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Lipidomic features of honey bee and colony health during limited supplementary feeding

Características lipidómicas de la abeja melífera y salud de la colonia durante dietas suplementarias restringidas

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Abstract

Honey bee nutritional health depends on nectar and pollen, which provide the main source of carbohydrates, proteins and lipids to individual bees. During malnutrition, insect metabolism accesses fat body reserves. However, this process in bees and its repercussions at the colony level are poorly understood. Using untargeted lipidomics and gene expression analysis, we examined the effects of different feeding treatments (starvation, sugar feeding and sugar + pollen feeding) on bees and correlated them with colony health indicators. We found that nutritional stress led to an increase in unsaturated triacylglycerols and diacylglycerols, as well as a decrease in free fatty acids in the bee fat body. Here, we hypothesise that stored lipids are made available through a process where unsaturations change lipid's structure. Increased gene expression of three lipid desaturases in response to malnutrition supports this hypothesis, as these desaturases may be involved in releasing fatty acyl chains for lipolysis. Although nutritional stress was evident in starving and sugar-fed bees at the colony and physiological level, only starved colonies presented long-term effects in honey production.

KEYWORDS
health, honey bee, malnutrition, unsaturations, untargeted lipidomics

INTRODUCTION

The Western honey bee, *Apis mellifera*, is among the most beneficial insects, providing pollination services to ecosystems and managed crops. The economic value of *A. mellifera* as a honey producer and agricultural pollinator is recognised worldwide, especially as the main pollinator of large-scale crop monocultures (Klein et al., 2007), such as almond, avocado, canola and coffee (Caron, 1977; Mcgkegok, 1971). However, honey bees and their essential services are at risk because of a decrease in health and survival attributed to various stressors, including the availability of adequate nutrition (Gilioli et al., 2019; Goulson et al., 2015). Adequate nutrition is integral to a properly functioning colony (Brodschneider & Crailsheim, 2010), but land fragmentation and alteration, loss of food resources, high colony densities and poor beekeeping management practices are responsible for nutritional stress (Goulson et al., 2015; Sperandio et al., 2019; Wright et al., 2018).

Honey bees' nutritional health depends on the availability and collection of nectar and pollen as well as their quantity and quality to meet the main nutritional requirements for carbohydrates, proteins, lipids, vitamins and minerals (Wright et al., 2018). The weight of the whole hive can be an indicator of colony growth and productivity and particularly food reserves (especially honey) (McLellan, 1977; Meikle et al., 2006). Nectar is the main component of honey and is the principal source of carbohydrates for bees, which is especially important in providing energy for flight (Suarez et al., 2005). Pollen is the primary source of protein and amino acids; however, it also provides lipids (including essential fatty acids and sterols), minerals and vitamins (Wright et al., 2018). Pollen provides nutrients for honey bee growth and development and for the rearing of the brood (Herbert et al., 1977; Loper & Berdel, 1980). The brood quantity is a standard indicator of the reproductive output and size of the colony (Harbo, 1986). Honey production decreases when pollen is scarce because of the reduction in forager bee numbers, causing the colony not to take full advantage of nectar flows (Black, 2006; Kleinschmidt & Kondos, 1979).

Among the nutrients bees obtain from pollen, lipids provide long-term energy reserves (Wright et al., 2018). Lipids are the major components of the fat body, an insect organ with the function of storing energy and with great biosynthetic and metabolic activity (Arrese & Soulages, 2010). Triglycerides (TGs) are synthesised from dietary carbohydrates, fatty acids, or proteins and are the main component of stored lipids (Arrese & Soulages, 2010). Most of the fatty acids acquired from the diet are transported to the fat body and integrated into TGs (Skowronek et al., 2021). Saturated fatty acids (SFAs) do not contain any double bonds, monounsaturated fatty acids (MUFAs) contain a single double bond and polyunsaturated fatty acids (PUFAs) contain more than one double bond. Unsaturated fatty acids are synthesised by the action of desaturases introducing double bonds in fatty acid chains (Los & Murata, 1998). The most important PUFAs for metabolism are linoleic acid (omega-6) and linolenic acid (omega-3), which are considered essential fatty acids for vertebrates, meaning that the body cannot produce them, but they must be taken up from the diet (Stanley-Samuelson et al., 1988). However, it has been demonstrated that many insects produce linoleic acid (Brandstetter & Ruther, 2016; de Renobales et al., 1987; Roelofs & Bjostad, 1984; Stanley-Samuelson et al., 1988). Moreover, Qin et al. (2019) showed that *A. mellifera* can synthesise oleic acid, linoleic acid and linolenic acid from palmitic acid by the action of elongases and desaturases for the production of pheromones (Qin et al., 2019).

To obtain a more comprehensive picture of malnutrition and its implications, honey bee physiology and colony health can be studied as coordinated factors. Lipids are involved in bee behavioural maturation, during which time the transition from nurses to forager bees encompasses a decrease in lipid storage in the fat body (Toth et al., 2005). Consequently, lipid depletion due to malnutrition in the colony causes an early transition of nurse bees to foragers at a younger age (Toth et al., 2005). An unbalanced diet of fatty acids also disturbs the capacity of bees to recognise unhealthy brood, which could negatively affect the hygiene and health of the colony (Bennett et al., 2022). Moreover, lipid unsaturation of membrane phospholipids changes with age and cast, although this might be linked to differential lipid uptake between casts (Hu et al., 2021; Martin et al., 2019). Hence, lipid restrictions affect the social organisation, health, hive duties and productivity of the whole colony, but bees appear to regulate their fat intake by a mechanism that is not yet completely known (Ament et al., 2010; Ament et al., 2011; Corby-Harris et al., 2021; Stabler et al., 2021).

During times of high energy demand, such as malnutrition, the insect's metabolism acquires energy from triacylglycerol stores in the fat body via lipolysis. Lipases are essential in the mobilisation of fat reserves from the fat body (Arrese & Soulages, 2010). Triacylglycerol lipases are enzymes that catalyse the hydrolysis of triacylglycerols, making fatty acids accessible (Arrese & Soulages, 2010; Nunes, 1986; Santana et al., 2017). In insects, triacylglycerol lipases resemble pancreatic lipases of mammals and lipids rich in unsaturations present increased lipase activity (Hoffman & Downer, 1979; Santana et al., 2017). Furthermore, desaturase enzyme activity seems crucial in the lipolysis process of TGs stores as they are the enzymes that desaturate fatty acid chains that then attract lipases (Hu et al., 2021; Los & Murata, 1998; Wang et al., 2020). Although differential expression of some desaturase genes has been found between fasted and fed bees (Corby-Harris et al., 2014), a change in unsaturation of lipids in response to starvation related to the expression of desaturases during starvation has not been previously reported for honey bees.

Characterising the molecular and colony implications of changes in lipid quality and quantity in bee diets, especially during malnutrition periods, provides opportunities to improve the development of supplementary feeds and nutritional practices in the beekeeping industry (Corby-Harris et al., 2021; Manning, 2001a; Toth et al., 2005). Nutritional disorders in the colony affect the first generation but also the subsequent ones (Brodschneider & Crailsheim, 2010). Nurse bees feed the worker larvae from the first day of hatching from the egg until before the cell is sealed. In worker bees, this is from day four to nine of larval development and the workers emerge another 12 days after being capped (Jay, 1963). Therefore, the effects of nutritional treatments on worker larvae can be assessed in newly emerged bees 12 days after exposure to the diet treatment. Here, we studied the effects of malnutrition on the lipid reserves of worker bee larvae related to unsaturation alterations in lipids. Recent lipidomic works have reported unsaturation index changes in worker bees associated with development and ageing

(Hu et al., 2021; Martin et al., 2019), but not during starvation and malnutrition in the fat body and at the colony level.

MATERIALS AND METHODS

Diet restriction field experiment

Set-up and build-up of beehives

The experiment consisted of 18 Langstroth wooden hives, with eight frames each. The frames in each hive consisted of two drawn combs, five foundation combs (combs with wax template) and a honeycomb (frame full of honey). Each hive was made up of one queen bee (genetically different from each other to exclude genetic bias) and 2 kg of worker bees. A raiser and a mat cover were placed under the lid. Bees and equipment for this experiment were provided by the apiary of The University of Western Australia in Perth.

After the establishment of the hives, we immediately placed them in a redgum (*Corymbia calophylla*) forest at Gidgegannup, Western Australia (31°47′59.6″ S 116°10′13.8″ E), allowing free foraging for 4 weeks. We moved them here in 2 batches of 10 colonies on January 31st 2019 and another eight colonies on February 5th 2019.

Experimental setup

After 4 weeks of free foraging, the hives were shifted to a burnt, unproductive site at Lancelin, Western Australia (30°47′11.8″ S, 115°16′48.7″ E) to limit foraging activity and to control feeding. We randomly assigned the colonies to three different nutritional treatments, with six replicate colonies each: (1) Starving-no food provided, (2) Sugar-colonies fed each week with 1 L of sugar syrup (commercial white cane sugar) 150% (w/v) and (3) Sugar + pollen-colonies were fed each week with a pollen patty of 250 g of redgum pollen mixed with 50 mL of 150% (w/v) sugar syrup and 1 L of sugar syrup 150% (w/v) was provided. Irradiated redgum pollen was purchased from Bees Neez Apiaries in Western Australia. The pollen patties were placed under the cover mat directly on top of the frames and the sugar syrup was placed in a 1.10 L bucket over the cover mat, using a riser under the lid to create enough space. We allowed the hives to settle for 1 week and considered week 1 measurements as control to the compared molecular changes within each treatment group. Each week, 27 newly emerged worker bees were collected from each hive, immediately placed on dry ice and stored at −80°C for biochemical analyses (Human et al., 2013). Newly emerged bees were identified as they emerged from the brood cell and by their appearance. As health indicators of the hives, we monitored the weight, temperature, humidity and quantity of brood.

Data and sample collection lasted for 4 weeks at the unproductive site when starvation indicators (lack of larvae and stored honey or pollen) were detected in the hives from the starvation and sugar treatments. The hives were moved to a honey flow in a whitegum (*Eucalyptus wandoo*) forest in Calingiri, Western Australia (31°09′05.0″ S, 116°18′05.1″ E) to allow the free foraging of all the colonies. Whitegum is a good and common source for honey production in Western Australia (Coleman, 1962; Manning, 2001b) flowering during late summer. The recovery and maintenance of health were monitored with the same variables for an additional 4 weeks and the sample collection continued. At week 12, chasing available floral sources, the hives were shifted to an open field surrounded by banksia woodland in Mimegarra, WA (30°51′11.4″ S 115°26′50.6″ E). Here, we kept tracking the colonies' productivity by hive weight once a month (at weeks 12, 16, 21 and 25) and assessing the capped brood quantity once more at week 21. Honey supers were added to each hive as needed. Biochemical analyses were carried out for the bees collected during the diet treatment period and the recovery period (weeks 1–8, Figure 1).

Newly emerged bees were used for this study, to represent the nutritional condition of the colony, at the time when nurse bees fed them (in their larval stage). Honey bee larvae are fed by nurse bees for 12 days, at which time the larvae are capped and develop into pupae and subsequentially adult bees. The baseline of the lipidomic analysis was set at week 1, when the newly emerged bees sampled had been fed by nurse bees in the colony that was freely foraging during their development (Figure 1). Subsequently, we used week 1 as the control for the lipidomic and qPCR analyses, which represented bees that were still fed at the end of the build-up period and not fed during the diet treatment period (Figure 1).

Hive health monitoring

Weight, temperature, humidity and quantity of the brood were measured each week. The brood box and eventually honey super box of each hive were weighed separately and the values were added. Weight was determined using a digital floor scale and hive weight was used as a measurement of general hive strength and honey storage (Human et al., 2013; Meikle et al., 2006). The weight for the wooden boxes (and additional equipment added to the boxes differentially during the experiment) was subtracted from the final weight of the hives. We measured temperature and humidity by placing a digital monitor (remote relative humidity/temperature monitor-800027, Sper Scientific) between the brood frames in each hive. To measure the quantity of the capped brood of each hive, we took photos of both sides of each frame (Canon PowerShot A3300 IS). The photos were processed in the software "Beestly" (https://cyency.com/products/beestly/index.html), which allows the easy identification and quantification of capped brood cells. Finally, the hives were fed according to the diet treatments at the end of each sampling day.

