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Evaluating late summer pollen substitutes on the growth and overwintering success of honey bee colonies and analyzing natural fall pollen nutrition in Nova Scotia, Canada

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ABSTRACT

The effect of feeding pollen substitutes in late summer to honey bee colonies (*Apis mellifera*) on colony growth and overwintering success was evaluated in Nova Scotia, Canada in 2018-2020. In 2018, 96 colonies were randomly assigned to 3 treatment feeding groups (Nutra Bee™, Ultra Bee™, or control) and in 2019, 96 colonies were randomly assigned to 2 treatment feeding groups (Ultra Bee™ or control). Treatment colonies received 3 pounds (lbs) of pollen substitute (or no pollen substitute for control colonies) over 3 feeding periods during the study, and colony growth was measured as seams of bees over 7 weeks in both years. Pollen traps were installed in a subset of colonies to gather pooled samples to determine which types and quality of pollen bees were collecting naturally in August and September. We found no significant difference in colony growth among treatment groups in 2018, and colonies receiving Ultra Bee™ had significantly less growth in 2019 compared to control colonies. There was no significant difference in overwintering success or spring strength. Goldenrod (Asteraceae) pollen was collected most frequently in both years, and the overall average crude protein from all pollen collected was 12.8%. We detected 19 amino acids in the pollen collected, including all 10 essential amino acids required for honey bee growth and development. Of the 10 required amino acids, only 4 were at adequate levels to support honey bee growth. Based on our study, there does not seem to be an economic or biological advantage to feeding honey bee colonies pollen substitute in the late summer under typical Maritime (New Brunswick, Nova Scotia, and Prince Edward Island) beekeeping conditions when there is an abundance of natural pollen available at this time.

INTRODUCTION

Proper nutrition, provided by pollen and nectar, is essential for the growth, development and survival of honey bee (*Apis mellifera*) colonies (Brodschneider and Crailsheim 2010). Honey bees require nectar as a carbohydrate source and pollen as a protein, amino acid, lipid, sugar, starch, vitamin, and mineral source to maintain hive function (Winston 1987; DeGrandi-Hoffman et al. 2008). The amount of protein colonies receive through pollen can impact bees physiologically and influence their survivability (Frias et al. 2016). Larvae reared under pollen-limited conditions may experience smaller weight gains, shorter lifespans, reduced foraging behaviour, and their ability to communicate effectively about food resources (e.g., through the waggle dance) may also be compromised (Scofield and Mattila 2015). In addition to protein, bees also need to consume 10 essential amino acids (De Groot 1953). Bees naturally access varying levels of protein and amino acids through pollen sources (Roulston et al. 2000), but not all pollen sources

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provide adequate nutrition for honey bee colonies in the form of crude protein and balanced amino acid profiles. The study region typically has abundant and diverse floral resources available from spring to fall, providing a diverse diet that may improve bee immunity (Alaux et al. 2010; Di Pasquale et al. 2013). However, with recent dry summers on record for the Maritime Provinces (New Brunswick, Nova Scotia, and Prince Edward Island) and changing land use, many apiary locations experience dearth conditions (shortage of floral resources to provide nectar and pollen). As a result, managing the nutritional requirements of honey bees is increasingly more challenging as beekeepers are concerned about adequate food resources for colonies as they prepare for overwintering.

Feeding honey bee colonies pollen substitute in late summer (August-September) is not a traditional practice in Canada for a variety of reasons, including additional cost and lack of research on the potential benefits. However, there may be biological advantages to this practice. Previous studies have demonstrated varying results of supplementing colonies with pollen in the late summer on bee population, overwintering survival, and subsequent spring success (Mattila and Otis 2007a,b; Martin 2009). These three studies fed pollen patties with real pollen, and Global™ pollen patties, respectively, whereas we were interested in comparing Nutra Bee™ and Ultra Bee™ pollen patties to control colonies fed no additional pollen.

