant difference in head weight favoring the use of *Chlorella* supplements, with similar results to hypopharyngeal gland size differences found in a previous studies using *Chlorella* (Jang et al., 2022, Jehlik et al., 2019). These results were also seen in the second physiology test among the bees measured on August 17th, with significant differences between the *Chlorella* diet and the control diet. However, the results were the opposite among Italian line bees with head weights being significantly higher among bees fed control diet vs the bees fed *Chlorella*. This was also reflected in the second set of bees measured on August 24th, with bees fed control diet having higher head weights compared to bees fed *Chlorella*, albeit not significantly. With these results though it should be of note that there was a significant difference in head weight between bees fed pollen and bees fed *Chlorella*, and no significance between bees fed pollen and bees fed control diet. This is opposite to the effects found in two previous experiments in the literature (Ricigliano et al., 2021; Ricigliano et al., 2022). However, it should be noted that Ricigliano et al., 2021 was using Spirulina in the diets fed instead of *Chlorella*. This potentially indicates that the species of algae, genetic lines, and portion of the lifespan of the bees could play a role on the overall impact algae diets have on hypopharyngeal gland stimulus.

For thorax weight, diets across the board were found to have roughly equal effects with no significance between diets, except for the second physiology test on August 24th showing a significant difference in thorax weight when bees fed diet with pollen was compared to bees either the control diet or diet with *Chlorella*. The results between the control diet and *Chlorella* are roughly similar to results in a previous literature result (Ricigliano & Simone-Finstrom, 2020), however it should be noted that the experiment used Spirulina instead of *Chlorella*. Of interest is the results of the second physiology test when compared to pollen showed similar results on August 17th when compared to the results from the previous experiments in the literature (Ricigliano et al., 2021; Ricigliano et al., 2022), but performed the opposite on August 24th to the previous literature results (Ricigliano & Simone-Finstrom, 2020; Ricigliano et al., 2021; Ricigliano et al., 2022). However, it should be noted that Ricigliano et al., 2021 and Ricigliano & Simone-Finstrom, 2020 was working with spirulina instead of *Chlorella*, and Ricigliano & Simone-Finstrom, 2020 was working with bees in during the spring instead of in the summer. Despite this, the results performing the opposite from Ricigliano et al., 2022 despite similar diets and conditions may indicate the lack of feeding between August 17th and August 24th may have played a role in the expression of weight, with pollen providing a means of weight retention longer during periods of dearth compared to current feed substitutes and algae-based feed substitutes.

In summary, our experiment answered the questions we set out to answer. Honey bees will consume algae-based diets, the diets are palatable when compared to current commercial substitutes, and physiological expressions did match some of the results found in the literature, albeit with some of the results being the opposite of those found in the literature. However, these results present evidence that algae may be a viable option for use in commercial feed substitutes performing at the same level as the current options available for beekeepers, with the added benefit of being a more sustainable and ecofriendly raw material when compared to current material in feed substitutes such as soy. Moving forward, additional experiments should be conducted using negative controls of sugar syrup, tests repeated over different lines at different times of the year, diets tested under different periods of dearth, different species tested in the same experiments, formulations refined, and meta-analysis taken among the results and current literature to begin determining the full potential and affects algae have as a feed substitute for honey bees.

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