Sample preparation for mass spectrometry analysis

For 20 bees per colony from weeks 1 to 8 (a total of 18 colonies), fat bodies were dissected following a common practice for extracting fat bodies and used in previous honey bee studies (Corby-Harris et al., 2019; Nunes et al., 2013; Wang et al., 2012). This consisted of removing the gut and the rest of the organs enclosed in the abdomen

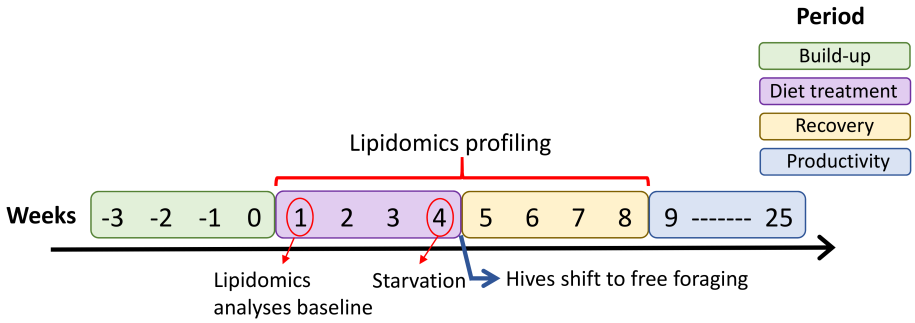

FIGURE 1 Schematic timeline of the differential nutrition protocol of honey bee hives by week. Colony build-up (−3–0 weeks); the diet treatment period (0–4 weeks); recovery period (5–8 weeks) and productivity period (9–25 weeks). The first red oval represents the approximate theoretical moment of the lipidomics baseline (considering the nourishing periods of larval development), and the second red oval indicates the starvation moment of the hives.

and conserving only the abdominal segment with the fat body attached at the dorsal region. This will be referred to as fat body hereafter.

Each sample consisted of 20 abdomens (∼180 mg). The samples were weighed and processed for analysis in two separate batches created by randomising hives and randomising samples within the batch. A solvent of 80% aqueous methanol (MeOH) was added at a concentration of 4:1 aqueous MeOH/bee material. The solvent contained a labelled internal standard at approximately 0.8 ppm of sphingosine-d9, tryptophan-d5 and taurodeoxycholic acid-d4. Samples were ground in 2 mL tubes containing ceramic beads (Precellys CKMix for tissue homogenization) using a tissue homogeniser (Precellys evolution, Bertin Instruments) at 6000 *g*, 2 × 20 s, at 0°C, with 15 s rest between cycles. This monophasic preparation was then split to be further processed for lipid extraction with chloroform and an aliquot was stored for future metabolite analysis (not reported in this study).

Untargeted lipidomic analysis by liquid chromatography high-resolution mass spectrometry (LC-HR-MS)

For untargeted lipidomic analysis, 100% chloroform ($CHCl_3$) was added to the extracts, reaching a final concentration of 1:1 aqueous MeOH/$CHCl_3$. Samples were vortexed for 10 s and subsequently incubated for 30 min at room temperature and 750 *g* in a Thermomixer. The samples were dried under nitrogen and resuspended in 100% isopropanol (IPA) according to weight, obtaining a concentration of 4:1 IPA/bee material. The samples were centrifuged at 9000 *g* and 4°C for 10 min and the supernatant was split into two separate tubes, where 200 µL was destined for lipidomic analysis and 200 µL was stored for fatty acid analysis. All the samples were dried under nitrogen and the samples destined for lipidomic analysis, using liquid chromatography high-resolution mass spectrometry (LC-HR-MS), were resuspended in 150 µL of IPA containing an internal standard of 1 ppm of deuterated cholesterol as a QC for sample extraction and instrument stability. The samples were vortexed and the supernatant was isolated and transferred to a glass vial ready for analysis. Quality control (QC) samples were prepared by pooling 90 µL of each sample from batch 1.

Samples were analysed by high-performance liquid chromatography (Dionex UltiMate 3000 RS) coupled to an Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific) with a heated electrospray ionisation probe (HESI) as the interface. Separation was achieved on a reversed-phase Supelco Analytical Titan C18 column (2.1 × 75 mm, 1.9 µm particle size). The mobile phase was 60:40 acetonitrile: water containing 10 mM ammonium formate and 0.1% formic acid (Solvent A) and 85.5:9.5:5 2-propanol (IPA): acetonitrile: water also containing 10 mM ammonium formate +0.1% formic acid (Solvent B). The elution gradient was as follows: isocratic with 20% solvent B for 0.5 min, followed by an increase to 100% solvent B (0.5–8.5 min) and maintenance at 100% B for 1 min. Then, the system reverted to the initial conditions (20% B) over 2 min and was equilibrated for 2.5 min before the next injection. The flow rate was 0.4 mL/min, the injection volume was 0.1 µL and the column oven temperature was 55°C. Full scans with data-dependent tandem mass spectrometry were acquired on the Orbitrap mass analyser. Full scans were acquired at a resolution of 70,000 at a mass-to-charge ratio (*m/z*) of 200 over the *m/z* range 150–2000 with the ESI conditions as follows: Capillary temperature: 350°C, sheath gas: 48 (arbitrary units), auxiliary gas: 15 (arbitrary units), ion spray voltage: +3.2 kV S-lens 60%. Tandem mass spectrometry analyses were performed at a resolution of 17,500 at *m/z* 200 on each sample with the collisional dissociation energy set at 40 eV. Data acquisition was carried out using Xcalibur software (Thermo Fisher Scientific).

Lipid data were analysed in MS-DIAL (version 4.18), where peak detection, identification and alignment were performed using the LipidBlast adjusted Fiehn lipidomics dataset for peak identification (Kind et al., 2013). Data normalisation and export were implemented as part of the MS-DIAL software as recommended by (Corby-Harris et al., 2019). To decrease the possibility of duplicated features and to increase the confidence of compound identification in the lipid analyses, only analytes that generated an MS fragmentation pattern were considered. We detected repeated diacylglycerol and triacylglycerol identities by sodium and ammonium adducts presenting the same results, independent of the adduct. Both adducts display a structurally informative fragmentation pattern (Han, 2016). Hence, to avoid duplications, we conserved the ammonium adducts, which consistently presented a higher area abundance.

Fatty acid methyl ester analysis

Previously dried lipid samples for fatty acid analysis were resuspended in 305 µL of extraction buffer [MeOH containing 2% H_2SO_4 (v/v)]. The tubes were sealed with microtube cap locks, mixed (using a vortex) for 10 s and incubated for 2 h at 80°C and 750***g*** in a Thermomixer. Samples were cooled to room temperature and 300 µL of NaCl [at 0.9%] and 300 µL of hexane were added. The samples were vortexed for 10 s and centrifuged at 15,700***g*** for 3 min at room temperature. Next, 100 µL of the upper layer was transferred into a glass vial and used for Fatty acid methyl ester (FAME) analysis by GC–MS. A QC sample was pooled from 90 µL of each sample from batch 1. The analysis was performed on a gas chromatograph (Agilent 6890 N) equipped with a Gas Mass Selective Detector, 5975 series (Agilent Technologies). Separation was completed on an Agilent VF-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) + 10 m EZ-Guard column. The sample was injected at an initial temperature of 280°C and held for 5 min with a solvent delay of 5 min. Helium was used as a carrier gas with a flow rate of 1 mL/min. The purge flow was set to start at 2 min at 20 mL/min. The oven temperature was programmed as follows: Initial temperature 70°C, hold for 2 min, then increase to 350°C at a rate of 7°C/min and a post-run hold time of 5 min at 280°C. The transfer line temperature was kept at 250°C and the ion source temperature was 230°C. The detector operated in scan mode from 40 to 600 Da. The MS Single Quad operated at 150°C and the collisional dissociation energy was set to 70 eV.

Fatty acid data were first analysed with Agilent MassHunter Qualitative Analysis Navigator (version B.08.00) to discard background peaks from blank samples. The fatty acids were identified by deconvoluting peaks with the Automatic Mass Spectral Deconvolution Identification System (AMDIS) (version 2.64, 2006) and accessing the Mass Spectral Search Program (NIST/EPA/NIH/Mass Spectral Library, Version 2.0.d, 2005) to identify each compound. Additionally, a hydrocarbon standard (C7-C30 Saturated Alkanes, 49451-U Sigma Aldrich) and three QC samples were run using a DB-5MS column (50 m × 0.2 mm, 0.23 µm film thickness), as described above, to calculate the retention indexes. Later, each fatty acid was identified using the calculated retention index by matching a NIST database (https://webbook.nist.gov). After the main compound features were identified and named, we used the program Agilent MassHunter Quantitative Analysis (version B.08.00) to align and quantify peak areas.

Desaturase gene primers, RNA isolation and quantitative real-time PCR

Desaturase genes of honey bees were found in the National Center for Biotechnology Information (NCBI) GenBank. We used primers previously reported for five acyl-CoA delta (11) desaturases (Falcon et al., 2014) and one more designed from Vernier et al. (2019). Using SnapGene 5.2 software (www.snapgene.com), we designed primers for three more acyl-CoA delta (11) desaturases or desaturases-like and one sphingolipid delta (4)-desaturase (Table S1). Arp1 (actin-related protein) and RpL32 (ribosomal protein 32) were used as housekeeping genes since they have been reported as stable housekeeping genes for honey bees (Wieczorek et al., 2020). The primer sequences used for each gene and specific information are listed in Table S1.

Bees from weeks 1, 3 and 4 were dissected as previously described for untargeted lipidomics analysis of the fat bodies and ground in liquid nitrogen using a mortar and pestle (using seven bees per sample and samples consisting of 3–6 hives per treatment). RNA isolation was performed with 15 mg of ground tissue from each sample using the SV Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer's specifications. RNA (1.2 µg) was used for cDNA synthesis using an iScript cDNA synthesis kit (Bio–Rad, 1708890). cDNA was further diluted and used for quantitative real-time PCR using a QuantiNova SYBR green PCR kit (Qiagen, 208056). Values were normalised against the housekeeping genes and the relative abundance of each gene by the sample was calculated by the comparative Ct method (Schmittgen & Livak, 2008) and ratios were calculated against week 1.

Statistical analysis

The Akaike information criterion (AIC) value was used to find a better-fitted model and distribution when analysing the data with linear models. For hive health data, we fitted generalised linear models with quasi-Poisson distribution for capped brood cell counts and gamma distribution for the remaining predictor variables (hive weight, temperature and humidity). We considered treatment (six hives per diet treatment), time (sampling week) and the second-order interaction of both as response variables. To determine significant differences between specific time points, we performed post-hoc least squares mean (LSM) comparisons, adjusting the *p*-value with the false discovery rate (FDR).