Winter bees are produced in the colony in late summer (Mattila et al. 2001). These winter bees have longer lifespans, enhanced fat bodies, and well-developed hypopharyngeal glands (Winston 1987). Fat bodies are cells that store fat, protein, and glycogen, while hypopharyngeal glands are used to produce key elements of larval food (e.g., proteins, vitamins, and lipids) as well as the enzyme invertase to process nectar into honey (Winston 1987). In contrast, summer bees have shorter lifespans, and in our temperate climate, do not require the same physiological adaptations as winter bees to perform their duties (Winston 1987). Nutrition during winter bee production could impact colony growth in late summer and subsequent overwintering success. For instance, winter bees are the cohort present during winter brood rearing (from February until summer bee production and turnover in the spring) and rely on stored food reserves in the hive as well as reserves stored in their fat bodies to contribute to brood rearing (Schneider 2015). By ensuring suitable nutrition through feeding pollen substitute during winter bee production, beekeepers could potentially positively influence overwintering success and subsequent spring build up. This is particularly important for beekeepers in the Maritimes as many beekeepers prepare colonies for early season pollination (e.g., wild blueberry (*Vaccinium angustifolium*) in May-June). Canada's 5-year honey bee colony loss average was 23.3% from 2015-19 (Canadian Association of Professional Apiculturists 2019), and any management practices to improve overwintering success, such as feeding pollen substitute in the late summer, could directly impact the number of colonies and therefore beekeeping revenue.

The objectives of our study were to evaluate the effect of feeding pollen substitute to colonies in the late summer on colony growth in preparation for winter, and overwintering success and colony strength the following spring. We also sampled naturally collected pollen (i.e., pollen collected from surrounding floral resources) collected by experimental colonies in August and September to determine the pollen source and to measure the protein and amino acid profile.

MATERIALS AND METHODS

Experimental Design

Honey bee colonies were studied from 2018-20 to evaluate the effect of feeding pollen substitute on colony growth, overwintering success, and colony strength the following spring. Additionally, composite pollen samples were collected from a subset of study colonies to determine which types of flowers bees were foraging upon, and to identify the pollen type, crude protein, and amino acid profile. In 2018, three treatment groups were studied: control, Nutra Bee™, and Ultra Bee™. In 2019, Nutra Bee™ was unavailable, so two treatment groups were studied: control and Ultra Bee™. Ultra Bee™ contains 18% crude protein, no natural pollen, and its protein source comes from plant protein products (Lamontagne-Drolet et al. 2019). No peer-reviewed or reputable nutritional analysis information was available for Nutra Bee™. Due to lack of funding and legal ramifications, we were unable to send Ultra Bee™ and Nutra Bee™ samples for protein analysis.

In August 2018, 96 honey bee colonies across 4 different apiaries located in Colchester County, Nova Scotia, were selected for the study. These 4 apiaries were a minimum of 5 km apart (5 km – 23 km range). The apiaries were in a similar geographical region in similar landscapes (mixture of agroecosystems and forests) with similar foraging opportunities (i.e., similar floral composition surrounding each yard). All treatments were present in each apiary; however, the design was unbalanced (i.e., different number of hives in each apiary). The colonies used for this study were all summer splits from the same

operation made in the same way using queens from the same source (Kona Queen Hawaii®) and managed according to commercial beekeeping practices in the area (including top and bottom entrances, 10-frame configuration in each box, Langstroth-style hive bodies, fall feeding of sugar syrup, and varroa mite treatment as required). The splits were made approximately one month before strength monitoring and feeding began (13 July 2018 and 12 July 2019). All colonies chosen for the trial were of similar strength (measured as seam counts) and housed in a double brood chamber configuration (i.e., 2 deep boxes). A complete schedule of research activities for experimental colonies is found in Table 1.

Table 1. Schedule of research activities for experimental honey bee colonies fed pollen substitute 2018-19 in Nova Scotia, Canada.

