Vairimorpha Ceranae Alters Honey Bee Microbiota Composition and Sustain the Survival of Adult Honey Bees

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Research

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Abstract

Background: *Vairimorpha (Nosema) ceranae* is the most common eukaryotic gut pathogen in honey bees, *Apis mellifera*. Infection is typically chronic but may result in mortality. Additional factors may be involved in mortality, including the honey bee gut microbiota. Previous studies of *V. ceranae* and gut microbiota identified positive associations between core bacteria and *V. ceranae* infection. These possibly synergistic or mutualistic associations are often disregarded because some core bacteria act as probiotic symbionts.

Methods: To clarify the effects caused by the positive associations, we added isomaltooligosaccharide (IMO), a prebiotic also found in honey, to alter the interactions between *V. ceranae* and gut bacteria. Mortality and sugar consumption of the caged bees were monitored. Infection intensities and gut bacteria were examed after 12 days post inoculation, the plateau phase of infection. The gut bacteria were evaluated using both qPCR and 16S rDNA sequencing.

Results: We confirmed that *V. ceranae* infections alone significantly enhance several core bacterial populations, including *Bifidobacterium* spp., *Snodgrassella alvi*, and *Gilliamella apicola* in the honey bee hindgut microbiota. Moreover, the qPCR results suggested that *V. ceranae* infected bees had significantly higher bacterial microbiota populations. In addition to the enhanced core bacteria, *Commensalibacter* and *Bartonella* were significantly increased in the fecal microbiome. Infected bees fed IMO had significantly higher *V. ceranae* spore counts but lower mortality; however, infected bees fed IMO did not have significant changes in gut bacteria populations compared to those fed only sucrose, but feeding IMO further reduced the fecal microbiome alpha-diversity.

Conclusions: The microbiota alterations caused by infection were similar to the microbiota differences found between summer bees and winter bees, the latter of which have longer lifespans, and feeding IMO increased this similarity. Our results indicated that the interactions between gut bacteria and *V. ceranae* not only enhanced both the pathogen and bacteria populations but also sustained the host survival. This mutualistic interaction potentially enhances disease transmission and avoids social immune responses of the honey bee hosts.

Introduction

Microsporidia are a group of intracellular eukaryotic microorganisms that are closely related to the Fungi. These naturally occurring pathogens often cause disease in laboratory animals that are reared in a dense population [1]. Honey bees are eusocial insects that intimately interact within densely populated colonies, creating a suitable environment for pathogens, including microsporidia, to thrive. There are two common microsporidian species, *Varirmorpha* (*Nosema*) *apis* and *Vairimorpha* (*Nosema*) *ceranae* (genus recently redefined; [2] identified in European honey bees, *Apis mellifera. V. ceranae* is the most common gut pathogen in adult honey bees [3].

V. ceranae was originally identified in the Asian honey bee, *Apis cerana*, and is usually described as an emerging pathogen in *A. mellifera* [4]. Early studies suggested that no host barrier exists in *A. mellifera* for *V. ceranae* infection [5]; however, the earliest *V. ceranae* infection in *A. mellifera* was traced back to 1979 in Brazil [6]. The pathogen may have adapted to the gut environment over the ensuing decades. Reports of pathogen survey in bumble bees [7, 8] and trials in different species of honey bees [9] have demonstrated that *V. ceranae* has a broad host-range that includes most corbiculate bees.

Chronic infections are commonly described for microsporidian species [10]. *V. ceranae* infections have characteristics of typical chronic infections [11], including hormonal and immune responses [12, 13] and subtle alterations of behavior [14]. Many studies suggest that *V. ceranae.* significantly reduces longevity [4], but this effect is not universally reported; differences among repeat trials made mortality insignificant in cages [15] and in hives [16]. In addition, feeding pollen, the major protein and polysaccharide source in the honey bee diet, increased longevity and *V. ceranae* infection intensity [17, 18]. Pollen feeding alters bee physiology, development, and gut microbiota [19].

Honey bee gut microbiota is simple and mainly consists of a small group of core bacterial species [20, 21]. The midgut, the site of *V. ceranae* infection, harbors few gut bacteria, possibly due to the constantly regenerated peritrophic membrane [22]. The hindgut has a narrowed ileum with complicated structures (observed in cross-section) and a balloon-like rectum for storing feces. Similar to most animals, the honey bee ileum and rectum harbor abundant gut microbes [20, 22]. Among the core bacteria, *Lactobacillus* Firm-4 and Firm-5, *Bifidobacterium* spp., *Gilliamella apicola*, and *Snodgrassella alvi*, dominate the hindgut environment. These bacteria affect various physiological functions of bees [20, 21]. These bacteria are recognized as necessary to maintain homeostasis and as beneficial symbionts in honey bee gut.

Independent studies have shown positive associations between *V. ceranae* infection and hindgut bacteria populations. *G. apicola* was significantly and positively associated with *V. ceranae* infections [23]. Our previous studies identified positive associations of *S. alvi* [24] and *Bifidobacteria* spp. [25]. The associations of bifidobacteria and *G. apicola* were recently confirmed in another independent study [26], and microsporidian infection was positively associated with *Snodgrassella* spp. in bumble bees as well [27]. These studies suggested positive associations between *V. ceranae* and the gut core bacteria species, except for *Lactobacillus* spp. Such positive associations seem counterintuitive, especially for *Bifidobacteria* spp. that are generally believed to be probiotics that possibly inhibit pathogenic bacteria [28]. Although most studies of microbiota/pathogen in animals aim to find agonistic associations, probiotic microbes provide benefits to the host and may not contradict the needs of a chronic pathogen causing chronic infections. Chronic pathogens rely on the infected host to tolerate the infection and remain active during the time the pathogen reproduces and is disseminated. Therefore, we hypothesized that *V. ceranae* may have adapted to the gut microbiota and possibly modulated them to maintain host homeostasis, leading to tolerance of the infection and benefiting both the pathogen and the gut bacteria.

To evaluate our hypothesis of the positive associations between *V. ceranae* and honey bee gut bacteria, we used prebiotics to enhance the gut bacteria populations. Isomaltooligosaccharide (IMO) was selected as a prebiotic because isomaltose (the disaccharide form of IMO) is a common sugar found in honey [29]. We found that feeding IMO to *V. ceranae* infected bees resulted in significantly higher *V. ceranae* infection intensity but significantly lowered mortality. Feeding IMO alone resulted in subtle changes in bacterial populations, but significantly reduced microbiome alpha diversity in infected bees. The positive associations between *V. ceranae* infection and specific core-bacteria that were previously reported were also confirmed in this study. In addition, we showed that *Commensalibacter* and *Bartonella* population ratios were increased in the fecal microbiome. These gut microbiota alterations were similar to those found in winter bees that have long lifespans [30]. We suggest that the positive associations between the pathogen and the gut microbiota serve to sustain the host survival, a benefit for both gut bacteria and *V. ceranae*. Moreover, these associations may prevent an apoptosis-like social immune response in which infected bees die prematurely to stop pathogen replication.

Materials And Methods

Honey bee rearing and inoculation

Honeybee colonies, commercially available hybrids with phenotypes of *Apis mellifera ligustica*, were locally purchased from an apiary in Fuzhou. The rearing method and conditions were identical to those of our previous work (Zhang et al., 2019; Huang and Solter 2013). Newly emerged workers were collected within 24 h and transferred into cages, 100 bees per cage. Six cages were prepared and randomly selected for inoculation with one of two sugar-water types, IMO and sucrose. The IMO solution contained 10% isomaltooligosaccharide (IMO), 45% sucrose, and 45% ddH2O, and the sucrose solution contained 50% sucrose and 50% ddH2O. IMO, purchased from Yuanye Bio (Shanghai), was food-grade fermented from starches and mainly resulted in disaccharides with the degree of polymerization up to five [31]. Artificial pollen patties and the sugar water solutions were fed *ad libitum*. The bees were held in a 34˚C growth chamber for five days until inoculation with *V. ceranae*.

