Nutrigenetic comparison of two *Varroa*-resistant honey bee stocks fed pollen and spirulina microalgae

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Abstract – We tested the influence of genetic variation on responses to natural and artificial diets in *Varroa*-resistant Pol-line and Russian honey bee stocks. Newly emerged workers from six colonies per stock were fed pollen, spirulina (blue-green microalgae), and sucrose-only diets in 144 total cages. Diet type had a strong effect on sugar intake, body weight, fat body lipid content, and *vitellogenin* (*vg*) expression. Spirulina consumption was approximately half that of pollen, but led to higher head weights, equivalent thorax weights and *vg* levels, and marginally reduced fat body lipids. Bee stock and colony had a significant impact on nutritional response. Despite equivalent diet intakes, Pol-line bees accumulated higher lipid levels and consumed less sugar overall than Russian bees. Furthermore, pollen-fed bees sourced from Pol-line colonies had significantly higher *vg* levels. These differences in nutrient and energy allocation may reflect life history-related physiological tradeoffs. Our results suggest that genotype-dependent nutritional responses are present in honey bees, with promising implications for breeding efforts and tailored approaches to diet and health in a changing global climate.

Apis mellifera / nutritional genetics, / breeding, / lipids, / vitellogenin

1. INTRODUCTION

Nutritional health is determined by interactions between environmental variables of supply, bio-availability, and consumption of diet components as well as the genetically controlled variables of digestion, absorption, transformation, and storage. Technical advances have accelerated the study of nutrition at the molecular level and inherited traits at the DNA level. This research has led to investigation of the roles of nutrients in gene and protein expression known as nutrigenomics, and concepts related to genetic variation and dietary response, known as nutrigenetics (e.g., different individual responses to the same diet leading to different levels of physiological biomarkers due to genetic variation, Simopoulos 2010). Nutrigenomics/

nutrigenetics has been successfully applied to increase growth rates and productivity in livestock (Scholtz et al. 2013; Benitez et al. 2017), and could provide a framework for breeding improved nutrient efficiency as well as identification of genotype-dependent supplements that promote health (Trujillo et al. 2006; Ferguson 2009; Tallentire et al. 2016). The honey bee is a promising model for such studies since its nutritional requirements are well characterized and a variety of genomic, transcriptomic, and proteomic resources are available (Alaux et al. 2011; Chakrabarti et al. 2019; Rutter et al. 2019; Ricigliano et al. 2020).

Floral nectar serves as an energy source while pollen provides all amino acids, lipids, and micronutrients necessary for honey bee development and reproduction (Brodschneider and Crailsheim 2010). The protein content of pollen and its relative abundances of essential amino acids are central to brood production and colony growth (De Groot 1953; Crailsheim 1990). Pollen

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also contains essential lipids (Ischebeck 2016) that are incorporated into abdominal stores associated with reproductive potential (Wegener et al. 2018; Martin et al. 2019). To supplement colony nutrition during periods of reduced natural forage, managed honey bee colonies are often fed artificial pollen substitute diets (Nabors 2000). This management practice has become more prevalent as commercial beekeeping has grown in scale, and landscapes increase in agricultural intensity, which reduces floral diversity and consequent nutritional value (Naug 2009). Artificial bee diets typically incorporate proteins derived from plant (e.g., soy, corn, or wheat), animal (e.g., milk or egg), and microbial sources (e.g., brewer's yeast). Additionally, microalgae have recently emerged as a promising pollen substitute due to their high macronutrient content and bioavailability (Ricigliano 2020).

A variety of bee responses to pollen and artificial diets have been described, but comparatively fewer studies have focused on molecular impacts of nutrition. For example, pollen and microalgae diets broadly upregulate metabolic processes associated with amino acids, lipids, and carbohydrates (Azzouz-Olden et al. 2018; DeGrandi-Hoffman et al. 2018; Ricigliano et al. 2020). These diets particularly upregulate protein expression associated with longevity and stress response, including *vitellogenin*, a multifunctional lipoprotein involved in nutritional storage, brood production, stress response, and overwintering (Amdam et al. 2003; Amdam et al. 2005; Smart et al. 2016). *Vitellogenin* titers are positively linked to abdominal lipid content and to the timing and quantity of diet consumption (Wegener et al. 2018). Therefore, *vitellogenin* expression at the mRNA and protein levels can be considered a biomarker of honey bee nutritional status (Alaux et al. 2011;).