Precision post-analysis tests were performed to clean the MS data for both FAME and lipidomic results, determining the relative standard deviation (RSD) and the dispersion ratio (D-ratio) for untargeted analysis and discarding the compounds that were over the acceptance criterion (RSD > 20% and D-ratio > 50%) (Broadhurst et al., 2018). The data were further reduced by retaining spectra with high confidence identifications; for ions, where the experimental precursor m/z < 1000, the calculated and found m/z values were within 0.003. For ions with m/z > 1000, we accepted an experimental value within 1 ppm of the calculated value. Lipidomics data were analysed with generalised linear mixed-effect models with gamma distribution, where the logarithm of each compound peak area was the predictor variable. The response variables were treatment, time and their interaction. The batch of laboratory analyses (two levels) was included as a random effect variable since we detected an effect in the raw data of the samples (Figure S1). Significance was tested with LSM comparisons using the FDR for adjusting the *p*-value. For closer examination of the lipidomics data, the software Perseus 1.6.15.0 was used to generate hierarchical clusters with the estimates of the models

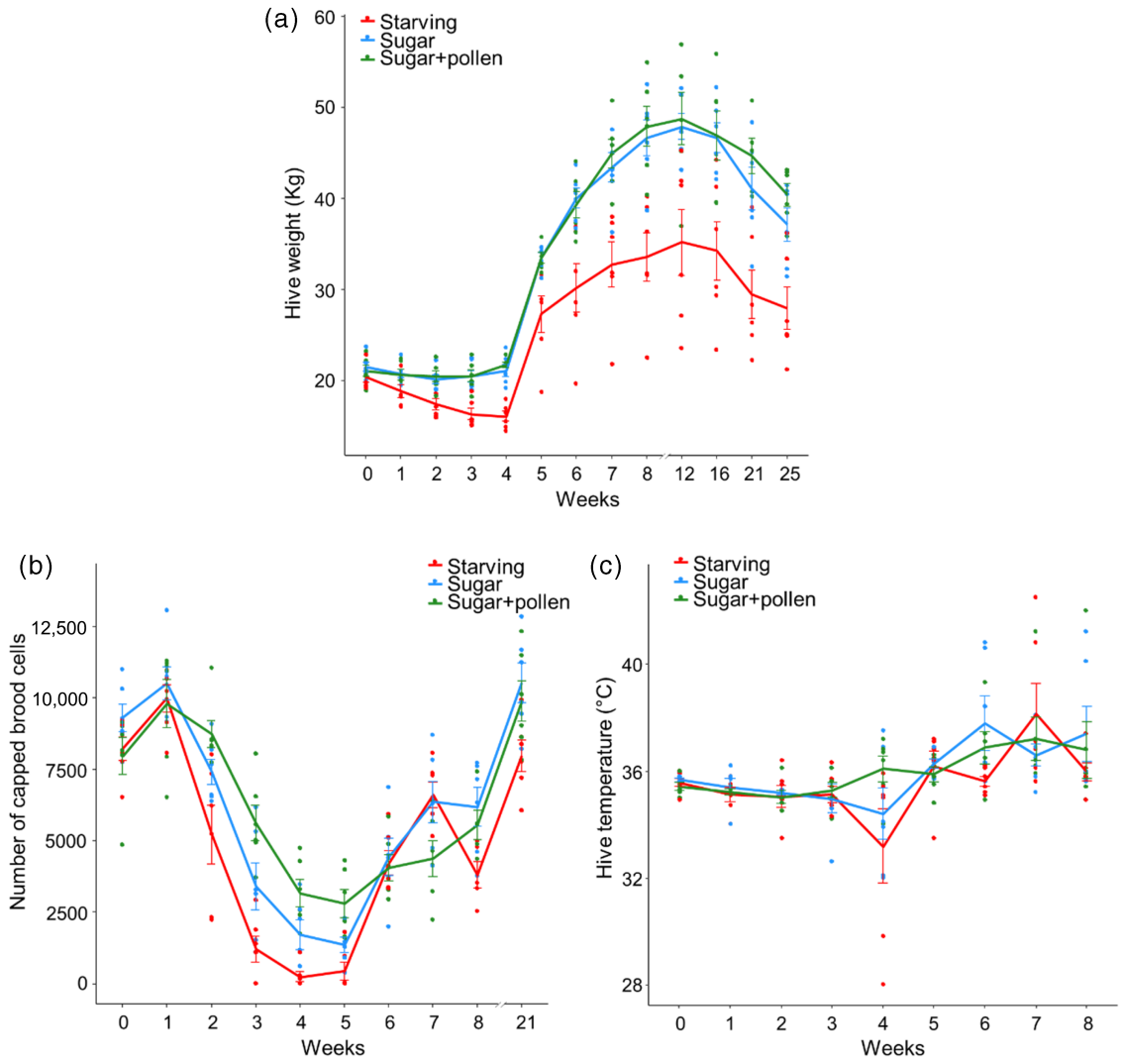

FIGURE 2 Hive health and performance indicators of colonies exposed to three diet treatments over time. (a) hive weight over 25 weeks; (b) capped brood cells over 21 weeks; (c) hive temperature over 8 weeks. Statistically significant differences given by analyses of deviance and LSM, FDR, ($p < 0.05$). Means with standard deviations are plotted. Coloured dots are the individual samples (n = six hives) corresponding to the diet treatments each week.

considering only the LSM significant compounds in each treatment ($p < 0.05$). A Pearson correlation was used to generate the clusters in each treatment, where a distance threshold of 0.81 was used for the generation of 4 clusters in the staving treatment group. A distance threshold of 0.67 in the sugar-fed treatment produced 3 clusters, while the sugar + pollen-fed treatment did not produce important clusters, so no clusters were annotated for this group.

The fatty acid results did not present an effect of batch laboratory analyses; however, the machine presented a performance decrease during the running of the samples. Subsequently, we performed QC-based random forest signal correction (QC-RFSC) using the statTarget package in R language (Luan et al., 2018; R Core Team, 2021). The values were normalised to week 1 by treatment and generalised linear mixed-effect models with gamma distribution were used to test the relative abundance of each compound per diet treatment against week 1, considering time as response variable and hive as a random effect variable. Significance was tested with analyses of deviance followed by post-hoc comparison with LSM and FDR adjustment.

The relative expression of desaturase genes was analysed with general linear models or generalised linear models with gamma distribution, depending on the distribution of the data. Mixed-effect models considering hive as a random effect were used when they resulted in a better-fitting model (according to the AIC value). We ran LSM comparisons using the FDR to test significance. In every analysis, assumptions of homogeneity of variance and dispersion were checked with Levene's test and residual distribution plots. Apart from Perseus software for the clustering analysis, the main statistical analyses and plots were performed in R version R-4.0.3 (R Core Team, 2021).

RESULTS

Different types of supplemental feeding affect malnutrition and honey bee colony health

Comparison of colony health between hives under different nutritional regimens showed differences between the diet treatments and time. The generalised linear model showed differences in the hive weight of the different diet treatments over time and between treatments (deviance = 6.143, $df = 24$, $p < 0.001$) (Figure 2a and Table S2). Hives of the starved group decreased in weight by week 3 (FDR, $p = 0.005$) and week 4 (FDR, $p = 0.002$) relative to week 0 (Figure 2a and Table S3). Conversely, hives fed supplementary diets (sugar and sugar + pollen) maintained their weight during the diet treatment period (Figure 2a and Table S3). All hives in all three treatments recovered when placed on a honey flow; however, the starvation-treated hives never reached the same weight as the supplementary-fed hives (Figure 2a and Table S3).

The differential nutritional regimens caused a difference in the capped brood quantity between treatments (deviance = 413.57, $df = 18$, $p < 0.001$) (Figure 2b and Table S2). All three treatments showed a significant decrease in the amount of capped brood at weeks 4–6 compared to week 0 (Figure 2b and Table S4), when the colonies were still foraging freely. The starved and sugar-fed colonies showed a decrease in the number of capped brood cells earlier (at week 3 compared to week 0) than the sugar + pollen-fed colonies (Figure 2b and Table S4). Moreover, no capped brood was found in three of the six hives at weeks 4 and 5 in the starvation-treated group. Due to this brood gap, no newly emerged bees were sampled from three of the six hives during week 4 and from five hives at weeks 5 and 6. Consequently, samples from weeks 5 and 6 were absent from the lipidomic results for the starvation treatment and week 4 consisted of sampling from three rather than six hives for the starvation treatment. The absence of newly emerged bees was also observed in the sugar-treated hives, where newly emerged bees were absent from one of six hives at week 4 and from two hives at weeks 5 and 6. Consequently, in the lipidomic statistical analysis, the sugar-fed treatment did not contain bees from those replicate hives at these time points.

The hive temperature was consistent between the three treatment groups (Figure 2c and Table S2) until week 4, when the starvation-treated hive temperatures decreased relative to week 0 (FDR, $p < 0.018$) and became more dysregulated than those of the feed treatments for the rest of the experiment (Figure 2c, and Table S5). Hive humidity did not differ between treatments or time.

Honey bee lipid stores change in quantity and quality during supplementary feeding and starvation

FAME analysis was performed on extracts from honey bee fat bodies to detect the global impact of supplementary feeding on fatty acid contents. In total, 14 fatty acids were quantitated and identified, 10 of which were SFAs, three MUFAs and one PUFA. A heatmap of the mean abundance of each fatty acid, normalised to week 1, shows the relative fatty acid abundances within and between each of the treatments over 8 weeks (Figure 3). The starving treatment had the greatest effect on the treated colonies as they observed a brood break and no emerging bees could be sampled for 2 weeks. The sugar treatment hives were the second most affected by malnutrition and showed the main observable abundance changes of fatty acids. Sugar treatment had a decrease in most of the measured during weeks 6–8 (Figure 3 and Tables S6 and S7).

Additionally, untargeted lipidomic analysis identified a total of 124 lipids belonging to eight different lipid classes with high confidence across all time points of all treatment groups (Table S8). Eighty triacylglycerols (TGs), six diacylglycerols (DGs), seven phosphatidylcholines (PCs), 10 lysophosphatidylcholines (LPCs), 12 phosphatidylethanolamines (PEs), five lysophosphatidylethanolamines (LPEs), one sphingomyelin (SM), one steryl ester (SE) and two unknowns were identified from the RIKEN P-VS1 database. The estimated relative abundance values for the interaction of treatment and time generated from linear models were plotted in a heatmap (Figure 4). The heatmap dendrogram clearly separated weeks 3 and 4 of the starving group from the rest of the samples. Samples from weeks 3 and 4 of the starving diet had increased levels of TGs, constituted by PUFAs, whereas another group of mainly TGs with SFAs and MUFAs showed decreased abundance (Figure 4). The total amount of lipids, calculated by a generalised linear model of the added abundances of each of the 124 lipids, showed no significant differences across time of treatment in any of the three treatment groups (deviance = 16.489, $df = 14$, $p = 0.284$, Figure S2).

When examining the molecular structure annotation in the names of the main lipids that changed in abundance, we observed clustering according to the number of double bonds in their fatty acyl chains (leading to degrees of unsaturation). To further examine these lipids, we generated hierarchical clusters of only significantly changing compounds per treatment over time (comparing every time point from week 1 to week 8 in each treatment) (LSM, FDR, $p < 0.05$). Out of 76 significantly changing lipids in the starvation treatment (Table S9), 4 clusters were produced (Figure S3a). Two of these clusters contained 86% of the lipids changing in abundance (Figure 5a). The first cluster consisted of 49% of the total lipids, which significantly decreased at weeks 3 and 4 of starvation and the group mainly consisted of TGs with three or fewer double bonds (Figure 5a). The second cluster of the starving group consisted of 37% of the lipids, most of which showed an increased abundance at week 4 and contained mainly TGs with more than 3 double bonds (Figure 5b).