Date	Research Activity
2018 Trial	
09 August 2018	Experimental colonies randomly assigned to treatment; initial colony strength assessment conducted; feeding of pollen substitute (if applicable)
19 August 2018	Second colony strength assessment conducted; second feeding of pollen substitute (if applicable)
27 August 2018	Pollen samples collected
29 August 2018	Third colony strength assessment conducted; third and final feeding of pollen substitute (if applicable)
12 September 2018	Fourth colony strength assessment conducted
27 September 2018	Fifth and final colony strength assessment conducted
06 May 2019	Overwintering mortality and colony strength assessments conducted
2019 Trial	
07 August 2019	Experimental colonies randomly assigned to treatment; initial colony strength assessment conducted; feeding of pollen substitute (if applicable)
17 August 2019	Second colony strength assessment conducted; second feeding of pollen substitute (if applicable)
27 August 2019	Third colony strength assessment conducted; third and final feeding of pollen substitute (if applicable)
09 September 2019	Pollen samples collected
10 September 2019	Fourth colony strength assessment conducted
23 September 2019	Fifth and final colony strength assessment conducted
30 April 2020	Overwintering mortality and colony strength assessments conducted

Late Summer Colony Growth

On 09 August 2018, hives in each apiary were randomly and evenly (n = 32) assigned to a pollen substitute treatment group: Ultra Bee™ (Mann Lake Ltd., MN), Nutra Bee™ (Jarrett Inc., CA) or control (no pollen substitute).

Initial colony strength was measured by conducting seam counts in each hive. Since the experimental colonies were housed in double brood chambers, the number of tops of frames in the bottom box and the number of bottoms of frames in the top box that were covered with bees were counted to the nearest one-half frame (Nasr et al. 1990). Seam counts relate to the overall cluster size and allow for colony size and growth to be measured. Each hive in the trial (except control colonies) was given a total of 3 lbs of pollen substitute, divided into 1 lb of pollen substitute per feeding over 3 feedings. Seam counts were conducted over a total of 7 weeks, 1 week longer than 2 complete brood cycles. This allowed us to determine if colony growth was impacted by feeding pollen substitute. Colonies receiving pollen substitute received 1 lb of either Ultra Bee™ or Nutra Bee™ between the 2 brood boxes during each feeding. Nutra Bee™ patties were massed to 1 lb (± 0.05 lbs) and placed on wax paper, similar to how Ultra Bee™ patties are sold commercially. Slits were cut into the wax paper for both pollen patties to aid in bee consumption. Control colonies did not receive any pollen substitute but were opened for the same duration of time during each feeding. All colonies in the trial were fed 2-4 L of 1:1 sugar syrup each week during the trial, but exact amounts and feeding schedule varied among individual hives. After approximately 10 days, the second round of feeding and seam count data collection were complete. By this time, most of the pollen substitute that was previously placed on the hives was completely consumed. In cases where the pollen substitute was not entirely consumed, the remaining pollen substitute was left in the hive. After another 10 days, the third and final round of feeding was complete, and seam counts were once again conducted. Two subsequent seam counts were taken after the final round of feeding of pollen substitute, each 14 days apart. In 2018, 27 September was the last colony assessment date until spring 2019.

In 2019, the study was repeated with the same beekeeping operation and modified to only compare control colonies and colonies fed Ultra Bee™. Ninety-six colonies (summer splits) of similar strength were used and managed the same as in 2018 (n = 48 for each treatment group). The 2019 design was balanced, where the study hives were equally distributed within 4 different apiaries. The feeding and strength assessment schedules were similar to 2018, beginning 7 August 2019 and ending 23 September 2019.

Overwintering Mortality and Spring Colony Strength

Colonies were overwintered in their respective test yards in groups of four hives per pallet and were communally wrapped with black plastic with insulation placed on top of each hive. Overwintering mortality and colony strength data were collected on 1 May 2019 and 30 April 2020 for each treatment group. For both study years, any failed colonies that were unlikely to overwinter successfully were omitted from the study whenever they were observed. Colonies continued to be managed by the participating beekeeper in the spring using common commercial beekeeping practices in the area (e.g., spring feeding of 2:1 sugar syrup).