Despite a sizable body of research pertaining to honey bee nutrition, relatively little is known about the effects of genetic variation on nutritional response. A number of honey bee stocks (isolated populations bred for specific traits) are produced by queen breeders and distributed throughout the US (Delaney et al. 2009; Saelao et al. 2020). Some of these stocks have been developed for specialized traits (e.g; mite resistance) or are the

product of research-based breeding programs (Page and Fondrk 1995; Spivak and Reuter 2001; Rinderer et al. 1997; Danka et al. 2016). Understanding honey bee genotype-dependent responses to nutrition could enable marker-assisted breeding for improved health outcomes or the development of tailored feed supplements. The effects of diet have been studied in other livestock animals with reference to effective breeding and their influence on production traits and health (Nowacka-Woszek 2020). The objective of this study was to test the effects of honey bee genetic variation on responses to natural and artificial diets. Two *Varroa*-resistant honey bee stocks, Pol-line and Russian, were selected for this study based on their closed breeding populations and genomic distinction. Using cage bioassays, nutritional response variables were measured in young worker bees fed bee-collected pollen and an artificial microalgae (spirulina)-based diet.

2. MATERIALS AND METHODS

2.1. Honey bees and experimental design

Experiments were conducted at the USDA-ARS honey bee lab in Baton Rouge, LA, USA. Six healthy colonies each of Pol-line and Russian honey bee stocks were selected. Newly emerged workers (<24 h old) were obtained by incubating sealed brood combs overnight at 35 °C and 50% RH. Bees from each colony were collected into separate containers and then assigned to diet treatment cages (50 bees per cage and 144 total cages, see Figure 1 for experimental design schematic). Bees were collected from each cage at day 7 for nutritional response measures. Dead bees were counted and removed from the cages daily. Mortality was less than 1% overall during the 7-day trial, so mortality was negligible.

2.2. Diet preparation and feeding assay

All cages were provided ad libitum access to drip feeders containing 50% (w/v) sucrose solution. Cages were fed ad libitum diets of bee-collected pollen, spirulina microalgae, or sucrose only. Pollen and spirulina diets were mixed into a paste with 50% sucrose (w/v) syrup containing

Figure 1. Schematic overview of the experimental design used in this study. Newly emerged workers from six colonies each of Pol-line and Russian honey bee stocks were used in cage feeding bioassays to test the effects of genetic variation on nutritional response.

5% glycerol (v/v; to improve pliability) and loaded into modified 1.7-mL microcentrifuge tubes (see Figure 1 for cage setup schematic), then stored at -20 °C before use. For the pollen diet, mixed corbicular pollen pellets were collected using entrance-mounted pollen traps in a USDA-ARS apiary in Baton Rouge and immediately frozen until ready for use (Mogren et al. 2018). Spirulina diet contained organic, dried spirulina (*Arthrospira platensis*) powder (Microingredients, California, USA). Approximately 1.5g of diet paste was provided to each cage. The amount of diet consumed by each cage was recorded on day 3, then the diet was refreshed with ~1.5g of new diet paste and consumption was measured again at day 7. As a control, diet samples were placed in cages without bees and weight loss was measured to determine the evaporation rate for each diet type. Diet consumption in each cage was adjusted for daily moisture loss and recalculated to give the total diet consumed over the 7 day period per bee. Sugar consumption was recorded and expressed as the total amount consumed over 7 days per bee for each cage.

2.3. Nutritional physiology measures

Bees were dissected into head, thorax (excluding legs and wings), and abdomens with guts removed. Then, dissected parts were collected into pools of 8. Average head and thorax weights per bee for each cage were determined by drying to a constant weight (60 °C for 48h) and recording to the nearest 0.1 mg. Average abdomen lipid content per bee for each cage was determined by homogenizing pooled abdomens in 800µl of TE buffer, centrifugation, then extracting 300µl of supernatant and combining it with 300µl of 2:1 of chloroform:methanol. Abdomen samples from pollen- and spirulina-fed bees required a further 1:1 dilution with chloroform. Samples were centrifuged and 250µl of the chloroform layer was added to glass tubes and incubated at 95°C to evaporate the chloroform. Next, 300µl of concentrated sulfuric acid (98%) was added and the sample was incubated for 20 min. Then, 100ul of sample was transferred in technical duplicates to a microplate. The remaining steps were carried out according to Cheng et al. 2011. Optical

reads of the lipid samples were compared with those measured for a canola oil standard ran on each plate. A standard curve was generated based on the average of standards ran on all plates and was used to calculate the lipid content of the samples. The R^2 value for the lipid standard curve was 0.99 (Online resource 1 Figure S1).