In the sugar treatment, a total of 53 lipids significantly changed in abundance over time (Table S10) and three clusters were identified (Figure S3b), while two clusters contained 52 of these 53 lipids. The first major cluster consisted of 79% of the lipids, which mainly decreased in abundance at week 6 of the sugar treatment and contained principally TGs with three or fewer double bonds (Figure 5c). The second cluster contained 19% of the lipids that mostly increased in abundance at week 4 of the sugar treatment and included mainly

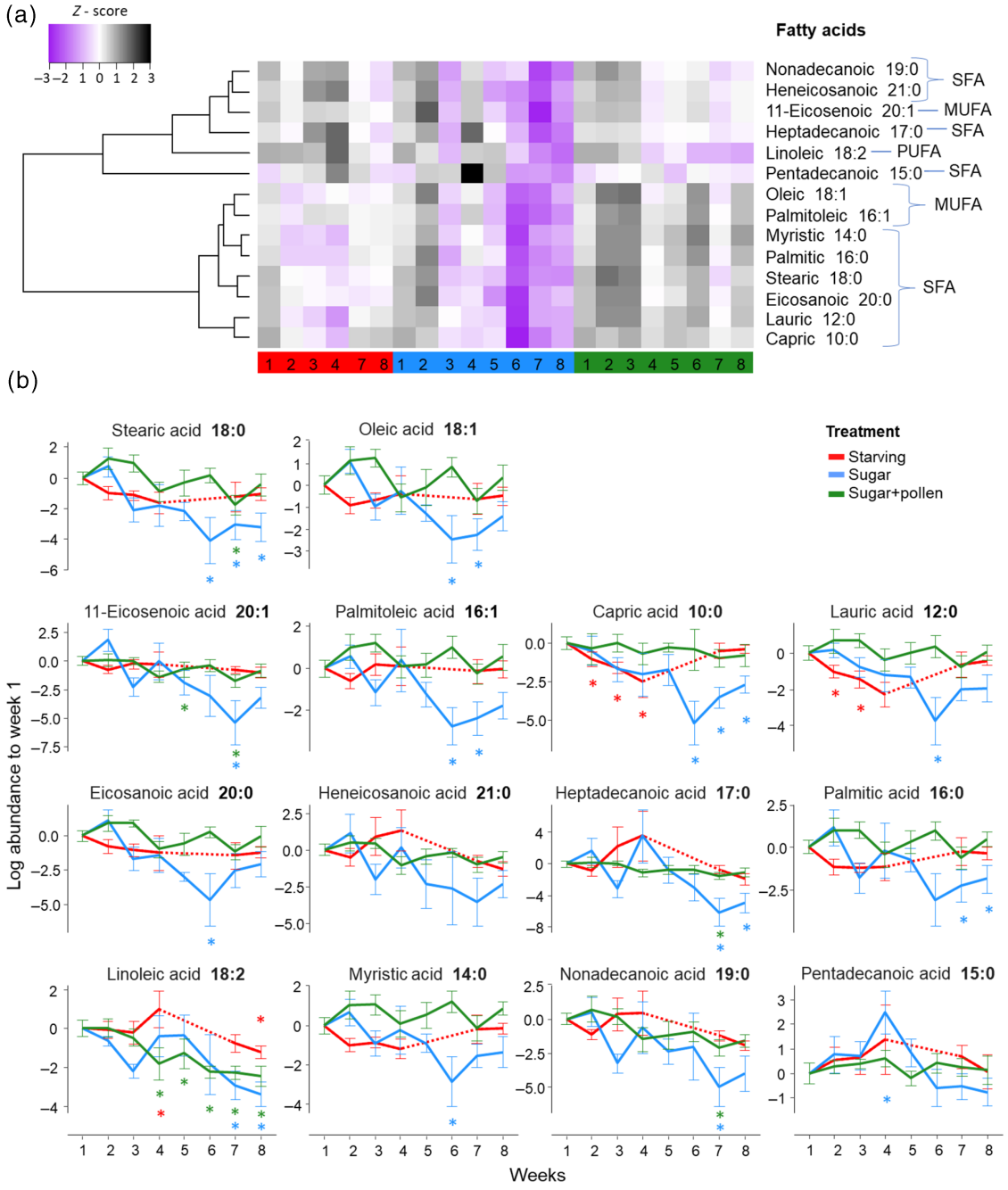

FIGURE 3 Identified fatty acids (as FAMEs) changing over time by treatment. (a) heatmap of all identified fatty acids across all samples, where purple indicates decreased abundance and black indicates increased abundance relative to week 1. Diet treatment is indicated represented by a colour code on the bottom and followed by treatment week indicated with numbers; (b) line plots of the mean of each identified fatty acid changing over time with standard deviations, asterisks (*) indicate significant differences (deviance, LSM FDR, $p < 0.05$) ($n = 3$–6 samples per week per fatty acid).

TGs with more than three double bonds (Figure 5d). In the sugar + pollen treatment, a total of 52 lipids significantly changed in abundance over time (Table S11), no distinct clusters were found (Figure S3c) and only a few compounds seemed to decrease over time (Figure 5e) all other remained unchanged. Mainly TGs with three or fewer double bonds were observed for the treatment group (Figure 5e).

Lipid unsaturation increases at starvation

More in-depth investigation of the data revealed several lipids, that changed in abundance, with the same number of carbons and fatty acid side chains, but with differing numbers of unsaturations. Our analysis could not identify the exact position of the double bonds; however, the number of unsaturations on each fatty acid side chain

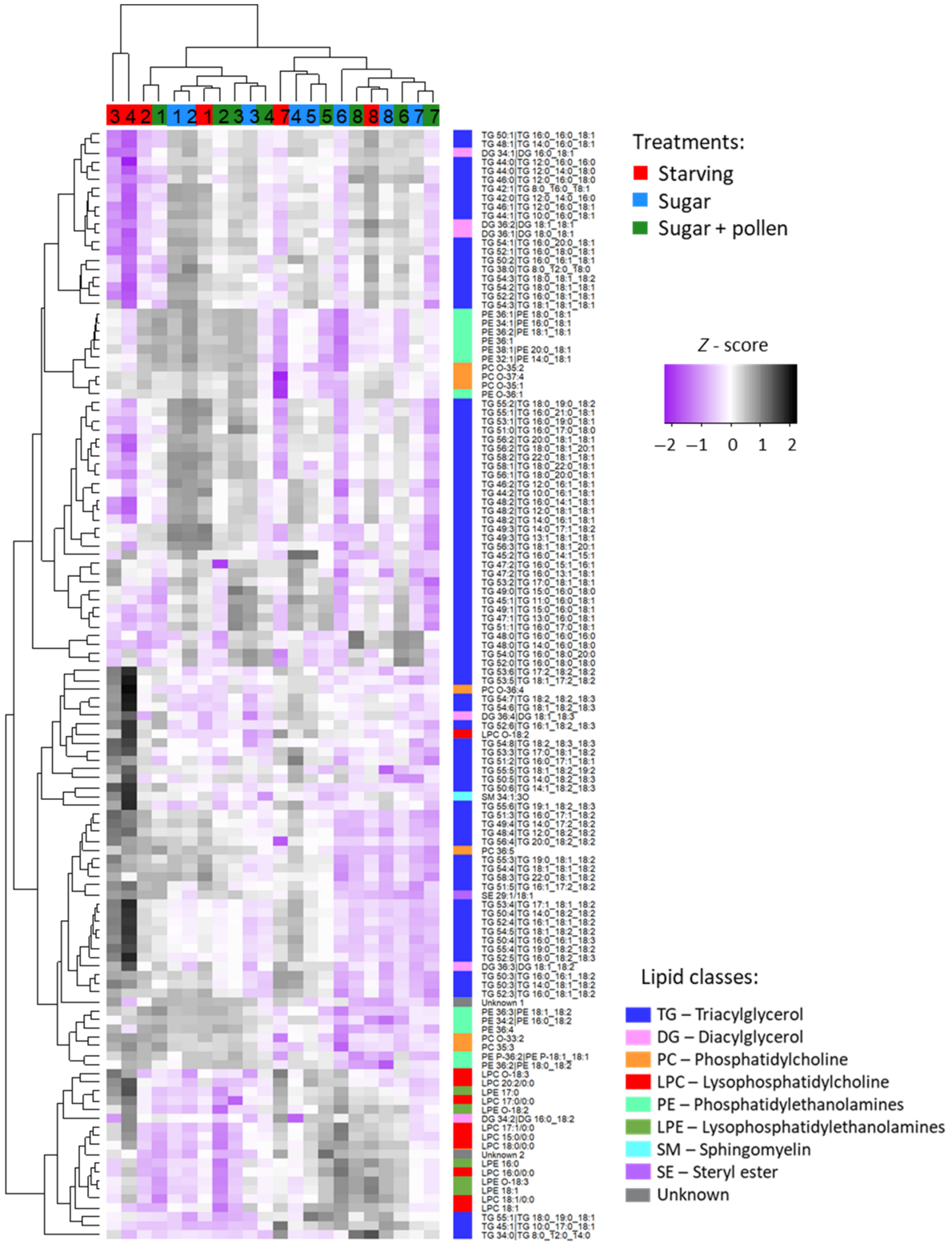

FIGURE 4 Heatmap of the relative abundance of different lipids identified by MS fragmentation pattern matches in the software MS-DIAL. Unsupervised clustering was performed for both the treatments and the lipids. Purple indicates decreased abundance and black indicates increased lipid abundance. A colour code represents lipid class in the right column and lipid abbreviations are listed on the right. The diet treatment group is indicated with a colour code at the top and the week of field experiment is indicated with numbers. Estimated values were determined with generalised linear mixed-effect models ($n = 3–6$ samples per week per treatment).

was determined from the MS fragmentation patterns and database searching. We grouped identified lipids with the same structure but a different number of double bonds in their fatty acyl chains (Figure 6). Based on this approach we found 11 triacylglycerols and two diacylglycerols, which showed increased abundance of fatty acids with a greater number of unsaturations during the starvation period (LSM,

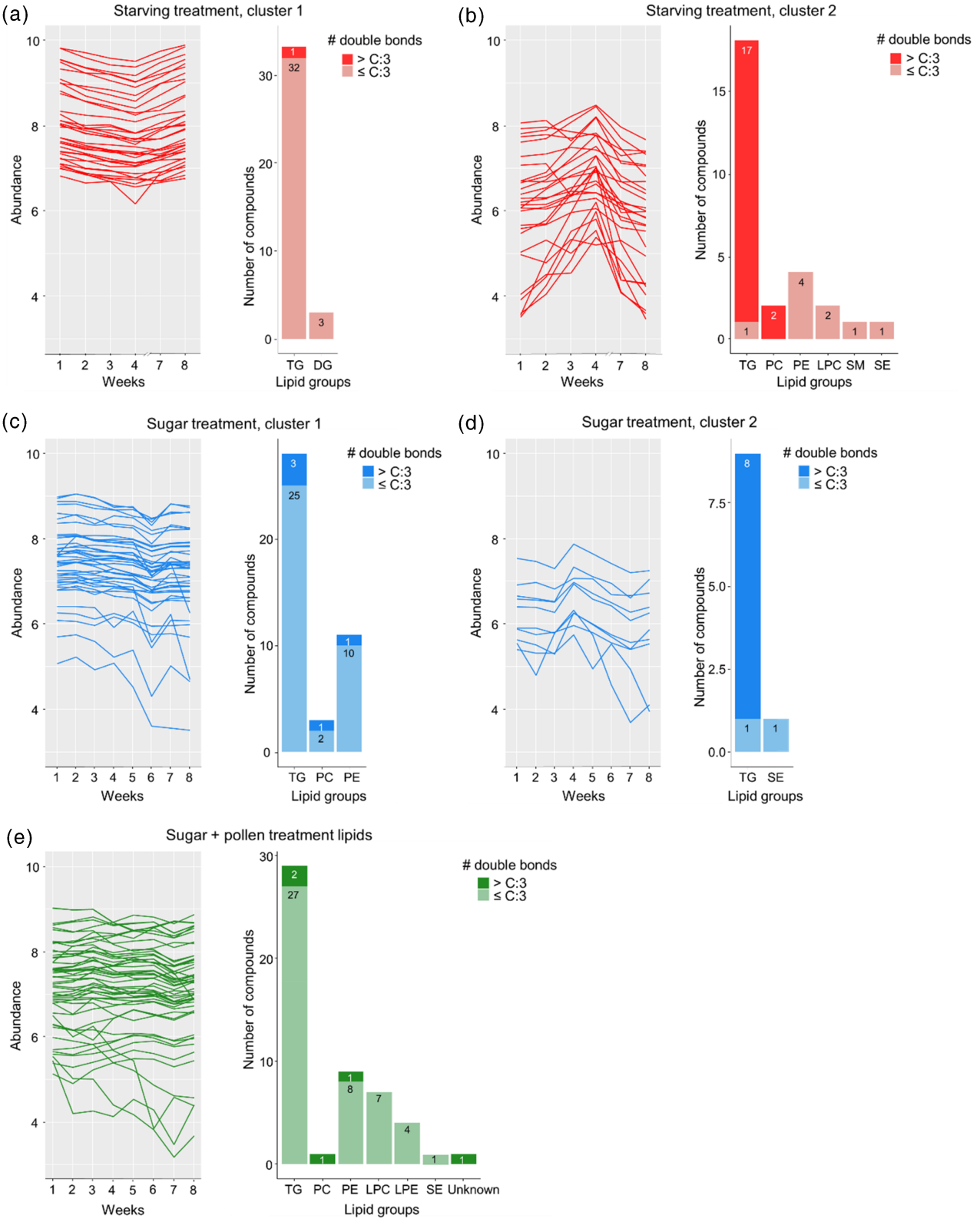

FIGURE 5 Legend on next page.