Naturally- Collected Pollen Sampling

Pollen samples were collected on 27 August 2018 and 09 September 2019 using bottom mount pollen traps (Pollen Depot, Port Hope, ON). One pollen trap was randomly assigned to each treatment group per bee yard for both years. In both years, pollen traps were deployed for 24 hours on days with high foraging potential (above 20˚C, no precipitation, low winds). All pollen samples were pooled to create 1 composite sample for each year of collection due to budgetary constraints. This pooled sample allowed for a general snapshot of which pollen sources are visited and collected by honey bees during the observation period. Pooled samples (1 per year) were sent for a complete amino acid profile and protein analysis (Research and Productivity Council Laboratory, Fredericton, New Brunswick, Canada) as well as for pollen identification (Laboratoire BSL, Quebec, Canada). This allowed us to determine which type of flowers the colonies were foraging on and the nutritional profile of the pollen. With this analysis we could determine if the natural pollen available at that time in Nova Scotia could properly support honey bee growth and development, based on protein and amino acid requirements by De Groot (1953).

Amino Acid Profile and Protein Analysis

Protein analysis was completed at the Research and Productivity Council Laboratory in Fredericton, New Brunswick, Canada using reference method AOAC 981.10 (Research and Productivity Council Laboratory 2020). Specific details of the standard operating procedure were unavailable from the laboratory due to corporate information protection, but the methodology used has been accredited by the Standards Council of Canada (2019).

Morphometric Pollen Identification

Pollen identification was determined morphometrically using microscopy by J. Parent from Laboratoire BSL. A 2 g pollen sample (composite sample pooled for each study year) was placed in a 50 mL centrifuge tube and distilled water was added to a volume of 40 mL. The sample was periodically shaken until dissolution was complete, then vortexed for 2 minutes. A small drop of sample was placed on a small cube of glycerine jelly stained with basic fuchsine on a microscope slide, warmed, and mixed until the solution melted and the preparation was homogeneous (Barth et al. 2010). A glass cover slip was put over the preparation, sealed with paraffin, and left to dry before beginning microscopy. Once dry, the slide was turned upside down, and a line was drawn through the center of the drop. Microscopy was performed at 1000x magnification; pollen identification began near the center line, moving in an "S" pattern until 500 pollen grains (or palynomorphs) were identified.

Statistical Analyses

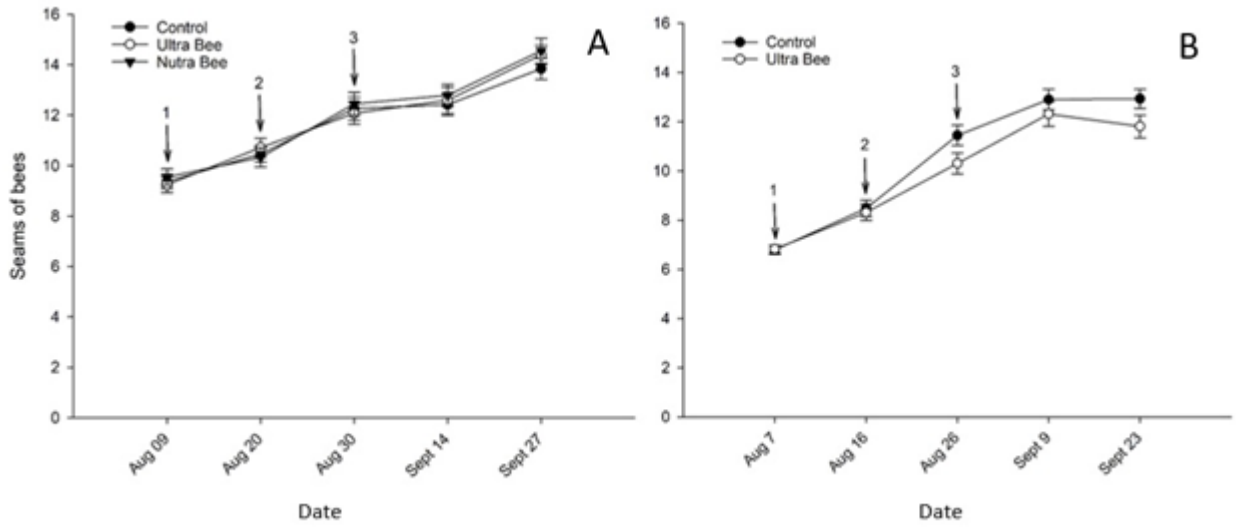
Due to strength assessments being carried out on the same hives multiple times over several weeks, we were interested in how hives grew as a function of time and if there was an interaction between treatment and time. For fall strength assessments, a repeated measures analysis of variance using a fitted general linear model was used. For both years, treatment, date, and the interaction between treatment and date were used as fixed effects, and apiary was used as a random blocking factor. Analysis of colony strength in the spring was done using a general linear model where treatment was a fixed factor and apiary was a random blocking factor. The unbalanced design of 2018, and the loss of experimental colonies throughout the study was not anticipated to affect the statistical analysis since only one factor (pollen substitute) was being studied. Assumptions of normality of error terms and constant variance of residuals were verified for analyses for both years and independence was assumed through randomization. Post hoc analysis of treatment effects were carried out using Tukey tests where appropriate. All statistical analyses were conducted in Minitab 17 (Minitab 2018).