Vairimorpha ceranae spores were freshly isolated from foragers collected in the field. Spores were isolated using the same method reported in our previous work (Zhang et al., 2019; Huang and Solter 2013) and used immediately to inoculate bees. Five-day-old bees from the same cage were divided into treated and control groups after anesthesia on ice, and then the treated groups were inoculated individually by feeding a dosage of 10^5 spores in 2 µl sugar water as previously published (Zhang et al., 2019; Huang and Solter 2013). At 30 min post-inoculation, bees were transferred to new cages, 45–50 bees per cage, and given the same sugar-water type and pollen patties that were fed before inoculation. Twelve cages were generated, three cages each for bees inoculated with *V. ceranae* and fed either IMO or sucrose, and two control groups of three cages each for bees fed IMO or sucrose without inoculation with the pathogen. The cages were held in a 30˚C growth chamber, 24 h dark, 70% relative humidity.

Examination of caged bees

The cages were checked daily after inoculation. The dead bees were removed and recorded for survival analysis (Kaplan-Meier method using SPSS23, IBM). Sugar-water consumption was recorded every 3 d when the feeders were replaced. To evaluate the infection intensity and bacterial microbiota, five bees were randomly collected from the cages at 12 d post-inoculation (dpi) and then every other day. Collected bees were dissected using the same method as previously described (Zhang et al., 2019). We separated the midgut, the hindgut and the feces for individual storage. The dissected hindgut was transferred into 50 µl TE buffer, and then the balloon-like rectum with the accumulated feces of the entire caged period was broken. The hindgut, ileum and broken rectum, were washed twice in TE buffer before storing. Midgut tissues were stored in 100 µl PBS and macerated for microscopic examination. The infected bees with midgut spore count < 10^7, the approximate plateau phase level of *V. ceranae* infection [32, 33] were not included in the following qPCR and 16S rDNA sequencing.

DNA extraction and quantification of bacteria of hindguts and feces

DNA of hindgut and feces was extracted by the Chelex method (Huang and Solter 2013) using the qPCR methods previously described (Zhang et al. 2019), including the amount of DNA used in the reactions. Primers for quantifying the core bacteria [34, 35] are listed in the supplementary table. Honey bee *actin* primer set [36] and the universal qPCR primer set for all bacteria [37] were used to normalize the bacteria qPCR results. The significance of the differences in results was calculated using the One-way ANOVA method (SPSS 23, IBM).

We noted that qPCR may not generate robust results from fecal samples due to the complex composition and effects of food debris [25]. To investigate if the prebiotic and *V. ceranae* infection affect the fecal microbiota, fecal samples were submitted for 16S rDNA sequencing using Illumina MisEq. We selected infected bees that had a fully developed infection (total spore count > 10^7); DNA samples from six bees of the same group were pooled and five pooled samples of each group were submitted for sequencing. The samples were PCR amplified using a universal primer set, 338f/806r, with adaptor sequence for the Miseq library and then sequenced on Miseq (Illumina) with the assistance from Majorbio (Shanghai, China). Briefly, the PCR was performed using Fastpfu DNA polymerase (TransGen, Beijing) in 30 cycles. PCR products were revealed in 2% gel-electrophoresis, and products in the expected size range were extracted using AxyPrepDNA kit (Axygen) and quantified using QuantiFluor™ (Promega). The PCR products were then processed using TruSeqTM DNA Sample Prep Kit (Illumina) before sequencing. The obtained data were processed using preset settings and analyzed on the Majorbio Cloud Platform (www.majorbio.com). Single reads were excluded from the results, and the operational taxonomic units (OTU) sharing more than 97% identity were recognized as the same species. Silva database (Release132, http://www.arb-silva.de) was used for the species annotations.

Results

The effects of *V. ceranae* infection and the prebiotic isomaltooligosaccharide (IMO) on the honey bee hindgut microbiota were investigated in this study. IMO alone (no infection) did not significantly affect

mortality and sugar water consumption. However, mortality in infected bees fed IMO was not different from the controls while mortality in infected bees fed sucrose was higher. Feeding IMO significantly increased *V. ceranae* infection intensities but did not significantly alter bacteria population ratios in either qPCR or microbiome analyses; nonetheless, feeding IMO significantly reduced microbiome alpha diversity and unevenness in the fecal microbiome of infected bees. These subtle changes in IMO-fed bees appeared to reduce mortality caused by *V. ceranae* infection. These results support our hypothesis that the specific gut bacteria were enhanced by the infection to maintain the host homeostasis.

Sugar consumption and mortality of the caged bees

No significant difference in consumption of sugar water was found between IMO and sucrose fed groups but *V. ceranae*-inoculated groups consumed significantly more sugar-water, both IMO and sucrose ($P = 0.034$, Fig. 1), than the uninfected controls. There was no difference in mortality between control groups fed the two sugars, however, mortality was significantly lower ($P < 0.001$) in the infected group fed IMO compared to the infected group fed only sucrose (Fig. 2). Mortality was not different for *V. ceranae*-inoculated bees fed IMO and uninfected control bees fed either solution.

Differences in *V. ceranae* infection intensities

To evaluate infection intensities in the midgut, five bees were analyzed by microscopy from each cage at 12 dpi and then every other day. The trial was terminated at 20 dpi because fewer than five bees survived in the cages. The inoculation resulted in a 100% infection rate in the collected bees, and the uninoculated bees were free of spores. Midguts of the same group had similar infection intensities collected in 12–20 dpi. *V. ceranae* infection intensities were slightly, but significantly, higher in IMO groups (Fig. 3). Individual differences and variation were found among the samples, and the bees fed IMO tended to have higher spore counts in the dissected midguts. Because we were investigating fully developed infections, infected bees with less than 10^7 spores were not included in the following analyses.

Quantification PCR results

To investigate bacterial microbiota alterations of hindgut linings and feces, we used qPCR to quantify the core-bacteria species with higher populations. *Lactobacillus* spp., *Bifidobacterium* spp., *Snodgrassella alvi*, and *Gilliamella apicola*, were included in the qPCR. We attempted to normalize the results using the honey bee Beta-actin gene and the universal bacteria primer set; however, both primer sets were problematic as the references for the bacteria qPCR results. Fecal samples yielded low (> 35) or no Ct values using the actin gene primer set. The universal bacteria primer set yielded slightly but significantly lower ($P < 0.001$) values in *V. ceranae* infected bees, both hindgut and fecal samples, whereas IMO feeding did not alter the values. The results suggested *V. ceranae* infection significantly increases bacteria populations, but the prebiotic, IMO, did not. Because this study aimed to investigate the microbiota alterations caused by the infection, we decided to present the hindgut results (Fig. 4A) normalized by the host Beta-actin to show the bacterial population alterations and the fecal results (Fig. 4B) normalized by the universal bacteria primer set to show the bacteria ratio alterations. Hindgut

sample results normalized with the universal bacteria primer set are provided in supplementary materials. In addition, we noted that not all fecal samples generated detectable Ct values within 40 cycles, especially in the qPCR of *S. alvi* and *G. apicola*. The correlation analysis suggested that detectable *S. alvi* and *G. apicola* Ct values in fecal samples were significantly correlated with *V. ceranae* infection ($P < 0.001$). To further clarify the alterations in fecal samples, we submitted randomly selected fecal samples for 16S rDNA sequencing.

Vairimorpha ceranae infection alone enhanced all quantified core-bacteria populations and ratios in hindgut and feces (Fig. 4A and B), except *Lactobacillus.* The hindgut samples used for DNA extraction and qPCR included no visible feces or food debris; therefore, *Snodgrassellla* and *Gilliamella* that attached to the ileum generated much lower values as expected (Fig. 4A). Overall, *Bifidobacterium*, *Snodgrassella* and *Gilliamella* were higher in infected bees; all alterations are significant in hindgut samples (Fig. 4A), and some in fecal samples (Fig. 4B).

Feeding IMO alone significantly increased the *Snodgrassella* population in hindgut samples and significantly decreased the *Lactobacillus* population ratio in fecal samples, which resulted in a marginally significant difference ($P = 0.053$) between the control and the infected groups fed IMO. For infected groups, IMO did not significantly alter bacteria populations in hindgut and fecal samples according to the qPCR results.

16S rDNA sequencing of fecal samples

The sequencing results of the fecal samples from the four groups showed significant differences. In the alpha diversity analyses (Fig. 5), the infected groups showed higher Shannon index values and lower Simpson index values, which suggested that *V. ceranae* infection significantly reduces the alpha diversity of the fecal microbiota. In addition, the infected group fed IMO showed the most distinctive changes in the analyses (Fig. 5). Feeding IMO alone did not result in any significant alteration in the microbiota (alpha diversity analyses), but the within-group differences were much higher than those in other groups (Fig. 5). Infected bees fed IMO showed the opposite results, the lowest within-group differences. Beta diversity analyses suggested that all the infected samples were grouped together in hierarchical clustering and Principal Co-ordinates Analysis (PCoA). In permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis method, *V. ceranae* infection alone significantly affected the microbiota composition ($P = 0.002$), and IMO feeding alone did not ($P = 0.488$).