FDR, $p < 0.05$). In contrast, the abundance of lipids with the same structure but a lower unsaturation index decreased at week 4 (Figure 6 and Table S12) compared to week 1. While we cannot exclude the possibility of other lipid modifications (such as oxidation or esterification) causing the decrease in lowly unsaturated lipids and the increase in the abundance of more unsaturated lipids here we group lipids of the same carbon structure but changing unsaturation indices.

The expression of specific desaturase genes is induced by malnutrition

To understand the molecular mechanism involved in the increase in lipid unsaturation during the starvation period in honey bees, as well as the effect of different dietary treatments over time, the gene expression level of the 10 annotated lipid desaturase genes from the honey bee genome was measured at weeks 1, 3 and 4 in each treatment group. LSM comparative analyses showed that three of these quantitated genes (GB195, LOC712 and GB236) showed increased expression during starvation (Figure 7 and Table S13). Gene expression of GB195 increased significantly at weeks 3 and 4 (LSM, FDR, $p = 0.002$) in starving bees relative to week 1 (Figure 7a). Moreover, bees fed sugar showed decreased expression at week 3 relative to week 1 (LSM, FDR, $p < 0.001$) but increased expression at week 4 relative to week 3 (LSM, FDR, $p = 0.002$) (Figure 7a). For the sugar + pollen-fed bees, the expression of this gene decreased at week 4 relative to week 1 (LSM, FDR, $p = 0.002$). The expression of LOC712 increased marginally significantly at week 4 relative to week 1 (LSM, FDR, $p = 0.086$) in the starvation treatment (Figure 7b). In contrast, for the starving + pollen-fed bees, this gene decreased expression at week 4 relative to week 1 (LSM, FDR, $p = 0.020$) (Figure 7b). The expression of GB236 also increased significantly in starved bees at week 4 relative to week 1 (LSM, FDR, $p = 0.043$) and did not change expression in either of the supplementary feeding treatments (Figure 7c).

DISCUSSION

Changes in the environment and beekeeping practices are increasingly putting honey bees under nutritional stress. Due to the loss of habitat and hive sites as well as intensive beekeeping, beekeepers are forced to rely increasingly on supplementary feeding to keep their bees alive and productive through periods of low availability of natural resources of pollen and nectar (Goulson et al., 2015; Paray et al., 2021). Here, we investigated the molecular and physiological effects of exposing honey bee colonies to three different diet regimens. The effects of starvation, sugar syrup and sugar + pollen feedings were assessed by measuring colony health and lipidomic changes in the accessibility of stored resources in newly emerged bees.

Hive health and performance indicators of malnutrition

Honey bee colonies under nutritional stress demonstrate several rescue behaviours for colony survival. One of the first signs of severe nutritional stress in colonies is the reduction of brood cells, where a decrease in rearing allows the colony to conserve resources (Schmickl & Crailsheim, 2002). During this process, bees cannibalise young larvae, obtaining the protein that they use to feed and maintain the older larvae until no more brood is produced when the queen stops laying eggs (Schmickl & Crailsheim, 2002; Schmickl & Crailsheim, 2004). The decreased number of capped brood cells and the absence of newly emerged bees in the starved hives during and after the starvation treatment and in some of the sugar-fed hives demonstrates that colonies of both treatment groups were nutritionally affected.

Colony productivity is generally measured as hive weight, representing the number of workers, developing brood and stored food reserves (McLellan, 1977; Meikle et al., 2006). Here, the reduction in hive weight observed in the starved hives reflects a loss of food reserves, workers and brood. After the recovery period, the number of workers and brood returned to initial levels in all treatments. However, while the feed-treatment hives increased colony weight during the recovery period, the starvation-treatment colony weight remained lower than the supplementary-fed colonies and did not fully recover by the end of the experiment. Showing long-term adverse consequences of the early starvation event. Possible explanations for the long-term effects of colony starvation are changes in the microbiota of the colony, alterations in the age in which bees transition in the division of labour and effects on the quality of the activities worker bees perform. A reduction in the abundance and diversity of gut

FIGURE 5 Main clusters of lipids significantly changed in abundance by treatment, with the number of lipids by lipid class and levels of unsaturation indicated in each cluster. (a,b), clusters of lipids in bees from the starvation treatment, where dark red on the bar plots represents the number of lipids with more than three double bonds (>C:3) and light red represents lipids with three or fewer double bonds (≤C:3); (c,d), clusters of lipids from the sugar treatment with dark blue in bar plots representing the number of lipids with more than three double bonds (> C:3) and the light blue number of lipids with three or fewer double bonds (≤C:3); (e) lipids from the sugar + pollen treatment with the number of lipids with more than three double bonds (>C:3) in dark green and the number of lipids with three or less double bonds (≤C:3) in light green in the bar plots. The lipid class is indicated as the abbreviated name at the bottom of each bar plot. TG-triacylglycerol, DG-diacylglycerol, PC-phosphatidylcholine, LPC-lysophosphatidylcholine, PE-phosphatidylethanolamine, LPE-lysophosphatidylethanolamine, SM-sphingomyelin, SE-steryl ester. Estimated values determined with generalised linear mixed-effect models ($n = 3$–6 samples in each week per lipid). Statistically significant lipids were determined by LSM comparisons with FDR adjustment ($p < 0.05$). Clusters were obtained using Pearson correlation in Perseus software.

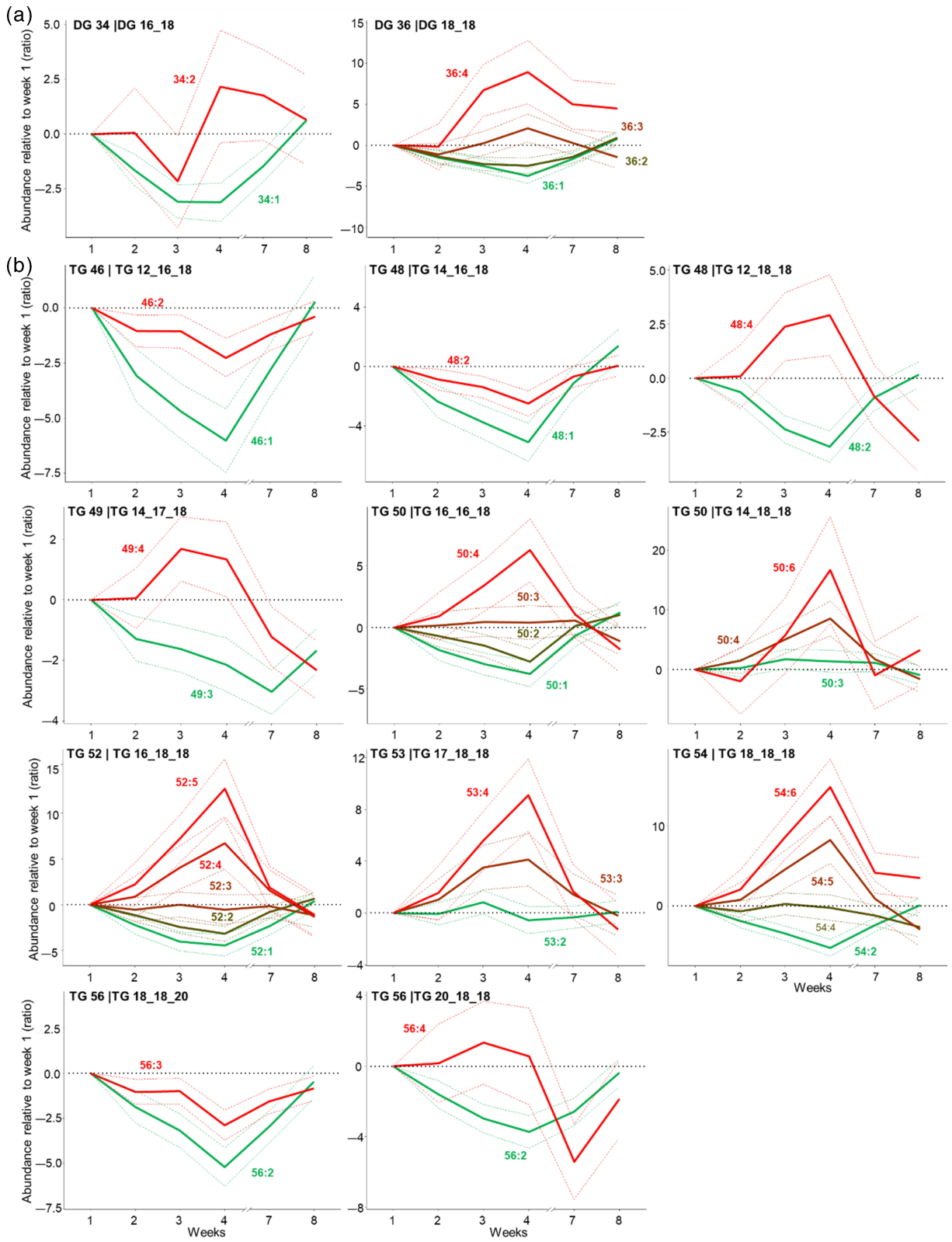

FIGURE 6 Abundance of different classes of storage lipids at starvation relative to week 1 and their number of unsaturations. (a) diacylglycerols (DGs); (b) triacylglycerols (TGs). The number of carbons and unsaturation index indicated in each line are plotted from green to red relative to the increase in double bonds. Contrast compared to week 1 was determined by LSM, comparisons with FDR adjustment are represented by solid lines ($n = 3$–6 in each week per lipid), while dashed lines represent standard errors.

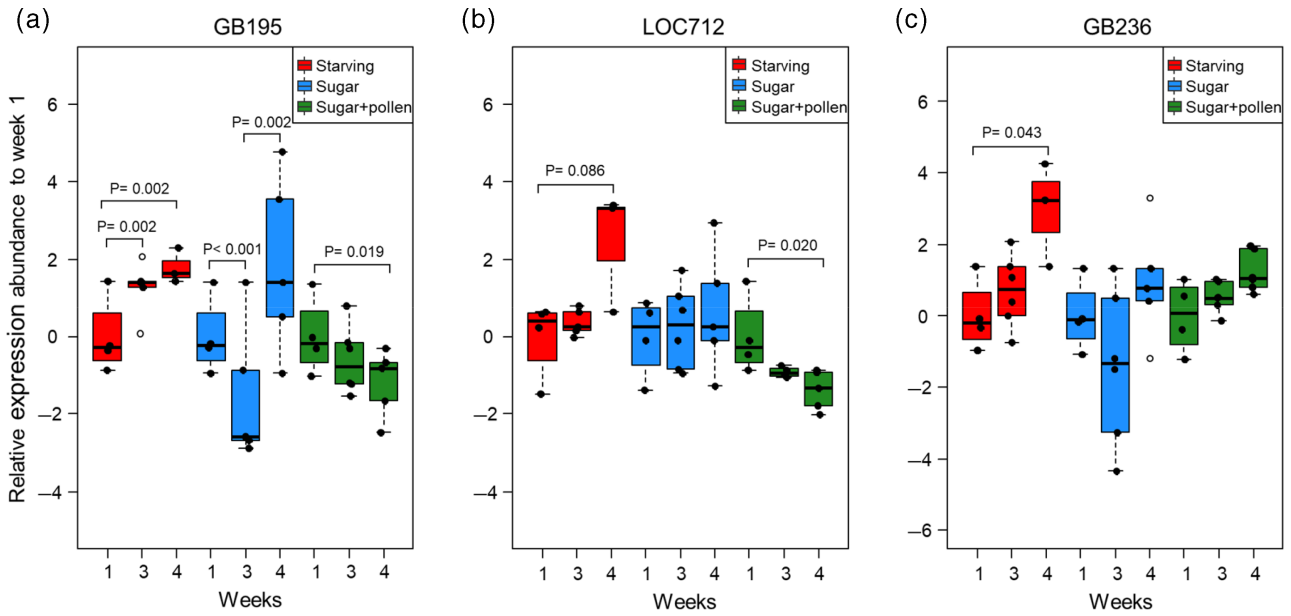

FIGURE 7 Boxplots of the relative gene expression of three desaturases over time in each diet treatment group. (a) GB195 desaturase; (b) LOC712 desaturase; (c) GB236 desaturase. Statistical significance (*p* values) indicated by LSM with FDR adjustment denoted above boxes. The boxplots show the median (line inside the box), interquartile range (box boundaries) and minimum and maximum values of the distribution. Dashed lines represent standard deviations and dots are the individual samples ($n = 3–6$) of each diet treatment over time.

bacteria due to poor nutrition increases bee mortality and susceptibility to pathogens (Maes et al., 2016; Raymann & Moran, 2018) a case in which colonies could take generations to recover a balanced microbiota. Furthermore, the division of labour and time the individuals spend in the jobs that sustain the colony are adjusted according to the colony's specific requirements and nutritional status (Seeley, 2009). For instance, worker bees transition from nurses to foragers earlier when they are nutritionally stressed (Schulz et al., 2002; Toth & Robinson, 2005) and the foraging efficiency of bees that developed from malnourished larvae can be compromised (Matilla & Otis, 2006).