RESULTS

Late Summer Colony Growth

During the observation period in 2018, we found no significant effect of feeding pollen substitute on colony growth in comparison to control colonies. There was no significant interaction between treatment and date of observation ($F_{8,455}$ = 0.28, P = 0.971) nor was there a significant difference between treatments ($F_{2,455}$ = 0.50, P = 0.606) or bee yard location ($F_{3,455}$ = 1.53, P = 0.205). There was a significant effect of time ($F_{4,455}$ = 65.0, P = 0.001) as all colonies grew during the observation period (Figure 1A). During the observation period, control colonies grew an average of 4.47 seams (sd = 2.75, range = 0 - 9.50, n = 32), colonies fed Ultra Bee™ grew an average of 5.03 seams (sd = 2.64, range = 0 - 9.50, n = 30), and colonies fed Nutra Bee™ grew an average of 5.05 seams of strength (sd = 2.75, range = 0 - 9.00, n = 29) during the 7 weeks that the hives were monitored for growth. All colonies displayed colony growth (i.e., the number of seams of bees increased throughout the study period), but there was no significant difference in colony growth among the treatments studied.

Figure 1. Growth of honey bee colonies measured as seams of bees throughout pollen substitute feeding trial, from 09 August to 27 September in Nova Scotia, 2018 (A) and from 07 August to 23 September in Nova Scotia, 2019 (B). The numbers 1, 2, and 3 correspond to the 3 feeding periods each year.



In 2019, there was no significant interaction between treatment and date of observation ($F_{2,438} = 1.12$, $P = 0.347$), however, there was a significant difference between treatments ($F_{1,438} = 6.73$, $P = 0.010$), bee yard ($F_{3,438} = 15.97$, $P = 0.001$) and time ($F_{4,438} = 103.86$, $P = 0.001$). Both treatment groups grew during the duration of observation, however, control colonies grew significantly more than colonies fed Ultra Bee™ (Figure 1B). During the 2019 observation period, control colonies grew an average of 6.03 seams (sd = 2.79, range = -1.00 – 12.00, n = 44) while colonies fed Ultra Bee™ grew an average of 5.02 seams (sd = 2.66, range = 0 – 11.0, n = 40) during the 7 weeks that the hives were monitored for growth.

Overwintering Mortality and Spring Colony Strength

In the spring of 2019, 12.5% (4/32) of colonies died in the control group, 16.7% (5/30) of colonies died in the Ultra Bee™ group, and 10.3% (3/29) of colonies died in the Nutra Bee™ group. There was no significant difference in colony strength among hives in the 3 treatment groups ($F_{2,73} = 0.04$, $P = 0.957$). Average strength of colonies in early May was 10.37 seams (sd = 4.26, range = 3.00 – 18.0, n = 28), 10.04 seams (sd = 4.22, range = 3.00 – 16.0, n = 25) and 10.27 seams (sd = 4.03, range = 3.00 – 18.0, n = 26) for control colonies, Ultra Bee™ colonies, and Nutra Bee™ colonies, respectively.

In the spring of 2020, 13.6% (6/44) percent of colonies died in the control group and 17.5% (7/40) percent of colonies died in the Ultra Bee™ group. There was no significant difference in colony strength among hives in the 2 treatment groups ($F_{1,73} = 0.59$, $P = 0.445$). The average strength of Ultra Bee™ colonies in late April was 9.19 seams (sd = 3.72, range = 2.00 – 16.5, n = 33), and the average strength of control colonies on the same date was 9.79 seams (sd = 3.19, range 4.00 - 15.0, n = 38).