2.4. *Vitellogenin* (*vg*) and *vg-like-A* expression

For gene expression analyses, flash frozen pools of 8 bee abdomens per cage were homogenized in 700 μ l of Maxwell® simplyRNA homogenization solution (Promega), centrifuged, and 400 μ l of the supernatant was transferred to Maxwell® RSC-48 cartridges (Promega). RNA extractions proceeded according to the Maxwell® RSC simplyRNA tissue kit protocol (Promega).

cDNA synthesis was carried out using 100 ng of DNAase (New England Biolabs)-treated RNA and QuantiTect Reverse Transcription Kits (Qiagen) according to the manufacturer's protocol. Quantitative PCR (qPCR) was carried out in triplicate using previously published primer pairs and cycling conditions to quantify expression levels of *vitellogenin* (*vg*) and its homologue *vg-like-A* (Salmela et al. 2016) using β -actin and RP-49 for normalization (Lourenço et al. 2008).

2.5. Statistical analyses

The effects of bee stock (Pol-line, Russian), colony (Pol-line 1-6, Russian 1-6), and diet type (sucrose only, pollen, spirulina) were evaluated using mixed-model ANOVA with post hoc contrasts of least squares mean differences as required (Figures 2, 3, 4 and 5). Dependent variables were evaluated for normality using fit statistics and probability plots. Variables with deviations from normality were re-evaluated after log transformation.

Secondary analyses were performed within groups of either pollen- or spirulina-fed bees to test the effects of bee stock and colony using nested ANOVA (Tables I, II, III and IV). Analyses were conducted in JMP v11 and Prism v7.

3. RESULTS

3.1. Diet and sugar consumption

Diet consumption was influenced by diet type ($F_{1, 92} = 218.5$, $P < 0.0001$) but not by stock or stock*diet interaction. Average consumption was significantly higher for the pollen diet (Pol-line: 41.60 mg/bee; Russian: 43.83 mg/bee) relative to the spirulina diet (Pol-line: 22.53 mg/bee; Russian: 24.14 mg/bee) (Figure 2).

Among pollen-fed bees, diet consumption was influenced by colony ($F_{10, 36} = 218.5$, $P = 0.0024$) (Table I) but not by stock. Among spirulina-fed bees, stock and colony did not significantly impact diet consumption (Table I).

Sugar consumption was higher in Russian bees ($F_{1, 142} = 16.3$, $P = 0.0024$) and was influenced by diet ($F_{2, 141} = 200.9$, $P < 0.0001$) but not by stock*diet interaction (Figure 2). Ranked by diet type, sugar consumption was as follows: sucrose (103.09 mg/bee) > spirulina (82.81 mg/bee) > pollen (63.54 mg/bee) (Figure 2).

Among pollen-fed bees, sugar consumption was higher in Russian stock ($F_{1, 46} = 34.9$, $P < 0.0001$) and was influenced by colony ($F_{10, 36} = 4.3$, $P = 0.0005$) (Table I). Among spirulina-fed bees, sugar consumption was also higher in Russian stock ($F_{1, 46} = 23.3$, $P < 0.0001$), but was not influenced by colony (Table I).

3.2. Head and thorax weight

Head weight was influenced by diet type ($F_{2, 138} = 99.3$, $P < 0.0001$) but not by stock or stock*diet interaction, although newly emerged Pol-line bees had higher head weights than Russian bees (Figure 3, Online resource 1 Figure S2). Average head weight was highest in spirulina-fed bees (Pol-line: 3.03 mg/bee DW; Russian: 2.91 mg/bee DW), followed by pollen-fed bees (Pol-line: 2.88 mg/bee DW; Russian: 2.73 mg/bee DW), followed by sucrose-fed bees (Pol-line: 2.37 mg/bee DW; Russian: 2.18 mg/bee DW) (Figure 3).