Interestingly, even though hives fed sugar showed early signs of malnutrition, such as the absence of newly emerged bees, honey production (measured by hive weight) after the recovery period was not affected. Furthermore, the temperature fluctuations within the starving colonies are a clear sign of malnutrition as described previously (Flores et al., 1996; Jay, 1963; Meikle et al., 2017; Tautz et al., 2003). The inability to thermoregulate is a consequence of reduced numbers of worker bees and/or a lack of coordinated behaviour in the colony, which can further affect the development of the brood (Meikle et al., 2017). In an environment rich in nutrients, carbohydrates are acquired mainly in the form of glucose that is stored as glycogen or converted into fats (Skowronek et al., 2021). Insect metabolism can rapidly degrade glycogen to access glucose to meet immediate energy needs, for example, during flight. For this reason, carbohydrates are considered the immediate energy source (Arrese & Soulages, 2010). Conversely, protein accumulates during the larval stage and is used during metamorphosis and protein is also used as an energy source during periods of starvation (Skowronek et al., 2021).

Differentially abundant lipids and their availability during malnutrition

During periods of adequate nutrition, lipids are stored in lipid droplets in the bee's fat body for long-term energy reserves in the form of TGs (Arrese & Soulages, 2010; Athenstaedt & Daum, 2006). During periods of energy demand such as starvation, the insect metabolism accesses these accumulated TGs via lipolysis (Arrese & Soulages, 2010). No significant change in the total abundance of lipids was detected in nutritionally stressed bees; however, an increase in unsaturations was detected in lipids of the fat bodies of malnourished bees. It seems that different stressors, such as diapause, parasitic infection and ageing can lead to an increase of unsaturation levels in insects (Hu et al., 2021; Vukašinović et al., 2013; Wang et al., 2020). An increase of unsaturations has previously been related to possible lipid mobilisation in honey bees, observed in the mandibular gland of bees during ageing (Hu et al., 2021). The addition of double bonds in fatty acid chains is known to lead to structural changes in lipids (van Kuiken & Behnke, 1994) and an increased affinity of insect triacylglycerol lipases to the unsaturated fatty acids chains (Hoffman & Downer, 1979; Santana et al., 2017). Furthermore, it is well known that an early step in the mobilisation of fat reserves, is the activity of triacylglycerol lipases that hydrolase TGs from the fat body (Arrese & Soulages, 2010; Skowronek et al., 2021).

In insects, lipolysis involves the release of DGs from TGs accumulated in the lipid droplets of the fat body to the haemolymph (the insect body fluid). DGs are then mobilised to somatic cells with the help of lipoproteins (Arrese & Soulages, 2010; van der Horst et al., 2002). Then, DGs are hydrolysed further into free fatty acids,

which through β-oxidation, supply energy in the absence of sufficient carbohydrate and protein resources (Arrese & Soulages, 2010; Canavoso et al., 2001). Our main results showed an increase of unsaturated TGs in the fat body of newly emerged honey bees during starvation, while saturated TGs decreased in abundance. We hypothesize that saturated TGs were converted to unsaturated TGs in response to starvation, as it has been previously suggested for honey bees during ageing; where an increase in desaturase activity was also reported (Hu et al., 2021). Increasing numbers of double bonds in the acyl chains of TGS can increase the fluidity of these lipids and their metabolism (Ohtsu et al., 1993; Vukašinović et al., 2013). At the same time, we observed increased levels of gene expression of three acyl-CoA desaturases, GB195, GB236 and LOC712. Honey bees have indeed been found to use desaturases to add double bonds to SFA, MUFA and PUFA fatty acid chains (Qin et al., 2019). Furthermore, it has been previously reported that up-regulation of acyl-CoA desaturases may result in an increase in unsaturated lipids under stress in insects (Hu et al., 2021; Wang et al., 2020). The GB195 gene in particular, showed increased expression in newly emerged bees starved during larval development and also in bees fed with sugar. Thus, the GB195 gene might have a more sensitive response to malnutrition even in the presence of carbohydrates. GB195 and GB236 desaturases have been reported to function as D9-desaturases in honey bees, inserting a double bond at the 9th position from the carboxyl group of fatty acid chains. In particular, the expression of the GB195 and GB236 genes have previously been found to increase in adult worker bees, exposed to a poor diet for eight days (Corby-Harris et al., 2014). Here, we show an increase in expression in nurse bees exposed to malnutrition during larval development.

Sugar-fed bees showed a decrease in the abundance of lowly unsaturated TGs weeks later than starved bees. Additionally, during the recovery period, we observed a decrease in abundance of most measured free fatty acids in sugar-fed bees. Many of these free fatty acids, including SFAs and MUFAs, have been previously reported in honey bees (Blum et al., 1967; Vyshchur et al., 2019; Zalewski et al., 2016). Initially, the sugar-fed bees may have been able to meet their immediate energy requirements, through access to carbohydrates, which serve as an immediate energy source (Arrese & Soulages, 2010). As the nutritional resources of lipids and protein became depleted in the sugar-fed bees, due to the absence of pollen, the stored lipids in the bees' fat bodies were potentially accessed and released (Arrese & Soulages, 2010; Skowronek et al., 2021). The individual free fatty acids that decreased in abundance in the sugar-fed bees were likely utilised to fulfil a variety of needs. For example, fatty acids, additional to having a crucial role in lipid storage, can serve as precursors in the synthesis of pheromones, eicosanoids, waxes and cell membrane phospholipids (Arrese & Soulages, 2010; Qin et al., 2019). Sugar feeding without pollen was shown to be less optimal at the molecular and colony level, but no long-term decrease in productivity was detected in sugar-fed colonies. Future studies, focused on colony behaviour, structure and productivity, need to address additional adverse effects of feeding sugar without a protein source, a common practice in the honey bee industry.

We detected a dysregulation in the abundance of a small number of lipids over time in the sugar + pollen-fed colonies and a decrease in the number of capped brood cells at week 4 (during the diet treatment), when compared to week 0. This indicates that free foraging and access to pollen from a variety of floral sources may be nutritionally more beneficial for developing honey bees, as has previously been suggested (DeGrandi-Hoffman et al., 2016; Schmidt & Hayden, 2011). Additionally, it has been demonstrated that diets high in lipids can increase brood production (Arien et al., 2020), but a high ratio of linoleic: linolenic acids (>5:1) negatively affects brood rearing, cognitive performance and survival (Arien et al., 2018; Arien et al., 2020). Beekeepers in Australia use eucalypts as their primary floral resource; although eucalypt pollen is typically low in lipids, honey bee colonies in Australia are some of the most productive in the world (Somerville, 2000). In Western Australia, the eucalypt 'redgum' (*Corymbia calophylla*) is especially preferred for bee management, being an excellent source for building up hive strength, according to beekeepers (Manning, 2001b). Redgum pollen contains a high concentration of myristic, linoleic and linolenic fatty acids, (with a ratio of linoleic: linolenic 3:1) (Manning, 2001b; Manning & Harvey, 2002) which could be beneficial for the bees, as these fatty acids have demonstrated antimicrobial activity (Feldlaufer et al., 1993). Whitegum pollen, also high linoleic acid concentrations, with a ratio 30:1 linoleic: linolenic (Manning, 2001b; Manning & Harvey, 2002) which exceeds the linoleic: linolenic ratio recommended by Arien et al. (2018) and Arien et al. (2020). Despite this excess, whitegum is a preferred sources for honey production in Western Australia after redgum (Manning, 2001b).

Although linoleic and linolenic acids both have been previously detected in honey bees (Blum et al., 1967; Corby-Harris et al., 2021; Vyshchur et al., 2019), we only detected linoleic and not linolenic acid in this study. Nevertheless, few studies have measured free fatty acids in newly emerged bees, previously, where linoleic and linolenic acids were detected in substantially lower concentrations (or only traces) in newly emerged bees compared with few days old adult bees (Manning, 2002; Robinson & Nation, 1970). One possible explanation for the low concentrations of linolenic acid in newly-emerged bees may be that the bees metabolism consumes these fatty acids at a higher rate during the emerging process, which agrees with the previously reported essentiality of these PUFAs for the successful emergence of the moth *Homona coffearia* (Sivapalan & Gnanapragasam, 1979).

CONCLUSIONS

According to our study, the honey bee response to starvation and malnutrition consists of a modification of triacylglycerols, which potentially involves the increase of double bonds in TGs fatty acid chains. The unsaturation index changes reported here, likely add knowledge to the lipolysis mechanism previously detected in the fat body of honey bees during nutritional stress (Corby-Harris et al., 2019). While this needs further investigation, three desaturases

are possibly involved in the early processes of lipolysis and could be potential biomarkers for honey bee nutritional stress. Recently, a kit that tests honey bee stress induced by a variety of factors through the measurement of lipolysis activity was developed (Chen et al., 2021). Following this idea, we propose that the desaturases reported here could be used to develop an assay for specifically assessing malnutrition stress levels. Moreover, earlier detection of malnutrition could be achieved due to the early action of desaturase enzymes in the lipolysis process. However, the expression of the reported desaturases will need to be tested with a larger sample size and under different conditions to confirm their specificity and measure their sensitivity to malnutrition. Furthermore, the long-term effects of starvation events on colony performance show a generational effect that can last for many weeks even when having the colonies in adequate nutrition. Future work will need to study whole colony effect of starvation to determine what caused the loss of productivity. Our study suggests that supplementary feeding in the hive cannot replace free foraging of pollen sources from a variety of flora without impacting the bees at a colony level and physiologically at an individual level.

AUTHOR CONTRIBUTIONS

Clara E. Castaños: Data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **Mary C. Boyce:** Methodology; investigation; resources; validation; supervision; writing – review and editing. **Tiffane Bates:** Conceptualization; investigation; project administration; resources. **A. Harvey Millar:** Validation; supervision; writing – review and editing. **Gavin Flematti:** Resources; investigation. **Nathan G. Lawler:** Investigation. **Julia Grassl:** Conceptualization; funding acquisition; investigation; methodology; resources; validation; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to be declared among the authors of the manuscript.

DATA AVAILABILITY STATEMENT

Lipidomics data to support the findings of this study will be deposited in a database for Metabolomics experiments and derived information MetaboLights (https://www.ebi.ac.uk/metabolights). The authors declare that the other data supporting the findings of this study are available within the paper and its supplementary information files.