Identification and Analysis of Nutritional Profile of Late Summer-Collected Pollen

Pollen collected naturally across treatment groups and yards was identified for late summer 2018 and late summer 2019 (Table 2). Goldenrod (Asteraceae) was collected most frequently in both years (78.6% and 65.4% in 2018 and 2019, respectively). The composite sample including pollen collections from both 2018 and 2019 contained 12.8% crude protein. Additionally, the amino acid analytes present in the composite pollen sample were analyzed (Table 3). The composite sample contained detectable amounts of 19 amino acids, including all 10 essential amino acids required for honey bee growth and development (De Groot 1953). Only 4 of the 10 essential amino acids present in the sample met the requirements for honey bees (Table 3).

DISCUSSION

Late Summer Colony Growth

There was no added benefit to feeding colonies pollen substitute in late summer based on the strength of the

Table 2. Pollen identification in Nova Scotia honey bee colonies on 27 August 2018 and 09 September 2019.

Collection Period	Plant (Family)	Plant (Genus)	Plant (Common Name)	Pollen Quantity (% per 500 grain sample)
18 August 2018	Asteraceae	*Solidago*	Aster/Goldenrod	393 (78.6%)
	Asteraceae	*Taraxacum*	Dandelion	54 (10.8%)
	Fabaceae	*Trifolium pratense*	Red clover	17 (3.4%)
	Fabaceae	*Trifolium hybridum*	Alsike clover	15 (3.0%)
	Balsaminaceae	*Impatiens*	Touch-me-not	14 (2.8%)
	Asteraceae	*Centaurea nigra*	Black knapweed	4 (0.8%)
	Vitaceae	*Parthenocissus*	Virginia creeper	2 (0.4%)
	Fabaceae	*Melilotus*	Sweet clover	1 (0.2%)
Trace amounts (fewer than 3 pollen grains each) of *Cirsium* (thistle) (Asteraceae), carrot (Apiaceae), and spotted lady's-thumb (Polygonaceae) detected on slides				
09 September 2019	Asteraceae	*Solidago*	Aster/Goldenrod	327 (65.4%)
	Rosaceae	*Fragaria*	Strawberry	128 (25.6%)
	Asteraceae	*Taraxacum*	Dandelion	23 (4.6%)
	Brassicaceae	*Brassica*	Mustard, turnip	16 (3.2%)
	Fabaceae	*Trifolium hybridum*	Alsike clover	5 (1.0%)
	Asteraceae	*Chrysanthemum*	Daisy	1 (0.2%)
Trace amounts (fewer than 3 pollen grains each) of *Zea mays* (corn) (Poaceae), *Trifolium pratense* (red clover) (Fabaceae), and *Picea* (spruce) (Pinaceae) detected on slides				

Table 3. Honey bee essential amino acids and crude protein present in late summer-collected pollen presented as measured units and as a ratio with threonine equal to 3 (De Groot 1953).

	Measured units (g/100g)	Bee requirement[a]	Late summer-collected pollen ratio
Threonine[c]	0.62	3.0	3.0
Phenylalanine[c]	0.53	1.5	2.6
Leucine[c]	0.84	4.5	4.1*
Isoleucine[c]	0.50	4.0	2.4*
Lysine[c]	1.04	3.0	5.0
Arginine[c]	0.55	3.0	2.7*
Histidine[c]	0.42	1.5	2.1
Valine[c]	0.57	4.0	2.8*
Methionine[c]	0.27	1.5	1.3*
Tryptophan[c]	0.14	1.0	0.7*
Alanine	0.71	0	3.4
Aspartic Acid	1.35	0	6.5
Glutamic Acid	1.31	0	6.3
Glycine	0.65	0	3.1
Serine	0.69	0	3.3
Tyrosine	0.32	0	1.5
Cystine	0.28	0	1.4
Proline	1.41	0	6.8
Hydroxyproline	0.76	0	3.7
Crude Protien (%)	12.8	20.0[b]	N/A