Bacterial population analysis at the genus level indicated that *Lactobacillus*, *Commensalibacter*, *Snodgrassella*, and *Bartonella* populations were significantly different among the four groups; *Bifidobacterium* and *Gilliamella* were marginally significantly different (Fig. 6). Comparing the infected and control groups fed IMO, all the core bacteria species were significantly altered. Similar to the qPCR results, there were no significantly altered bacterial populations between the infected groups fed sucrose and IMO. Neither was there a significant alteration in bacterial population ratios between control groups fed sucrose and IMO, although the mean value was different (Fig. 6), possibly because of the high diversity and deviation of the control group fed IMO, shown in Fig. 5.

Discussion

Vairimorpha (*Nosema*) *ceranae* is the most common eukaryotic pathogen of *A. mellifera*. *A. mellifera* foragers can support tens of millions *V. ceranae* spores and still perform daily activities. Such tolerance for *V. ceranae* infection is surprising because the spores occupy an enormous part of the midgut cells. Positive associations between *V. ceranae* and several core bacteria symbionts, including *Bifidobacterium* spp., *Snodgrassella alvi*, and *Gilliamella apicola* that are generally considered to be probiotics, led to the hypothesis that these enhanced symbiotic bacteria may positively affect host homeostasis and longevity. Probiotics are expected to add health benefits to the hosts, including protection from pathogens. But probiotics that have no pathogen protection effects may be able to form synergistic relationships with pathogens. To test this hypothesis, we added IMO as a prebiotic to alter the interactions. Although results suggested that infected bees have increased microbiota populations and reduced microbiome alpha diversity, and feeding IMO may intensify these alterations, only alpha diversity was significantly impacted by IMO. However, the intensifications seemed to be enough to diminish the mortality effect caused by *V. ceranae* infections.

Modulated bacterial populations may have distinct functions that could enhance *V. ceranae* infection, host tolerance, or both, but the current knowledge about the enhanced bacteria and physiological changes in bees provides only associations, not causation. For example, the population numbers of *Bifidobacterium* and *Snodgrassella* spp. in the rectum were associated with longevity in comparison of worker and queen bees [38]. The increase of *Commensalibacter* and *Bartonella* population ratios and the reduced microbiome alpha diversity in *V. ceranae*-infected bees coincided with results found in the comparisons between summer and winter foragers [30]. Winter foragers that have longer lifespans had overall higher bacterial populations [30], which is similar to our findings in *V. ceranae*-infected bees. *Bifidobacterium* spp. affects juvenile hormone titer, an effect that also has been reported in *V. ceranae* infection [39]. Interestingly, *Lactobacillus* spp., the only core bacteria that showed a negative association with *V. ceranae* infection, has no known specific physiological function in the bees [21], but the metabolites they produced could reduce *V. ceranae* infection intensity [40].

Similar results to ours were reported in *V. ceranae* studies evaluating the effects of feeding pollen; infection intensity increased [17, 41] and mortality was reduced [17], but the possible microbiota alterations were not analyzed in these studies. Because pollen is the main polysaccharide source in the honey bee diet, we assumed that pollen feeding and *V. ceranae* infections altered the bee microbiota in those studies. Similar results were obtained when indigenous gut bacteria were gavaged into bees with microsporidian infection [42], but neither did this study evaluate microbiota alterations. Feeding antibiotics and *V. ceranae* to bees showed the opposite results in that mortality increased [43]. Overall, these independent studies generated similar results that support the concept that gut microbiota can reduce the negative impacts caused by *V. ceranae* infection. However, only live bees were analyzed in the studies, including the work described here and there is no evidence to suggest that bees dying prematurely from the *V. ceranae* infection have unaltered microbiota. One study suggested that the modulations and the positive associations may not exist in early infection stages [44].

Vairimorpha ceranae infection may modulate the host's core bacteria by affecting polysaccharide digestion, in addition to the hypothesized immune modulations [45]. Polysaccharides are digested in the honey bee midgut in varying degrees. The isomaltooligosaccharides (IMO) we used are mostly small oligos, and honey bees have enzymes to digest such small oligos [46]. We found pronounced prebiotic effects only in *V. ceranae* infected bees, and IMO appeared only to decrease diversity and unevenness in the microbiome of infected bees. *V. ceranae* infection may have impaired or altered polysaccharide digestion in the midgut, but the expression of some related genes was upregulated [47] and proteomic levels were differentially regulated [48]. Interestingly, fumagillin, the antibiotic treatment for *Nosema* disease in bees, also affected modification of polysaccharide digestion enzymes and enhanced intensity of *V. ceranae* infection at low levels [49]. Nonetheless, digestion impairments caused by *V. ceranae* cannot fully explain all of the microbiota alterations. Both lactobacilli and bifidobacteria have the genes needed for metabolizing IMO [29], but lactobacilli populations were still lower in infected bees fed IMO in our fecal microbiome analysis. Although niche competition among the core bacteria might explain the decrease of *Lactobacillus* spp., additional modulations are probably involved. Immune response or other physiological functions modulated by *V. ceranae* infection may involve the core bacteria, and inhibiting immune responses could benefit some bacteria species, especially *S. alvi* and *G. apicola* that are not immune neutral [45].

Establishing mutualistic interactions with the gut microbiota appears to be a reasonable function for *V. ceranae*, which has a broad host-range among apid bees. Because the microbiota is a significant part of the gut environment to which *V. ceranae* needs to adapt, establishing mutualistic interactions with gut bacteria may facilitate *V. ceranae* infection in other host species that share similar gut microbiota. The gut microbiota in corbiculate bees are nearly identical [22, 50], and *V. ceranae* infects most corbiculate bees, including bumble bees [7, 8] and different species of honey bees. The mutualistic interactions between *V. ceranae* and the gut microbiota may have contributed to the broader host range and allowed host-switching in the field [51]. Although not all bees developed the high infection intensities found in honey bees, the possibility that the pathogen can use wild bees as reservoirs has possibly increased the difficulty of managing the disease in managed honey bees.

The results of our study suggest a synergistic and potentially mutualistic interaction between honey bee gut bacteria and *V. ceranae*: In infected hosts, the populations of both *V. ceranae* and the associated bacteria are higher and host lifespan is not affected. Preserving the host lifespan could be a strategy to avoid social immune reactions of the honey bee colony as a super-organism [52, 53]. A social immune response includes the premature death of infected individuals to stop the spread of disease, similar to apoptosis by infected or damaged cells. Because social immune response in honey bees may be efficient enough to drastically reduce immune-related genes in honey bee genome [54], it could also be the selection force that leads to the synergistic associations between *V. ceranae* and gut bacteria.

Conclusion

We demonstrated that the interaction of symbiotic bacteria and pathogens is not unidirectional, only favoring the hosts. Symbiotic bacteria and pathogens can establish mutualistic associations if there are no obvious harmful effects for the hosts. In this case, the *V. ceranae* infected honey bees with enhanced symbiotic gut bacteria had mortality rates similar to the uninfected controls, and populations of specific gut bacteria and *V. ceranae* were all increased. Honey bees are important pollinators and some reports have suggested pathogens, including *V. ceranae*, are having serious negative impacts on honey bee populations. An understanding of the success (high infection rates and intensities) of *V. ceranae* in honey bees is a step toward better control of the disease. The mutualistic interactions between gut microbiota and *V. ceranae* may lead to finding solutions for nosema disease, e.g. finding the optimal gut bacteria for gene-modifications [55] as treatments. We believe this interaction is not a unique case. *V. ceranae* is not the first microsporidium identified that can affect gut bacteria [56] and microsporidia are not the only pathogens that can destroy or affect the host microbiota [57]. Honey bees have a simple gut microbiota that provides an opportunity to identify these interesting mutualistic associations.

Declarations

♣ Ethics approval and consent to participate

Not applicable.

♣ Consent for publication

Not applicable.

♣ Availability of data and material

Please contact author for data requests.