Among pollen-fed bees, head weight was higher in Pol-line stock ($F_{1, 46} = 7.8$, $P = 0.0084$) and was influenced by colony ($F_{10, 36} = 2.9$, $P = 0.0085$) (Table II). Among spirulina-

Figure 2. Ad libitum diet and sugar consumption by the two honey bee stocks after 7 days. Plot whiskers show minimum and maximum values with the lower and upper edge of each box denoting the 25th to 75th percentiles, median as a horizontal bar, and “+” as the mean. Main effects determined by mixed model ANOVA are indicated in red.

fed bees, head weight was influenced by colony ($F_{10, 35} = 2.5$, $P = 0.0208$) but not by stock (Table II). There was a significant, positive correlation between head weight and diet consumption in Pol-line but not Russian stock (Online resource 1 Figure S3).

Thorax weight was influenced by diet type ($F_{2, 138} = 71.3$, $P < 0.0001$), but not by stock or by stock*diet interaction (Figure 3). Newly emerged Pol-line bees had higher thorax weights than Russian bees (Online resource 1 Figure S4). Average

thorax weight was not significantly different between bees fed pollen (Pol-line: 9.73 mg/bee DW; Russian: 9.23 mg/bee DW) and spirulina (Pol-line: 9.85 mg/bee DW; Russian: 9.33 mg/bee DW) but was lowest in sucrose-fed bees (Pol-line: 8.93 mg/bee DW; Russian: 8.09 mg/bee DW).

Among pollen-fed bees, thorax weight was higher in Pol-line stock ($F_{1, 46} = 24.1$, $P = 0.0018$) and was influenced by colony ($F_{10, 36} = 2.5$, $P = 0.0207$) (Table II). A significant, positive correlation was determined

Figure 3. Head and thorax weights of the two honey bee stocks after 7 days of diet consumption. Plot whiskers show minimum and maximum values with the lower and upper edge of each box denoting the 25th to 75th percentiles, median as a horizontal bar, and “+” as the mean. Main effects determined by mixed model ANOVA are indicated in red.

between thorax weight and pollen consumption in Russian but not Pol-line stock (Online resource 1 Figure S5). Among spirulina-fed bees, thorax weight was higher in Pol-line stock ($F_{1, 46} = 11.4$, $P < 0.0001$) and was influenced by colony ($F_{10, 36} = 3.5$, $P = 0.0030$) (Table II).

3.3. Abdominal lipid content

Abdomen lipid content was higher in Pol-line bees ($F_{1, 139} = 8.7$, $P = 0.0144$) and was influenced by diet ($F_{2, 138} = 44.8$, $P = 0.0144$) but not stock*diet interaction (Figure 4). Ranked by diet, average lipid content was as follows: pollen-fed bees (591.48 $\mu\text{g}/\text{bee}$) > spirulina-fed bees (511.79 $\mu\text{g}/\text{bee}$) > sucrose-fed bees (310.21 $\mu\text{g}/\text{bee}$).

Figure 4. Abdominal lipid accumulation in the two honey bee stocks after 7 days of diet consumption. Plot whiskers show minimum and maximum values with the lower and upper edge of each box denoting the 25th to 75th percentiles, median as a horizontal bar, and “+” as the mean. Main effects determined by mixed model ANOVA are indicated in red.

Among pollen-fed bees, lipid content was higher in Pol-line stock ($F_{1, 46} = 25.5$, $P < 0.0001$) and was influenced by colony ($F_{10, 36} = 4.3$, $P = 0.0006$) (Table III). Among spirulina-fed bees, lipid content was also higher in Pol-line stock ($F_{1, 46} = 12.1$, $P = 0.0014$) and influenced by colony ($F_{10, 36} = 6.3$, $P < 0.0001$) (Table III).

3.4. Vitellogenin (vg) and vg-like-A expression

Vitellogenin (vg) expression was influenced by diet ($F_{2, 139} = 179.9$, $P < 0.0001$) but not by stock or stock*diet interaction (Figure 5). Vg expression levels were not significantly different between bees fed pollen and spirulina, but were lowest in sucrose-fed bees.

Among pollen-fed bees, vg expression was higher in Pol-line stock ($F_{1, 46} = 7.6$, $P < 0.0095$) and was influenced by colony ($F_{10, 35} = 7.9$, $P < 0.0001$) (Table IV). A significant positive correlation was determined between diet consumption and vg expression in Pol-line stock, and trended toward significance in Russian stock (Online resource 1 Figure S6). Among spirulina-

fed bees, vg levels were influenced by colony ($F_{10, 35} = 3.0$, $P = 0.0071$) but not stock (Table IV).