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REFERENCES

Ament, S.A., Chan, Q.W., Wheeler, M.M., Nixon, S.E., Johnson, S.P., Rodriguez-Zas, S.L. et al. (2011) Mechanisms of stable lipid loss in a social insect. *The Journal of Experimental Biology*, 214, 3808–3821. Available from: https://doi.org/10.1242/jeb.060244

Ament, S.A., Wang, Y. & Robinson, G.E. (2010) Nutritional regulation of division of labor in honey bees: toward a systems biology perspective. *Wiley Interdisciplinary Reviews. Systems Biology and Medicine*, 2, 566–576. Available from: https://doi.org/10.1002/WSBM.73

Arien, Y., Dag, A. & Shafir, S. (2018) Omega-6:3 ratio more than absolute lipid level in diet affects associative learning in honey bees. *Frontiers in Psychology*, 9, 1001. Available from: https://doi.org/10.3389/fpsyg.2018.01001

Arien, Y., Dag, A., Yona, S., Tietel, Z., Lapidot Cohen, T. & Shafir, S. (2020) Effect of diet lipids and omega-6:3 ratio on honey bee brood development, adult survival and body composition. *Journal of Insect Physiology*, 124, 104074. Available from: https://doi.org/10.1016/j.jinsphys.2020.104074

Arrese, E.L. & Soulages, J.L. (2010) Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*, 55, 207–225. Available from: https://doi.org/10.1146/annurev-ento-112408-085356

Athenstaedt, K. & Daum, G. (2006) The life cycle of neutral lipids: synthesis, storage and degradation. *Cellular and Molecular Life Sciences*, 63, 1355–1369. Available from: https://doi.org/10.1007/s00018-006-6016-8

Bennett, M.M., Welchert, A.C., Carroll, M., Shafir, S., Smith, B.H. & Corby-Harris, V. (2022) Unbalanced fatty acid diets impair discrimination ability of honey bee workers to damaged and healthy brood odors. *The Journal of Experimental Biology*, 225, jeb244103. Available from: https://doi.org/10.1242/jeb.244103

Black, J.L, (2006) *Honeybee nutrition: review of research and practices*. Publication No. 06/052. Canberra, Australia: Rural Industries Research and Development Corporation.

Blum, M.S., Bumgarner, J.E. & Taber, S. (1967) Composition and possible significance of fatty acids in the lipid classes in honey bee semen. *Journal of Insect Physiology*, 13, 1301–1308. Available from: https://doi.org/10.1016/0022-1910(67)90132-1

Brandstetter, B. & Ruther, J. (2016) An insect with a delta-12 desaturase, the jewel wasp nasonia vitripennis, benefits from nutritional supply with linoleic acid. *The Science of Nature*, 103, 1–4. Available from: https://doi.org/10.1007/s00114-016-1365-0

Broadhurst, D., Goodacre, R., Reinke, S.N., Kuligowski, J., Wilson, I.D., Lewis, M.R. et al. (2018) Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics*, 14, 72. Available from: https://doi.org/10.1007/s11306-018-1367-3

Brodschneider, R. & Crailsheim, K. (2010) Nutrition and health in honey bees. *Apidologie*, 41, 278–294. Available from: https://doi.org/10.1051/apido/2010012

Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E. & Wells, M.A. (2001) Fat metabolism in insects. *Annual Review of Nutrition*, 21, 23–46. Available from: https://doi.org/10.1146/annurev.nutr.21.1.23

Caron, D.M. (1977) Insect pollination of cultivated crop plants. *Bulletin of the Entomological Society of America*, 23, 104. Available from: https://doi.org/10.1093/besa/23.1.104

Chen, Y., Heerman, M.C., Cook, S.C. & Evans, J.D. (2021) Detection of lipase activity in honey bees.

Coleman, R.S. (1962) Bee farming: honey flora of Western Australia. *Journal of the Department of Agriculture, Western Australia, Series 4*, 3, Article 21. Available from: https://researchlibrary.agric.wa.gov.au/cgi/viewcontent.cgi?article=3361&context=journal_agriculture4

Corby-Harris, V., Bennett, M.M., Deeter, M.E., Snyder, L., Meador, C., Welchert, A.C. et al. (2021) Fatty acid homeostasis in honey bees (*Apis mellifera*) fed commercial diet supplements. *Apidologie*, 52, 1195–1209. Available from: https://doi.org/10.1007/S13592-021-00896-0/FIGURES/4

Corby-Harris, V., Jones, B.M., Walton, A., Schwan, M.R. & Anderson, K.E. (2014) Transcriptional markers of sub-optimal nutrition in developing *Apis mellifera* nurse workers. *BMC Genomics*, 15, 1–16. Available from: https://doi.org/10.1186/1471-2164-15-134

Corby-Harris, V., Snyder, L. & Meador, C. (2019) Fat body lipolysis connects poor nutrition to hypopharyngeal gland degradation in *Apis mellifera*. *Journal of Insect Physiology*, 116, 1–9. Available from: https://doi.org/10.1016/j.jinsphys.2019.04.001

de Renobales, M., Cripps, C., Stanley-Samuelson, D.W., Jurenka, R.A. & Blomquist, G.J. (1987) Biosynthesis of linoleic acid in insects. *Trends in Biochemical Sciences*, 12, 364–366. Available from: https://doi.org/10.1016/0968-0004(87)90167-8

DeGrandi-Hoffman, G., Chen, Y., Rivera, R., Carroll, M., Chambers, M., Hidalgo, G. et al. (2016) Honey bee colonies provided with natural forage have lower pathogen loads and higher overwinter survival than those fed protein supplements. *Apidologie*, 47, 186–196. Available from: https://doi.org/10.1007/S13592-015-0386-6/FIGURES/6

Falcón, T., Ferreira-Caliman, M.J., Franco Nunes, F.M., Tanaka, É.D., do Nascimento, F.S. & Gentile Bitondi, M.M. (2014) Exoskeleton formation in *Apis mellifera*: cuticular hydrocarbons profiles and expression of desaturase and elongase genes during pupal and adult development. *Insect Biochemistry and Molecular Biology*, 50, 68–81. Available from: https://doi.org/10.1016/j.ibmb.2014.04.006

Feldlaufer, M.F., Knox, D.A., Lusby, W.R. & Shimanuki, H. (1993) Antimicrobial activity of fatty acids against *Bacillus larvae*, the causative agent of American foulbrood disease. *Apidologie*, 24, 95–99. Available from: https://doi.org/10.1051/apido:19930202

Flores, J.M., Ruiz, J.A., Ruz, J.M., Puerta, F., Bustos, M., Padilla, F. et al. (1996) Effect of temperature and humidity of sealed brood on chalkbrood development under controlled conditions. *Apidologie*, 27, 185–192. Available from: https://doi.org/10.1051/apido:19960401

Gilioli, G., Sperandio, G., Hatjina, F. & Simonetto, A. (2019) Towards the development of an index for the holistic assessment of the health status of a honey bee colony. *Ecological Indicators*, 101, 341–347. Available from: https://doi.org/10.1016/j.ecolind.2019.01.024

Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347, 1255957. Available from: https://doi.org/10.1126/science.1255957

Han, X. (2016) *Fragmentation patterns of Glycerolipids. Lipidomics: comprehensive mass spectrometry of lipids*. Hoboken, NJ: John Wiley & Sons, Inc. Available from: https://doi.org/10.1002/9781119085263.ch9

Harbo, J.R. (1986) Effect of population size on brood production, worker survival and honey gain in colonies of honeybees. *Journal of Apicultural Research*, 25, 22–29. Available from: https://doi.org/10.1080/00218839.1986.11100687

Herbert, E.W., Shimanuki, H. & Caron, D. (1977) Optimum protein levels required by honey bees (HYMENOPTERA, APIDAE) to initiate and maintain brood rearing. *Apidologie*, 8, 141–146. Available from: https://doi.org/10.1051/apido:19770204

Hoffman, A.G.D. & Downer, R.G.H. (1979) End product specificity of triacylglycerol lipases from intestine, fat body, muscle and haemolymph of the American cockroach, Periplaneta Americana L. *Lipids*, 14, 893–899. Available from: https://doi.org/10.1007/BF02533502

Hu, X., Zhang, W., Chi, X., Wang, H., Liu, Z., Wang, Y. et al. (2021) Non-targeted lipidomics and transcriptomics analysis reveal the molecular underpinnings of mandibular gland development in *Apis mellifera* ligustica. *Developmental Biology*, 479, 23–36. Available from: https://doi.org/10.1016/J.YDBIO.2021.07.016

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J.D., Forsgren, E. et al. (2013) Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research*, 52, 1–53. Available from: https://doi.org/10.3896/IBRA.1.52.4.10

Jay, S.C. (1963) The development of honeybees in their cells. *Journal of Apicultural Research*, 2, 117–134. Available from: https://doi.org/10.1080/00218839.1963.11100072

Kind, T., Liu, K.H., Lee, D.Y., Defelice, B., Meissen, J.K. & Fiehn, O. (2013) LipidBlast in silico tandem mass spectrometry database for lipid identification. *Nature Methods*, 10, 755–758. Available from: https://doi.org/10.1038/nmeth.2551

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. et al. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274, 303–313. Available from: https://doi.org/10.1098/rspb.2006.3721

Kleinschmidt, G.J. & Kondos, A.C. (1979) Colony management for low quality pollens. *Australas Beekeep*, 81, 5–6. Available from: http://agris.fao.org/agris-search/search.do?recordID=US201302097883

Loper, G.M. & Berdel, R.L. (1980) A nutritional bioassay of honeybee brood-rearing potential. *Apidologie*, 11, 181–189. Available from: https://doi.org/10.1051/apido:19800208

Los, D.A. & Murata, N. (1998) Structure and expression of fatty acid desaturases. *Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids*, 1394, 3–15. Available from: https://doi.org/10.1016/S0005-2760(98)00091-5

Luan, H., Ji, F., Chen, Y. & Cai, Z. (2018) statTarget: a streamlined tool for signal drift correction and interpretations of quantitative mass spectrometry-based omics data. *Analytica Chimica Acta*, 1036, 66–72. Available from: https://doi.org/10.1016/J.ACA.2018.08.002

Maes, P.W., Rodrigues, P.A.P., Oliver, R., Mott, B.M. & Anderson, K.E. (2016) Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and Nosema disease in the honeybee (*Apis mellifera*). *Molecular Ecology*, 25, 5439–5450. Available from: https://doi.org/10.1111/mec.13862

Manning, R. (2001a) Fatty acids in pollen: a review of their importance for honey bees. *Bee World*, 82, 60–75. Available from: https://doi.org/10.1080/0005772X.2001.11099504

Manning, R. (2001b) *Pollen analysis of eucalypts in Western Australia*. RIRDC Publication No. 01/53. Canberra, Australia: Rural R&D Corporation.

Manning, R. (2002) *Body nutrient changes to honey bees when fed pollen or pollen supplementary feedstuffs in the field in Western Australia*. Perth, Australia: Department of Agriculture, Government of Western Australia.

Manning, R. & Harvey, M. (2002) Fatty acids in honeybee-collected pollens from six endemic Western Australian eucalypts and the possible significance to the Western Australian beekeeping industry. *Australian Journal of Experimental Agriculture*, 42, 217–223. Available from: https://doi.org/10.1071/EA00160

Martin, N., Hulbert, A.J., Brenner, G.C., Brown, S.H.J., Mitchell, T.W. & Else, P.L. (2019) Honey bee caste lipidomics in relation to life-history stage and the long life of the queen. *The Journal of Experimental Biology*, 222, 1–11. Available from: https://doi.org/10.1242/jeb.207043

Matilla, H.R. & Otis, G.W. (2006) The effects of pollen availability during larval development on the behaviour and physiology of spring-reared honey bee workers. *Apidologie*, 37, 533–546. Available from: https://doi.org/10.1051/apido:2006037

Mcgkegok, S.E. (1971) Insect pollination of crops. *Bulletin of the Entomological Society of America*, 17, 164. Available from: https://doi.org/10.1093/besa/17.3.164

McLellan, A.R. (1977) Honeybee Colony weight as an index of honey production and nectar flow: a critical evaluation. *Journal of Applied Ecology*, 14, 401. Available from: https://doi.org/10.2307/2402553

Meikle, W.G., Holst, N., Mercadier, G., Derouané, F. & James, R.R. (2006) Using balances linked to dataloggers to monitor honey bee colonies. *Journal of Apicultural Research*, 45, 39–41. Available from: https://doi.org/10.1080/00218839.2006.11101311

Meikle, W.G., Weiss, M., Maes, P.W., Fitz, W., Snyder, L.A., Sheehan, T. et al. (2017) Internal hive temperature as a means of monitoring honey bee colony health in a migratory beekeeping operation before and during winter. *Apidologie*, 48, 666–680. Available from: https://doi.org/10.1007/s13592-017-0512-8

Nunes, C.S. (1986) Adaptation of pancreatic lipase to the amount and nature of dietary lipids in the growing pig. *Reproduction, Nutrition, Development*, 26, 1273–1280. Available from: https://doi.org/10.1051/RND:19860905