♣ Competing interests

The authors declare that they have no competing interests.

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♣ Authors' contributions

WFH conceptualized the study; WFH and YZ designed the trials; YZ, MS, and LW completed the bench works; WFH, YZ, and MS analyzed the results; YZ and WFH created the figures; SH, SS, and WFH coordinated the trials and obtained the fundings; WFH wrote the manuscript.

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Figures

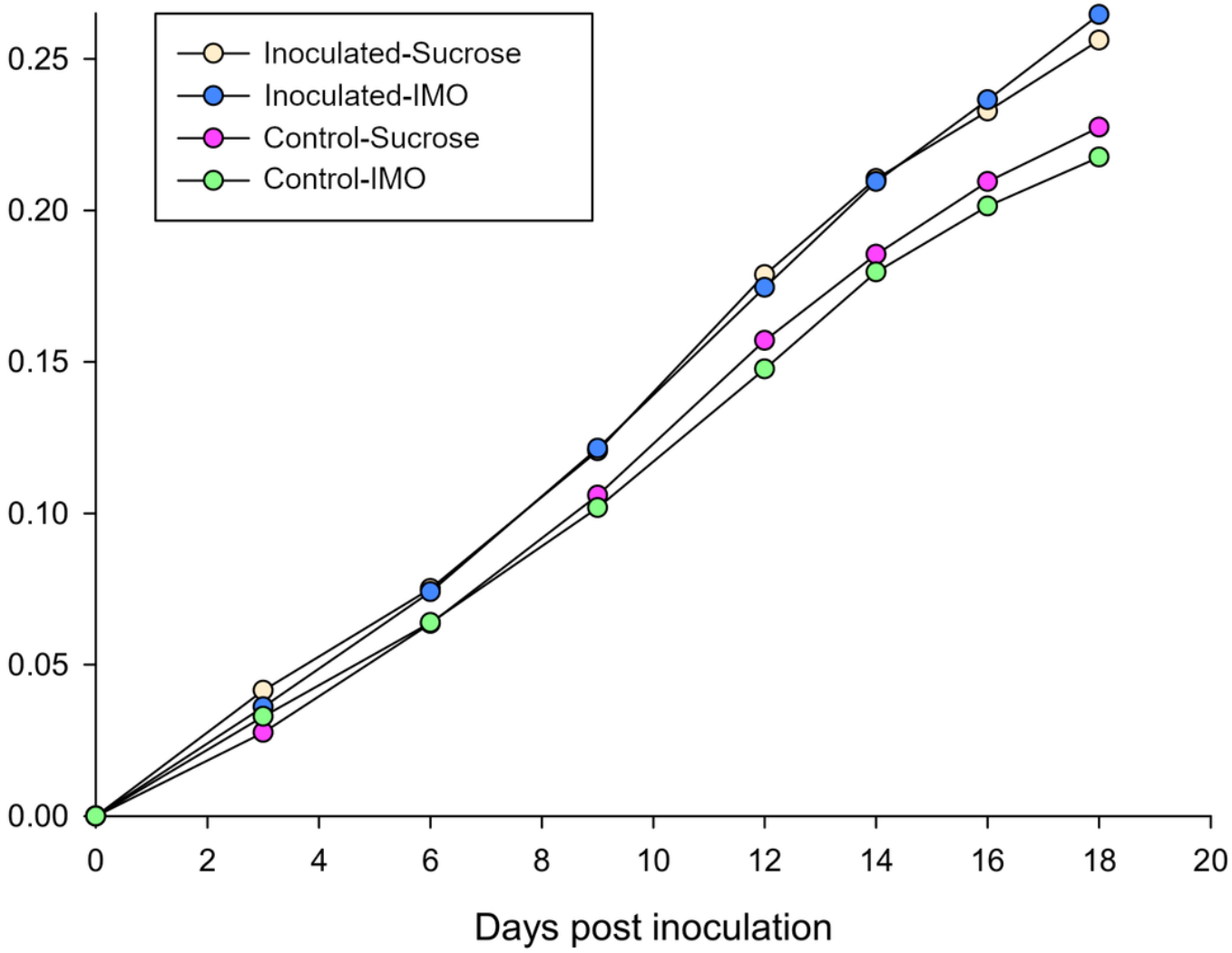
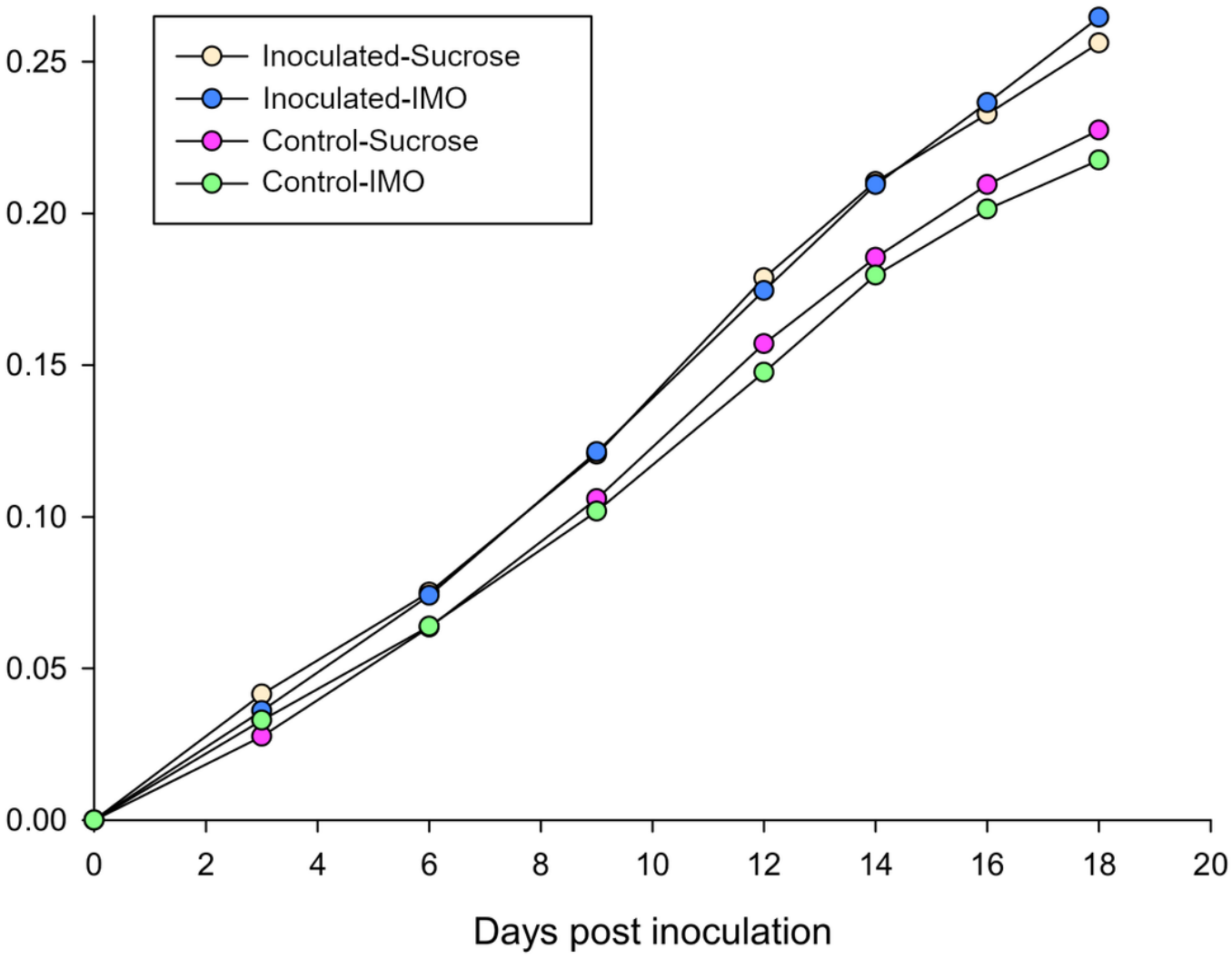


Figure 1

Accumulated sugar water consumption of the four honey bee treatment groups. Y-axis indicates the accumulated sugar-water consumption in grams. The four treatment groups were generated by two variables: IMO/sucrose and V. ceranae inoculation/control.