Vg-like-A expression was influenced by diet ($F_{2, 140} = 68.9$, $P < 0.0001$) but not by stock or stock*diet interaction (Figure 5). *Vg-like-A* levels in bees fed pollen and spirulina were not different from each other, but were significantly higher than sucrose-fed bees (Figure 5).

Among pollen-fed bees, *vg-like-A* expression was higher in Russian stock ($F_{1, 46} = 4.6$, $P = 0.0398$) and was influenced by colony ($F_{10, 35} = 7.4$, $P < 0.0001$) (Table IV). Among spirulina-fed bees, *vg-like-A* expression was not influenced by stock or colony (Table IV).

4. DISCUSSION

This study compared nutritional responses of two honey bee stocks fed pollen and spirulina, a microalgae-based artificial feed. The Pol-line stock was developed by USDA-ARS via introgression of the *Varroa* Sensitive Hygiene trait (Harbo and Harris 2005) into Italian bees managed by several US commercial beekeepers (Danka et al. 2016). The Russian honey bee

Figure 5. Relative mRNA expression levels of *vitellogenin* (*vg*) and its homologue *vg-like-a* in the two honey bee stocks after 7 days of diet consumption. Plot whiskers show minimum and maximum values with the lower and upper edge of each box denoting the 25th to 75th percentiles, median as a horizontal bar, and “+” as the mean. Main effects determined by mixed model ANOVA are indicated in red.

breeding program was established by USDA-ARS via importation of queens from eastern Russia for the purpose of genetic improvement of *Varroa* resistance (Rinderer et al. 1997). Pol-line and Russian stocks were chosen for this study based on closed breeding programs and recent evidence confirming their genomic distinction (Saelao et al. 2020). We identified genotype-dependent nutritional responses, and these findings warrant further investigation of additional

honey bee populations at individual and colony levels. Genetic and environmental factors influence nutrient and energy allocation for specific physiological functions at different life stages of homometabolous insects (Nestel et al. 2016). A range of metabolic regulatory mechanisms can affect all developmental stages, and ultimately fitness (Kaspi et al. 2002). Organization of the honey bee colony is often referred to as a super-organism, in which workers perform all functions

Table I. Average diet and sugar consumption by newly emerged workers from Pol-line and Russian colonies after 7 days

Colony	Feed consumption (mg/bee $\pm$ SE)					Sugar consumption (mg/bee $\pm$ SE)			
	Pollen-fed		Spirulina-fed			Pollen-fed		Spirulina-fed	
Pol-1	44.54 $\pm$ 3.37	abc	26.27 $\pm$ 0.98	a		56.20 $\pm$ 1.46	bc	73.58 $\pm$ 2.06	ab
Pol-2	48.17 $\pm$ 2.25	a	25.75 $\pm$ 2.06	a		52.88 $\pm$ 2.76	c	71.93 $\pm$ 3.54	b
Pol-3	35.14 $\pm$ 1.05	c	19.48 $\pm$ 1.66	a		59.84 $\pm$ 3.36	bc	70.59 $\pm$ 2.57	b
Pol-4	44.40 $\pm$ 2.11	abc	23.80 $\pm$ 2.94	a		52.46 $\pm$ 8.11	c	80.47 $\pm$ 4.49	ab
Pol-5	36.05 $\pm$ 1.61	bc	19.35 $\pm$ 1.35	a		60.53 $\pm$ 5.31	bc	74.30 $\pm$ 6.64	ab
Pol-6	41.27 $\pm$ 1.55	abc	23.07 $\pm$ 1.54	a		58.63 $\pm$ 3.29	bc	75.48 $\pm$ 4.17	ab
Rus-1	47.08 $\pm$ 1.55	ab	22.08 $\pm$ 4.75	a		71.93 $\pm$ 0.83	abc	94.52 $\pm$ 9.95	ab
Rus-2	45.35 $\pm$ 2.33	abc	19.36 $\pm$ 2.18	a		60.94 $\pm$ 3.15	bc	86.55 $\pm$ 9.43	ab
Rus-3	45.97 $\pm$ 0.57	abc	24.62 $\pm$ 1.23	a		60.62 $\pm$ 1.37	bc	86.47 $\pm$ 2.78	ab
Rus-4	42.80 $\pm$ 0.53	abc	27.01 $\pm$ 2.40	a		64.84 $\pm$ 1.78	bc	83.69 $\pm$ 2.51	ab
Rus-5	37.88 $\pm$ 5.19	abc	26.90 $\pm$ 1.14	a		73.40 $\pm$ 2.17	ab	94.15 $\pm$ 7.18	ab
Rus-6	43.95 $\pm$ 0.91	abc	20.78 $\pm$ 2.18	a		90.20 $\pm$ 6.56	a	102.1 $\pm$ 8.93	a