Nunes, F.M.F., Ihle, K.E., Mutti, N.S., Simões, Z.L.P. & Amdam, G.V. (2013) The gene vitellogenin affects microRNA regulation in honey bee (*Apis mellifera*) fat body and brain. *The Journal of Experimental Biology*, 216, 3724–3732. Available from: https://doi.org/10.1242/JEB.089243/257681/AM/THE-GENE-VITELLOGENIN-AFFECTS-MICRORNA-REGULATION

Ohtsu, T., Katagiri, C., Kimura, M.T. & Hori, S.H. (1993) Cold adaptations in drosophila. Qualitative changes of triacylglycerols with relation to overwintering. *The Journal of Biological Chemistry*, 268, 1830–1834. Available from: https://doi.org/10.1016/s0021-9258(18)53929-6

Paray, B.A., Kumari, I., Hajam, Y.A., Sharma, B., Kumar, R., Albeshr, M.F. et al. (2021) Honeybee nutrition and pollen substitutes: a review. *Saudi Journal of Biological Sciences*, 28, 1167–1176. Available from: https://doi.org/10.1016/j.sjbs.2020.11.053

Qin, Q.H., He, X.J., Barron, A.B., Guo, L., Jiang, W.J. & Zeng, Z.J. (2019) The capping pheromones and putative biosynthetic pathways in worker and drone larvae of honey bees *Apis mellifera*. *Apidologie*, 50, 793–803. Available from: https://doi.org/10.1007/s13592-019-00686-9

R Core Team. (2021) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raymann, K. & Moran, N.A. (2018) The role of the gut microbiome in health and disease of adult honey bee workers. *Current Opinion in Insect Science*, 26, 97–104. Available from: https://doi.org/10.1016/j.cois.2018.02.012

Robinson, F.A. & Nation, J.L. (1970) Long-chain fatty acids in honeybees in relation to sex, caste, and food during development. *Journal of Apicultural Research*, 9, 121–127. Available from: https://doi.org/10.1080/00218839.1970.11100258

Roelofs, W. & Bjostad, L. (1984) Biosynthesis of lepidopteran pheromones. *Bioorganic Chemistry*, 12, 279–298. Available from: https://doi.org/10.1016/0045-2068(84)90011-7

Santana, C.C., Barbosa, L.A., Basílio Júnior, I.D., do Nascimento, T.G., Dornelas, C.B. & Grillo, L.A.M. (2017) Lipase activity in the larval midgut of *Rhynchophorus palmarum*: biochemical characterization and the effects of reducing agents. *Insects*, 8, 8. Available from: https://doi.org/10.3390/insects8030100

Schmickl, T. & Crailsheim, K. (2002) How honeybees (*Apis mellifera* L.) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. *Behavioral Ecology and Sociobiology*, 51, 415–425. Available from: https://doi.org/10.1007/s00265-002-0457-3

Schmickl, T. & Crailsheim, K. (2004) Inner nest homeostasis in a changing environment with special emphasis on honey bee brood nursing and pollen supply. *Apidologie*, 35, 249–263. Available from: https://doi.org/10.1051/APIDO:2004019

Schmidt, J.O. & Hayden, C. (2011) Feeding preferences of *Apis mellifera* L. (Hymenoptera: Apidae): individual versus mixed pollen species. *Journal of the Kansas Entomological Society*, 572, 323–327.

Schmittgen, T.D. & Livak, K.J. (2008) Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, 3, 1101–1108. Available from: https://doi.org/10.1038/nprot.2008.73

Schulz, D.J., Vermiglio, M.J., Huang, Z.Y. & Robinson, G.E. (2002) Effects of colony food shortage on social interactions in honey bee colonies. *Insectes Sociaux*, 49, 50–55. Available from: https://doi.org/10.1007/s00040-002-8279-x

Seeley, T.D. (2009) *The wisdom of the hive: the social physiology of honey bee colonies*, Vol. 40. Cambridge, MA: Harvard University Press, pp. 304–306. Available from: https://doi.org/10.1353/pbm.1997.0016

Sivapalan, P. & Gnanapragasam, N.C. (1979) The influence of linoleic acid and linolenic acid on adult moth emergence of Homona coffearia from meridic diets in vitro. *Journal of Insect Physiology*, 25, 393–395. Available from: https://doi.org/10.1016/0022-1910(79)90005-2

Skowronek, P., Wójcik, Ł. & Strachecka, A. (2021) Fat body—multifunctional insect tissue. *Insects*, 12, 547. Available from: https://doi.org/10.3390/insects12060547

Somerville, D. (2000) *Honey bee nutrition and supplementary feeding*. Orange, Australia: Agnote 481/53, NSW Agriculture, pp. 1–8.

Sperandio, G., Simonetto, A., Carnesecchi, E., Costa, C., Hatjina, F., Tosi, S. et al. (2019) Beekeeping and honey bee colony health: a review and conceptualization of beekeeping management practices implemented in Europe. *Science of the Total Environment*, 696, 133795. Available from: https://doi.org/10.1016/J.SCITOTENV.2019.133795

Stabler, D., Al-Esawy, M., Chennells, J.A., Perri, G., Robinson, A. & Wright, G.A. (2021) Regulation of dietary intake of protein and lipid by nurse-age adult worker honeybees. *The Journal of Experimental Biology*, 224, 1–9. Available from: https://doi.org/10.1242/jeb.230615

Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, G.J. & de Renobales, M. (1988) Fatty acids in insects: composition, metabolism, and biological significance. *Archives of Insect Biochemistry and Physiology*, 9, 1–33. Available from: https://doi.org/10.1002/ARCH.940090102

Suarez, R.K., Darveau, C.A., Welch, K.C., O'Brien, D.M., Roubik, D.W. & Hochachka, P.W. (2005) Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *The Journal of Experimental Biology*, 208, 3573–3579. Available from: https://doi.org/10.1242/jeb.01775

Tautz, J., Maier, S., Groh, C., Rössler, W. & Brockmann, A. (2003) Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 7343–7347. Available from: https://doi.org/10.1073/pnas.1232346100

Toth, A.L., Kantarovich, S., Meisel, A.F. & Robinson, G.E. (2005) Nutritional status influences socially regulated foraging ontogeny in honey bees. *The Journal of Experimental Biology*, 208, 4641–4649. Available from: https://doi.org/10.1242/jeb.01956

Toth, A.L. & Robinson, G.E. (2005) Worker nutrition and division of labour in honeybees. *Animal Behaviour*, 69, 427–435. Available from: https://doi.org/10.1016/j.anbehav.2004.03.017

van der Horst, D.J., van Hoof, D., van Marrewijk, W.J.A. & Rodenburg, K.W. (2002) Alternative lipid mobilization: the insect shuttle system. *Molecular and Cellular Biochemistry*, 239, 113–119. Available from: https://doi.org/10.1023/A:1020541010547

van Kuiken, B.A. & Behnke, W.D. (1994) The activation of porcine pancreatic lipase by cis-unsaturated fatty acids. *Biochimica et Biophysica Acta (BBA) – Lipids and Lipid Metabolism*, 1214, 148–160. Available from: https://doi.org/10.1016/0005-2760(94)90039-6

Vernier, C.L., Krupp, J.J., Marcus, K., Hefetz, A., Levine, J.D. & Ben-Shahar, Y. (2019) The cuticular hydrocarbon profiles of honey bee workers develop via a socially-modulated innate process. *eLife*, 8, 1–27. Available from: https://doi.org/10.7554/eLife.41855

Vukašinović, E.L., Pond, D.W., Worland, M.R., Kojić, D., Purać, J., Blagojević, D.P. et al. (2013) Diapause induces changes in the

composition and biophysical properties of lipids in larvae of the European corn borer, Ostrinia nubilalis (lepidoptera: Crambidae). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 165, 219–225. Available from: https://doi.org/10.1016/j.cbpb.2013.05.001

Vyshchur, V.Y., Gutyj, B.V., Nyschemenko, N.P., Kushnir, I.M., Salata, V.Z., Tarasenko, L.O. et al. (2019) Effect of industry on the content of fatty acids in the tissues of the honey-bee head. *Ukrainian Journal of Ecology*, 9, 174–179. Available from: https://doi.org/10.15421/2019_76

Wang, J., Jin, H., Schlenke, T., Yang, Y., Wang, F., Yao, H. et al. (2020) Lipidomics reveals how the endoparasitoid wasp Pteromalus puparum manipulates host energy stores for its young. *Biochimica et Biophysica Acta – Molecular and Cell Biology of Lipids*, 1865, 158736. Available from: https://doi.org/10.1016/j.bbalip.2020.158736

Wang, Y., Brent, C.S., Fennern, E. & Amdam, G.V. (2012) Gustatory perception and fat body energy metabolism are jointly affected by vitellogenin and juvenile hormone in honey bees. *PLoS Genetics*, 8, 8. Available from: https://doi.org/10.1371/JOURNAL.PGEN.1002779

Wieczorek, P., Frąckowiak, P. & Obrępalska-Stęplowska, A. (2020) Evaluation of the expression stability of reference genes in *Apis mellifera* under pyrethroid treatment. *Scientific Reports*, 10, 1–15. Available from: https://doi.org/10.1038/s41598-020-73125-w

Wright, G.A., Nicolson, S.W. & Shafir, S. (2018) Nutritional physiology and ecology of honey bees. *Annual Review of Entomology*, 63, 327–344. Available from: https://doi.org/10.1146/annurev-ento-020117-043423

Zalewski, K., Zaobidna, E. & Zóltowska, K. (2016) Fatty acid composition of the parasitic mite varroa destructor and its host the worker prepupae of *Apis mellifera*. *Physiological Entomology*, 41, 31–37. Available from: https://doi.org/10.1111/phen.12121

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Principal component analyses (PCA) plot of laboratory batch effect from raw data of lipidomic samples. Grey dots are samples from batch 1 and blue dots are samples from batch 2.

Figure S2. Total abundance of lipids identified by MS fragmentation pattern matched by treatment and time. The line plots show the sum of the added abundance of lipids changing over time with standard errors ($n = 3$–6 samples per week).

Figure S3. Heatmap representation of each treatment hierarchical clustering, using Pearson correlation in Perseus software. (a) the starving treatment separated into 4 clusters, (b) sugar treatment into 3 clusters and (c) sugar + pollen lipids. The number of clusters is indicated by different colours in the dendrogram.

Table S1. Primers sequences and information used for quantitative real-time PCR.

Table S2. Analyses of deviance for hive health indicators according to treatment, time and their interaction.

Table S3. LSM comparisons with FDR adjustment of weight. Contrast of week 0 relative to further weeks.

Table S4. LSM comparisons with FDR adjustment of capped brood cells. Contrast of week 0 relative to further weeks.

Table S5. LSM comparisons with FDR adjustment of hive temperature. Contrast of week 0 relative to further weeks.

Table S6. Analyses of deviance from generalised mixed effects linear models of each treatment over time.

Table S7. LSM comparations with FDR adjustment of fatty acids significantly changing in abundance in generalised mixed effects linear models (deviance, $p < 0.05$) of each treatment over time. Contrasts of different weeks relative to week 1. SE, standard error.

Table S8. Lipids identified by MS fragmentation pattern matches in the software MS-Dial. Data from left to right include alignment ID of each compound (column A); the name of each lipid (column B); the fitted expression estimates of the interaction treatment and time for each lipid obtained from generalised mixed effects linear models (columns C to X); the raw values obtained from MS-Dial software (columns Y to GK) and different parameters obtained from the software MS-Dial (columns GL to HP) S: Starving treatment, SF: Sugar feeding treatment, SP: Sugar + pollen feeding treatment, T: Time (sample week).

Table S9. LSM comparations with FDR adjustment of lipids significantly changing in abundance in the starving treatment. SE: standard error.

Table S10. LSM comparations with FDR adjustment of lipids significantly changing in abundance in the sugar treatment. SE: standard error.

Table S11. LSM comparations with FDR adjustment of significant lipids significantly changing in abundance in the sugar + pollen treatment. SE: standard error.

Table S13. LSM comparisons with FDR adjustment of desaturase genes expression of lipids significantly changing in abundance over time. SE: standard error.

Table S12. LSM comparisons with FDR adjustment of lipids grouped according to unsaturation increases over time in the staving treatment, relative to week 1.

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