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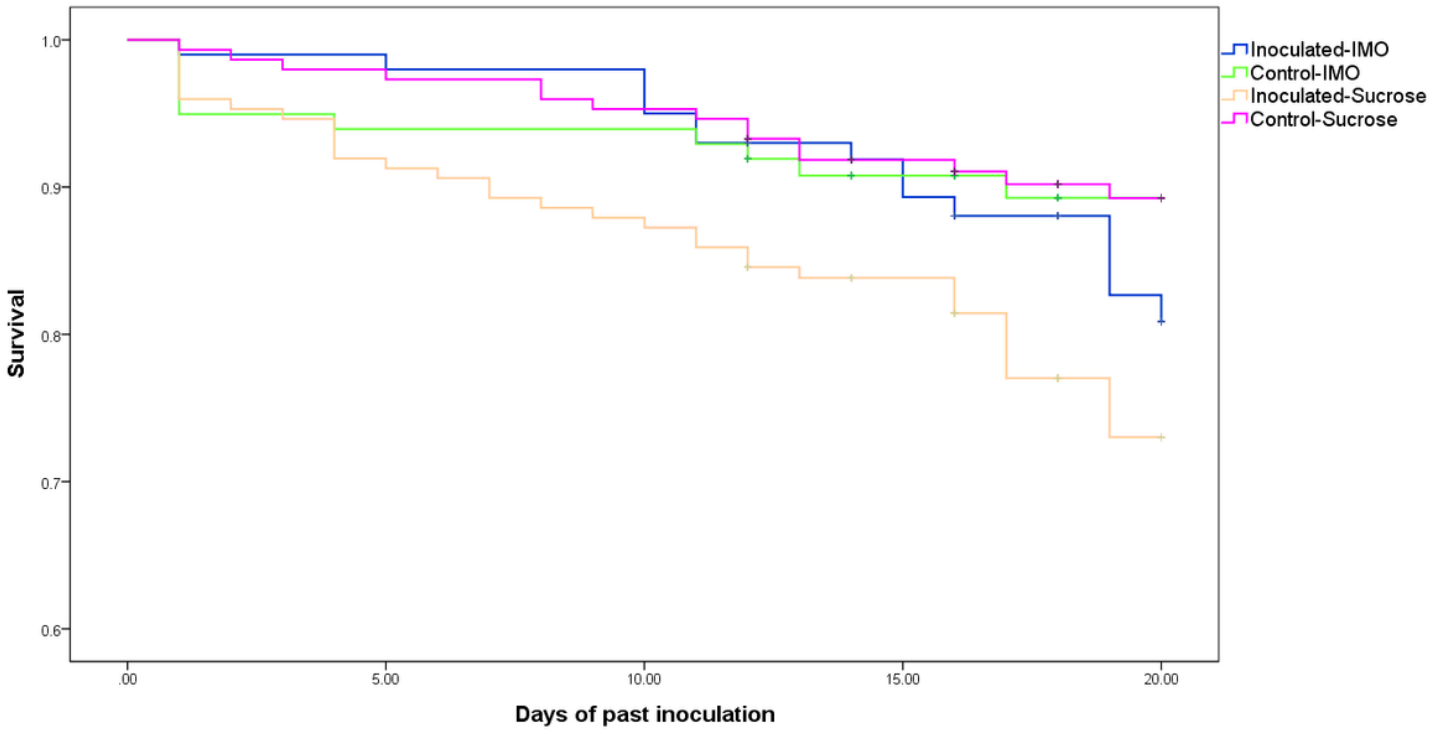
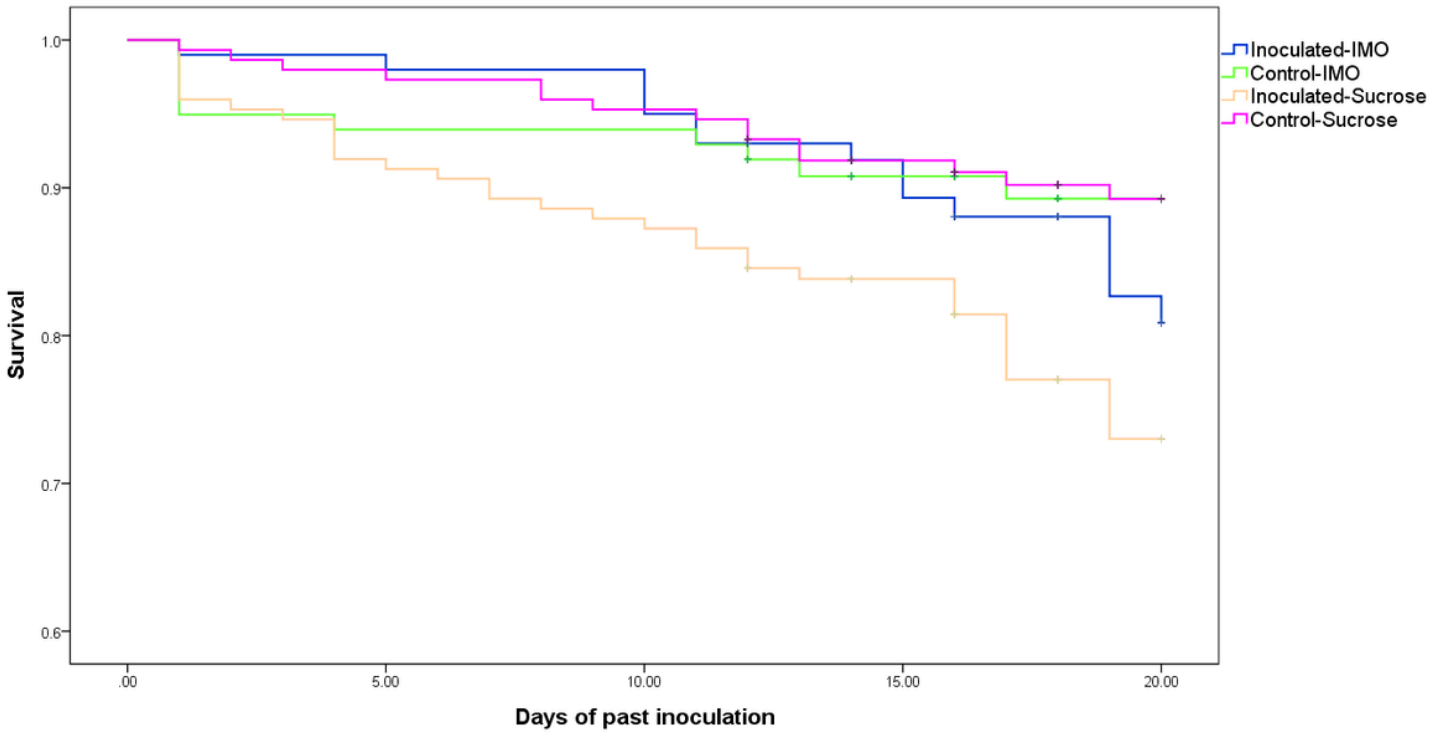


Figure 2

Survival analysis using the Kaplan-Meier method of the four treatment groups. Only the group inoculated with sucrose showed a significant increase in mortality.