Values within a column which have no letters in common are significantly different at $\alpha = 0.05$

related to colony maintenance as they transition through different life history stages (Hölldobler and Wilson 2009). The superorganismal nature of a bee colony is particularly apparent in the context of nutrition. Young adult workers (nurse

bees, <14 days old) play a central role since this age cohort of individuals assimilates, stores, and redistributes pollen-derived nutrition (Crailsheim et al. 1992). As such, we focused on nutritional responses of newly emerged workers after 7 days,

Table II. Average head and thorax weights of newly emerged workers from Pol-line and Russian colonies after 7 days of pollen and spirulina consumption

Colony	Head weight (mg/bee DW $\pm$ SE)					Thorax weight (mg/bee DW $\pm$ SE)			
	Pollen-fed		Spirulina-fed			Pollen-fed		Spirulina-fed	
Pol-1	2.78 $\pm$ 0.15	ab	3.05 $\pm$ 0.07	a		9.18 $\pm$ 0.05	bc	9.24 $\pm$ 0.11	bc
Pol-2	2.90 $\pm$ 0.13	ab	2.95 $\pm$ 0.08	a		9.66 $\pm$ 0.26	abc	9.42 $\pm$ 0.14	abc
Pol-3	2.65 $\pm$ 0.06	ab	2.88 $\pm$ 0.17	a		9.82 $\pm$ 0.12	abc	9.49 $\pm$ 0.20	abc
Pol-4	3.18 $\pm$ 0.15	a	2.98 $\pm$ 0.20	a		10.2 $\pm$ 0.29	ab	9.75 $\pm$ 0.15	abc
Pol-5	2.73 $\pm$ 0.08	ab	2.98 $\pm$ 0.08	a		9.85 $\pm$ 0.19	abc	10.1 $\pm$ 0.36	ab
Pol-6	3.09 $\pm$ 0.09	a	3.35 $\pm$ 0.18	a		10.4 $\pm$ 0.26	a	10.4 $\pm$ 0.21	a
Rus-1	2.98 $\pm$ 0.08	ab	3.31 $\pm$ 0.14	a		9.47 $\pm$ 0.15	abc	9.60 $\pm$ 0.23	abc
Rus-2	2.68 $\pm$ 0.11	ab	2.67 $\pm$ 0.18	a		9.04 $\pm$ 0.27	c	8.98 $\pm$ 0.26	c
Rus-3	2.72 $\pm$ 0.07	ab	3.08 $\pm$ 0.16	a		9.33 $\pm$ 0.31	abc	9.24 $\pm$ 0.14	bc
Rus-4	2.64 $\pm$ 0.05	ab	2.69 $\pm$ 0.07	a		9.06 $\pm$ 0.24	c	9.13 $\pm$ 0.08	bc
Rus-5	2.76 $\pm$ 0.06	ab	2.93 $\pm$ 0.09	a		9.55 $\pm$ 0.15	abc	9.87 $\pm$ 0.31	abc
Rus-6	2.52 $\pm$ 0.09	b	2.70 $\pm$ 0.13	a		8.87 $\pm$ 0.18	c	9.02 $\pm$ 0.22	bc

Values within a column which have no letters in common are significantly different at $\alpha = 0.05$

Table III. Average abdomen lipid accumulation in newly emerged workers from Pol-line and Russian colonies after 7 days of pollen and spirulina consumption