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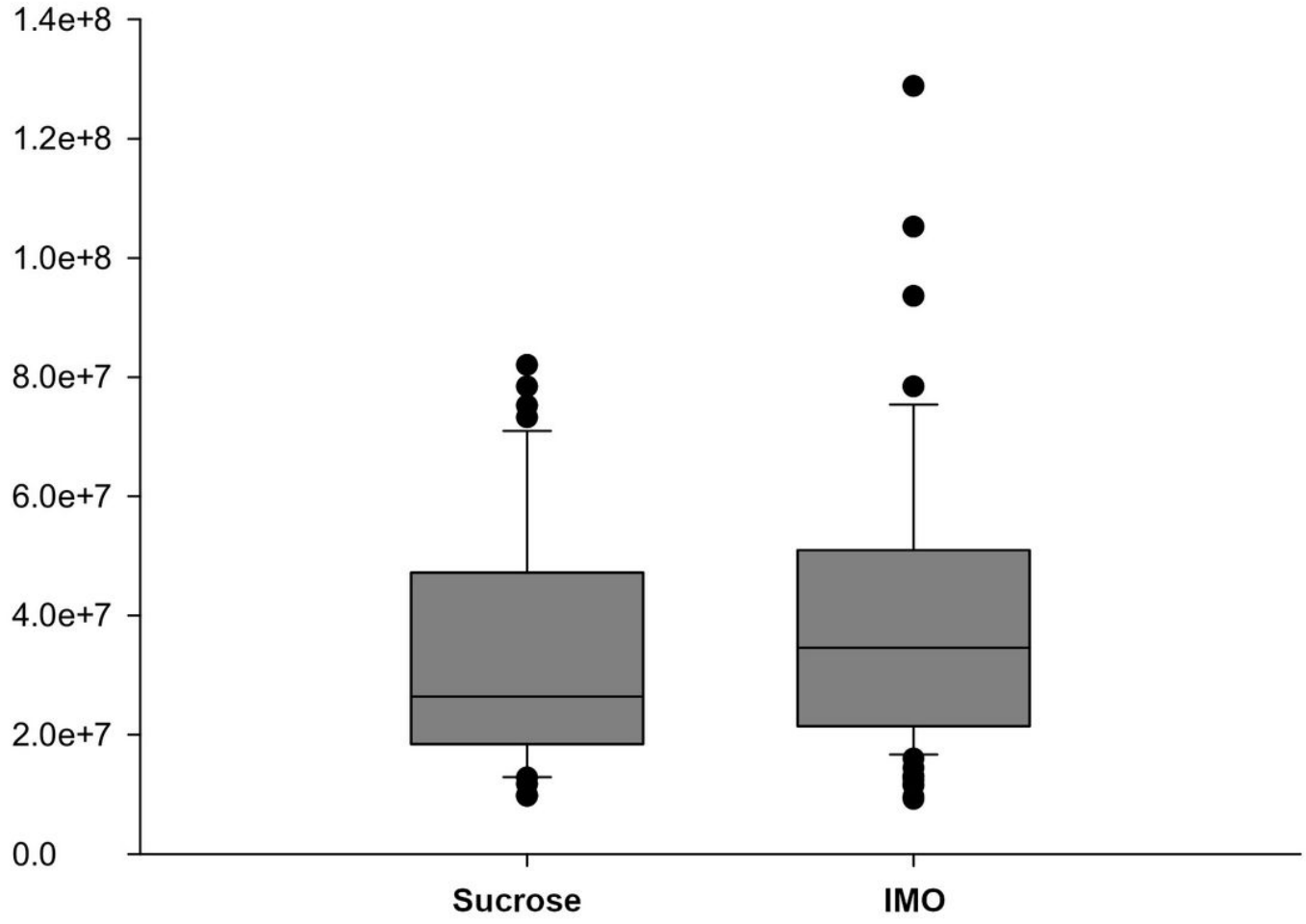
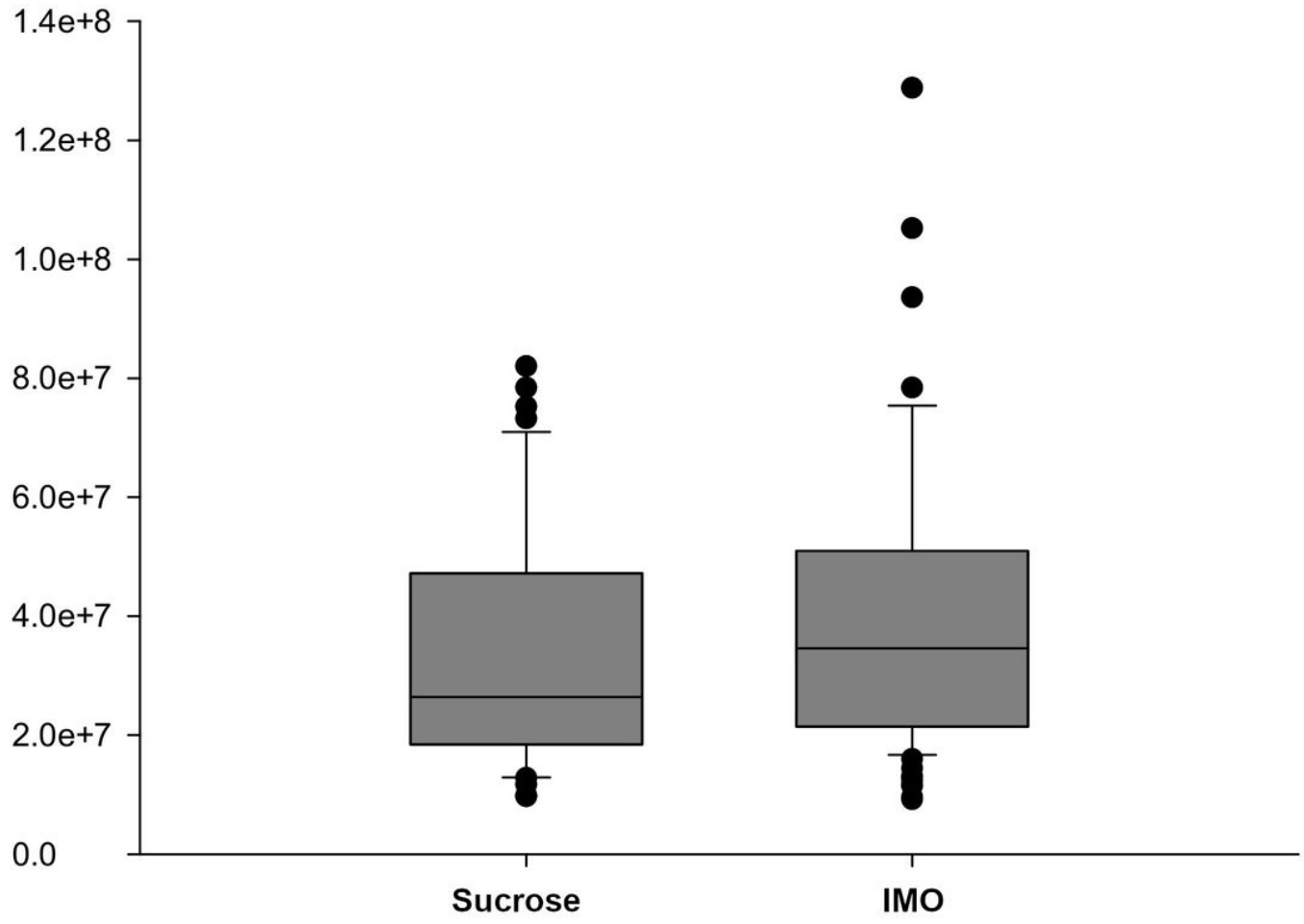


Figure 3

Infection intensities of the inoculated groups fed sucrose and IMO sugar water. The Y-axis indicates the spore counts. Each bee midgut was individually processed and spores counted under a phase contrast microscope. Control bees were free of spores.



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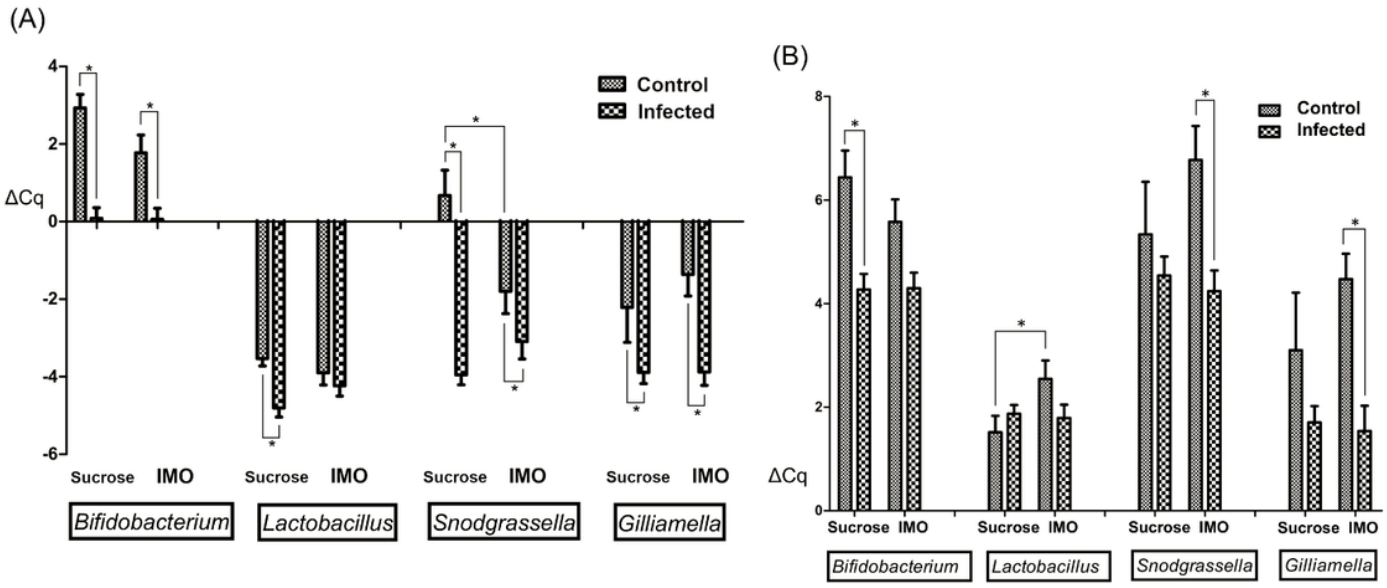
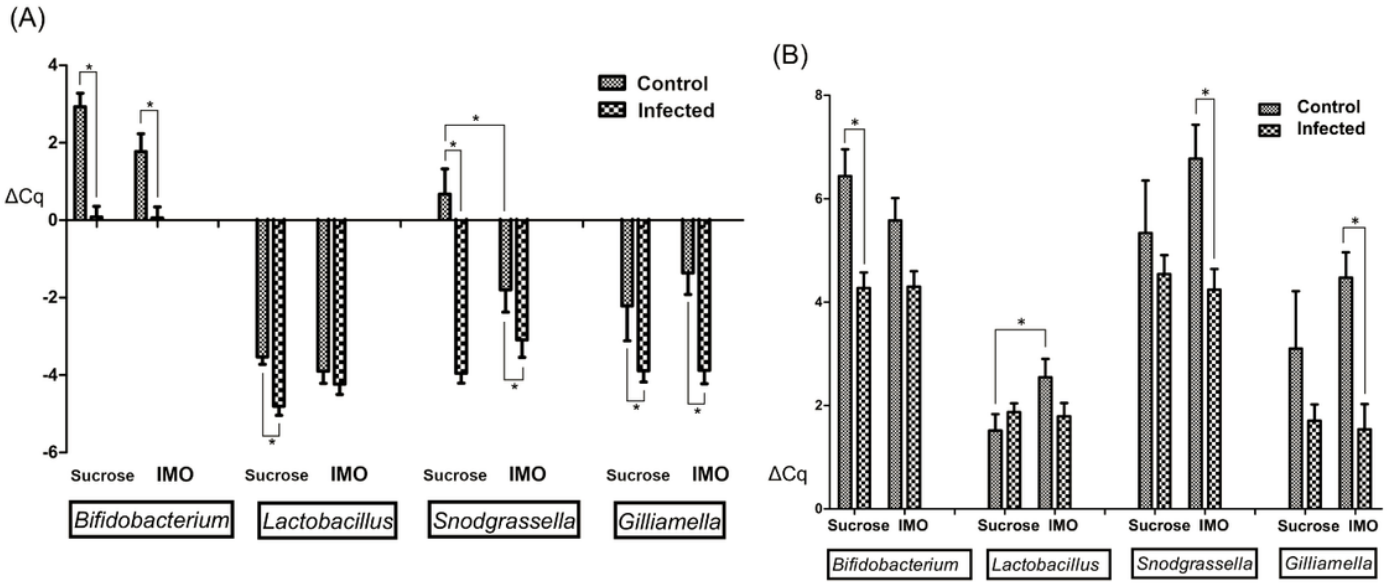


Figure 4

Relative qPCR results of the hindgut and the fecal samples. The lower ΔCq values indicate higher bacteria populations. (A) Hindgut samples qPCR results, the relative quantification results normalized by the honey bee Beta-actin gene. V. ceranae infection significantly increased bacterial populations. Feeding IMO significantly increased Snodgrassella ($P<0.001$) and marginally increased Bifidobacterium ($P=0.058$) populations in control bees; however, IMO caused no significant bacteria population differences in infected bees. (B) Fecal sample qPCR results normalized by the universal bacteria primer set results. Similar to the hindgut results, but Snodgrassella and Gilliamella were not significantly altered in sucrose feeding groups. IMO also caused no significant alterations in bacterial population ratios in infected groups.



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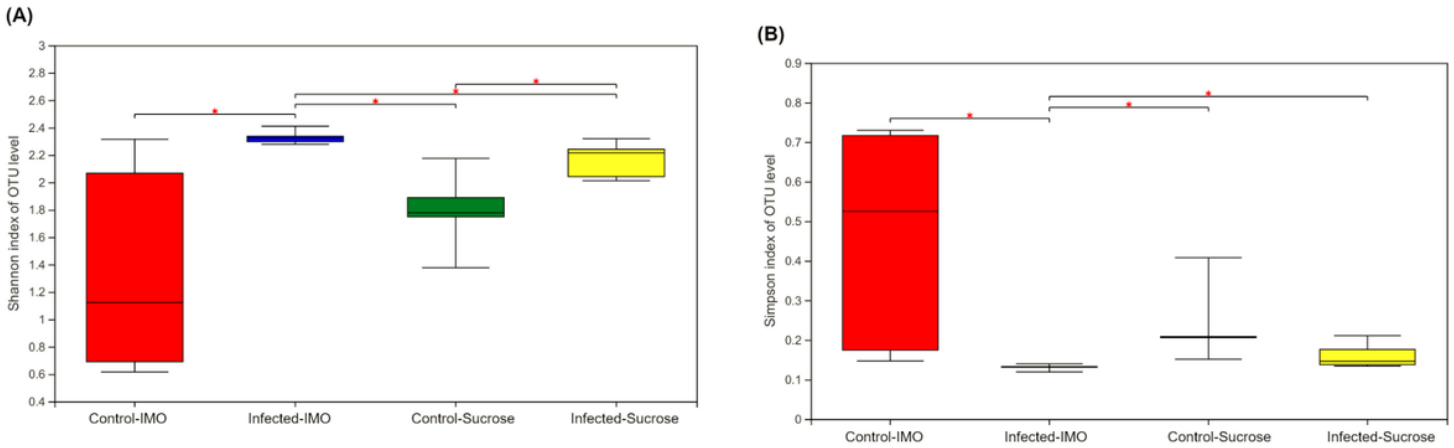
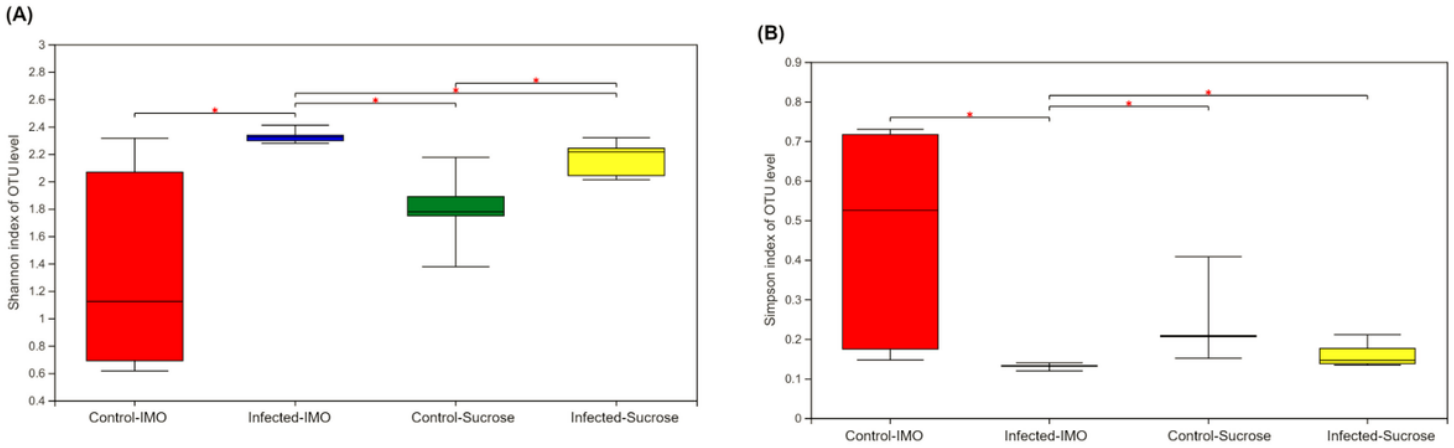


Figure 5

Alpha diversity analyses. (A) Shannon index. (B) Simpson index. The infected group receiving IMO had a significantly higher Shannon index value (2.329, P=0.012) and lower Simpson index value (0.130, P=0.012).