Colony	Abdomen lipids (µg/bee ± SE)				
	Pollen-fed			Spirulina-fed	
Pol-1	515.3 ± 29.1	bcde		502.5 ± 46.1	abc
Pol-2	671.0 ± 21.6	abc		571.9 ± 28.4	ab
Pol-3	729.9 ± 16.0	a		503.8 ± 44.0	abc
Pol-4	629.6 ± 26.5	abcde		653.7 ± 38.0	a
Pol-5	640.7 ± 63.1	abcd		531.0 ± 41.5	abc
Pol-6	695.2 ± 17.2	ab		560.5 ± 18.9	ab
Rus-1	659.6 ± 62.8	abc		548.0 ± 28.7	ab
Rus-2	449.2 ± 28.7	e		330.7 ± 51.7	c
Rus-3	613.3 ± 29.1	abcde		372.4 ± 48.9	bc
Rus-4	509.7 ± 56.6	cde		490.8 ± 67.0	abc
Rus-5	537.8 ± 39.9	bcde		391.6 ± 49.1	bc
Rus-6	447.9 ± 31.6	de		686.9 ± 13.8	a

Values within a column which have no letters in common are significantly different at $\alpha = 0.05$

which approximates nurse bee physiology in a colony. While this study is based on experiments with laboratory-reared bees and as such provides some insight into effects on workers, the findings

warrant further study on the impacts of genetic variation on nutritional response at the colony level.

Pollen intake was approximately twice as high as spirulina in both stocks. This was consistent with reduced spirulina consumption relative to pollen and pollen substitute diets observed before (Ricigliano and Simone-Finstrom 2020). Ad libitum sugar consumption was highest in bees fed only sucrose and lowest in pollen-fed bees. Honey bees exclusively use aerobic carbohydrate catabolism for energy, which is well-characterized during flight (Beenakkers 1969; Harrison and Roberts 2000), and also during walking and resting (Rothe and Nachtigall 1989). Overall sugar consumption by Russian bees was higher than Pol-line with no differences in feed (protein and lipid) intake. This result may be indicative of an increased basal metabolic rate. Environment and genetics influence flight metabolic rate (Harrison and Fewell 2002), which likely extends to activity and basal metabolism under laboratory conditions. Consistent with our findings, bee genotype influences the average number of daily flights, the total number of foraging flights, and total flight duration, in which Russian honey bees outperformed Italian honey bees (de Guzman et al. 2017).

Table IV. Average *vitellogenin* (*vg*) and *vg-like-A* expression in newly emerged workers from Pol-line and Russian colonies after 7 days of pollen and spirulina consumption

Colony	Relative <i>vg</i> expression (± SE)				Relative <i>vg-like-A</i> expression (± SE)			
	Pollen-fed		Spirulina-fed		Pollen-fed		Spirulina-fed	
Pol-1	4.73 ± 0.98	ab	0.51 ± 0.18	b	0.004 ± 0.001	bc	0.009 ± 0.004	a
Pol-2	5.36 ± 1.13	a	1.45 ± 0.25	ab	0.009 ± 0.003	a	0.032 ± 0.011	a
Pol-3	1.91 ± 0.11	bc	0.74 ± 0.33	ab	0.003 ± 0.001	c	0.001 ± 0.0003	a
Pol-4	2.52 ± 0.47	bc	1.76 ± 0.47	ab	0.006 ± 0.002	abc	0.012 ± 0.005	a
Pol-5	1.28 ± 0.38	c	2.51 ± 0.14	ab	0.011 ± 0.007	c	0.001 ± 0.0004	a
Pol-6	0.80 ± 0.09	c	2.80 ± 0.59	a	0.020 ± 0.010	c	0.001 ± 0.0003	a
Rus-1	1.94 ± 0.39	bc	1.84 ± 0.54	ab	0.015 ± 0.003	ab	0.031 ± 0.006	a
Rus-2	2.78 ± 0.43	bc	1.70 ± 0.36	ab	0.009 ± 0.006	abc	0.021 ± 0.006	a
Rus-3	1.76 ± 0.22	bc	1.76 ± 0.48	ab	0.005 ± 0.002	c	0.004 ± 0.001	a
Rus-4	1.02 ± 0.20	c	1.26 ± 0.22	ab	0.011 ± 0.002	c	0.005 ± 0.003	a
Rus-5	1.24 ± 0.18	c	2.74 ± 0.28	a	0.005 ± 0.002	c	0.002 ± 0.0003	a
Rus-6	1.21 ± 0.52	bc	2.22 ± 0.79	ab	0.011 ± 0.004	c	0.002 ± 0.001	a

Changes in head and thorax mass represent dietary protein assimilation into brood food-producing hypopharyngeal glands (Crailsheim 1990; DeGrandi-Hoffman et al. 2010) and flight muscles (Hendriksma et al. 2019), respectively. Despite reduced consumption, spirulina-fed bees from both stocks had higher head weights and equal thorax weights compared to pollen-fed bees. This is consistent with high protein bioavailability of spirulina diets determined in commercial Italian bee stock (Ricigliano and Simone-Finstrom 2020). Although newly emerged Pol-line bees had higher head and thorax weights than Russian bees, the stocks experienced equivalent fold increases in weight after consuming pollen and spirulina diets.