(A)
Shannon index of OTU level
Control-IMO
Infected-IMO
Control-Sucrose
Infected-Sucrose
(B)
Simpson index of OTU level
Control-IMO
Infected-IMO
Control-Sucrose
Infected-Sucrose

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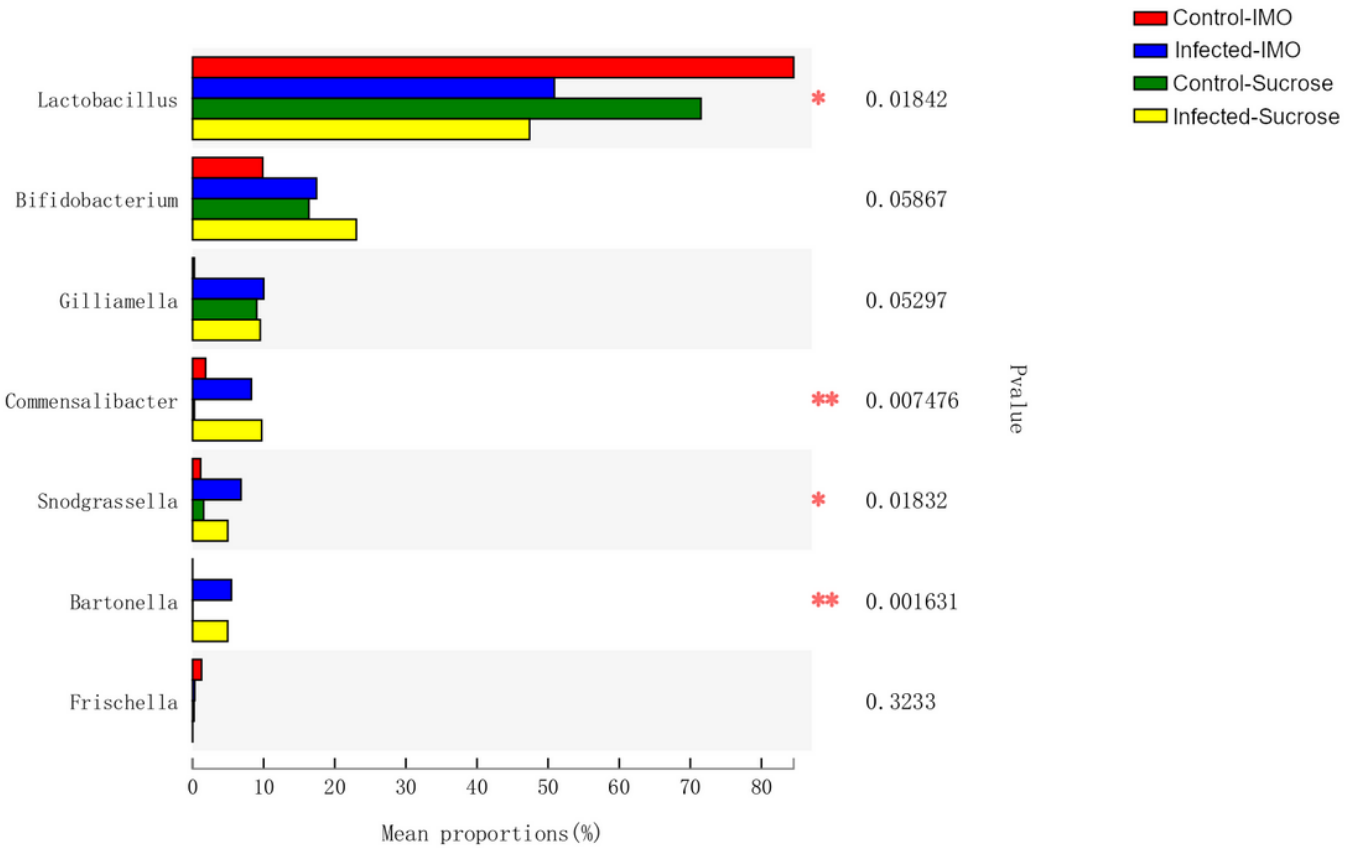
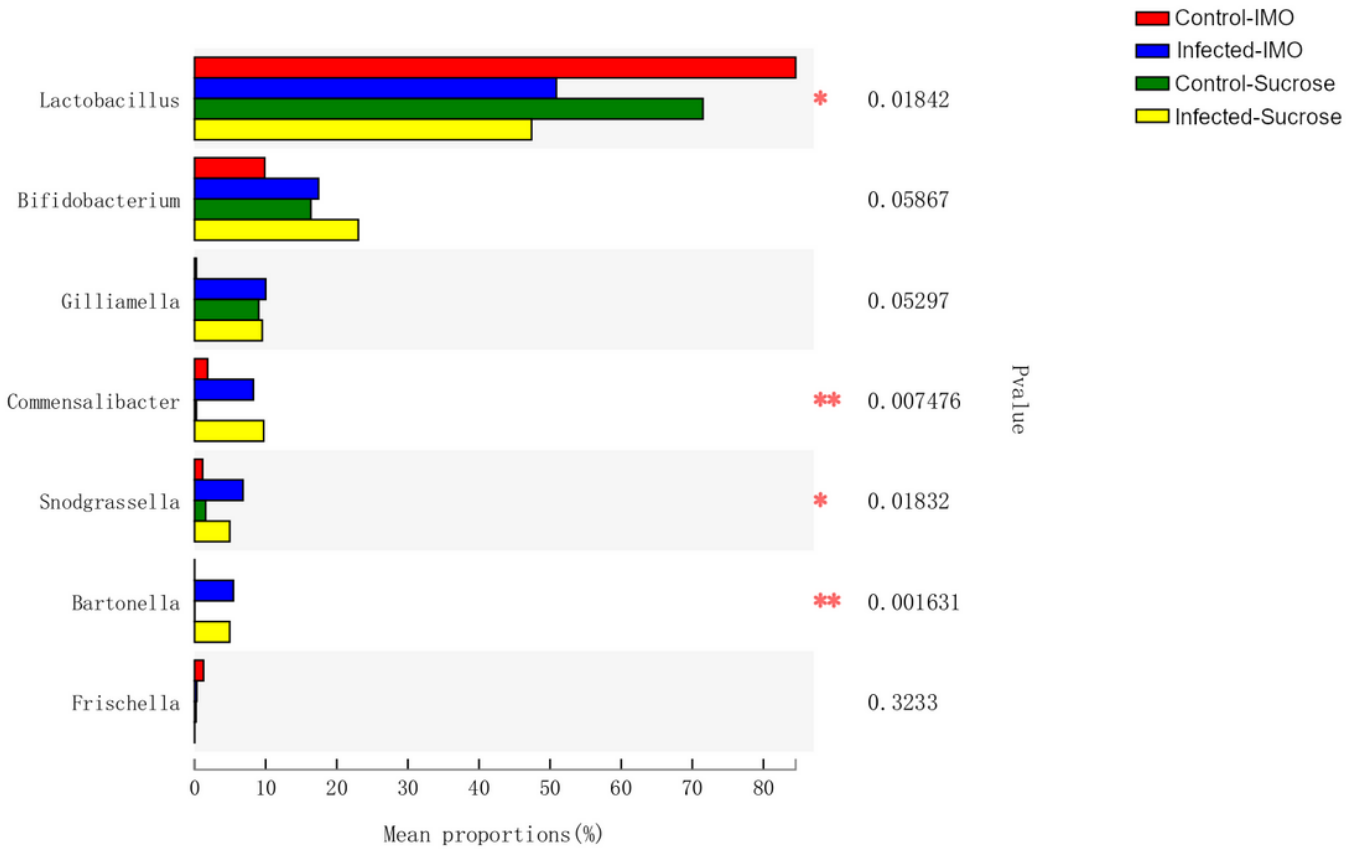


Figure 6

The differences in bacterial population ratios among the treatment groups. The multiple group comparisons were calculated using the Kruskal-Wallis H test with the False Discovery Rate (FDR) approach and the Welch's posthoc method. The comparisons were made at the bacterial genus level.



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Supplementary Files

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