The abdominal fat body is the main site of insect nutrient storage and metabolic activities, especially protein synthesis and lipid metabolism (Li et al. 2019). In honey bees, the fat body is most developed in nurses, with decreases in lipid and *vitellogenin* levels shown to precede the transition to foraging (Amdam et al. 2003; Toth and Robinson 2005). Pollen consumption increases fat body lipid stores and polyunsaturated fatty acid (PUFA) abundance, which are positively linked to *vg* expression (Wegener et al. 2018). Pollen-fed bees had higher lipid and equivalent *vg* levels relative to spirulina-fed bees. Despite significantly reduced consumption, spirulina led to 88–90% of the pollen diet-induced lipid content in both stocks. This high bioavailability is consistent with a variety of functional lipids occurring in spirulina (Ricigliano and Simone-Finstrom 2020) including linoleic acid and alpha-linoleic acid, two PUFAs that are considered essential for bees (Avni et al. 2014; Arien et al. 2015). Overall, Pol-line accumulated more abdomen lipids and consumed less sugar than Russian bees. Further, Pol-line bees fed pollen had 1.8-fold higher *vg* levels than pollen-fed Russian bees. These differences may reflect life history-related physiological trade-offs between reproduction and flight capacity. Pol-line is derived from introgression of *Varroa* resistance into commercial Italian bee stock, which was selected for the favorable

management characteristics of colony size and honey production (Delaney et al. 2009). Reproductive potential is generally linked to nutritional stores in social Hymenoptera (Wheeler 1986) and so reduced lipid accumulation in Russian bees is consistent with their colonies tending to have smaller bee populations than Italian colonies (Danka et al. 2006).

The health implications of studying genotype-nutrition interactions has been realized in humans and other model animals over the past decade (Mathers 2017). Nutrigenomics applied to livestock agriculture can be effective in increasing growth rate, productivity, nutrient efficiency, disease resistance, and reproduction (Scholtz et al. 2013; Benitez et al. 2017). Since feed costs comprise a significant expense in animal production, nutrigenomics has potential to bridge the gap between genetic profile and nutrient conversion into augmented productivity. This is best exemplified by the poultry industry, where artificial selection for meatier broiler chickens has facilitated the rapid development of chickens with improved digestive efficiency, favorable body composition, and energy utilization for growth and metabolic activities (Tallentire et al. 2016). One example of how genotype-dependent variation in nutrient efficiency could impact honey bee health is differential capacities to convert available nutrition into *vitellogenin* or other protein and lipid constituents essential to colony- and individual-level processes. Furthermore, genetic variation may influence gut microbiome dynamics, which are implicated in nutrient assimilation and feeding behaviors (Ricigliano and Anderson 2020). There are potentially thousands of genetic polymorphisms that may result in variations in nutritional biochemistry influencing honey bee health. While honey bee nutrition is an area of significant research focus, the impact of genetic variation on nutritional response is largely unexplored. Future contrasts between genetic stocks may lead to the identification of metabolic and physiological phenotypes for breeding bees with improved nutrient efficiency and robustness as well as genotype-specific nutritional supplements.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

VAR conceived this research and designed the experiments. VAR, KEI, and STW performed the experiments and analyzed the data. VAR, KEI, and STW wrote the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

CODE AVAILABILITY

Not applicable

DECLARATIONS

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

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Comparaison nutriginétique de deux souches d'abeilles mellifères résistantes au *Varroa* nourries au pollen et à la microalgue spirulina.

Apis mellifera / génétique nutritionnelle / élevage / lipide / vitellogénine.

Nutrigenetischer Vergleich zweier *Varroa*-resistenter Honigbienenstämme, die mit Pollen und Spirulina-Mikroalgen gefüttert wurden.

Apis mellifera / Ernährungsgenetik / Zucht / Lipide / Vitellogenin.

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