[a]Data from DeGroot, 1953; [b]Data from Somerville, 2005; [c]Essential amino acids; *Level lower than required minimum

colonies in late summer, overwintering survival, or colony strength the following spring. In fact, during the 2019 late summer feeding trial, colonies that were fed pollen substitute grew significantly less during the 7-week observation period in the late summer than control colonies. Mattila and Otis (2007a) similarly found that supplementing colonies with natural pollen in the late summer did not improve winter survival, spring growth and brood rearing efficiencies compared to control colonies and colonies that were partly deprived of pollen in late summer. Colonies that were fed additional pollen in the late summer reared more workers that season, however, these workers were short lived and died before winter (Mattila and Otis 2007a). In Quebec, however, colonies fed pollen substitute in late summer had significantly greater spring build up (Martin 2009). Mattila and Otis (2007b) found that additional pollen supply in late summer reared more workers, but the resulting winter bee population was the same compared to colonies that were supplemented with pollen and those that were not. A key difference between these studies and our study is that we supplemented colonies with pollen substitute (Nutra Bee™ or Ultra Bee™), while Mattila and Otis (2007a,b) supplemented colonies with protein patties made from real pollen, and Martin (2009) used Global™ pollen patties.

We did not find any benefit in late summer colony growth in 2018 from feeding colonies pollen substitute, and surprisingly saw negative effects in 2019 where late summer fed colonies had a lower population by the end of September compared to control colonies. One possible explanation for this is that bees reared from pollen substitute may have shorter lifespans than natural pollen-reared workers; Lamontagne-Drolet et al. (2019) demonstrated that worker bees reared from pollen free substitute (Ultra Bee™) lived a significant shorter life span than worker bees reared from natural pollen. Since we also found a significant effect of bee yard location in 2019, it is possible that factors within the bee yard contributed to the difference observed between control and Ultra Bee™ colony seam counts. The difference in colony strength observed between control and Ultra Bee™ colonies in late summer 2019 did not result in any difference in overwintering survival or colony strength the following spring (2020).

Overwintering Mortality and Spring Colony Strength

It is possible we did not detect a significant benefit from feeding pollen substitute to colonies in the late summer on overwintering success because of the participating operation and their overall best management practices in place. The 5-year Canadian honey bee colony loss average from 2015-19 was 23.3% (ranging from 16.4-32.6%) (Canadian Association of Professional Apiculturists 2019), yet the colonies we tested for overwintering mortality were well below this national average (13.2% across the 3 treatment groups in 2019; 15.6% 2020). Overwintering mortality in our study colonies was also below the Nova Scotia 5-year colony loss average of 16.2% (ranging from 13.2-19.8%) (Canadian Association of Professional Apiculturists 2019). It is therefore conceivable that had we worked with beekeeping

operations with higher historical average winter losses and other limiting management practices, feeding pollen substitute may have masked or buffered the overwintering losses we predicted initially in control colonies.

Identification and Analysis of Nutritional Profile of Late Summer-Collected Pollen

The major pollen source collected from in both years of the study was goldenrod. This was not an unexpected result because in Nova Scotia there is an abundance of goldenrod blooming in late summer, and this plant is known to provide plentiful nectar and pollen (Jachuła et al. 2020). The documented protein content of goldenrod ranges from 13-29% (Frias et al. 2016; Jachuła et al. 2020). Nutritional shortcomings have been noted in other plants in the Asteraceae family (e.g., sunflowers (*Helianthus annuus*) (Nicolson and Human 2013) and dandelion (*Taraxacum officinale*) (Herbert et al. 1970)), and the colonies in our study did collect considerable amounts of dandelion pollen (10.8% in 2018 and 4.6% in 2019). Although goldenrod did comprise the largest percentage of the pollen collected, the bees in our study sourced pollen from a diversity of sources, although not to the same magnitude as goldenrod. A diversity of pollen sources can buffer poor pollen quality and nutrition from a particular plant source (Alaux et al. 2010; Di Pasquale et al. 2013).

Although the protein content of naturally collected pollen in our study was only 12.8%, all 10 of the amino acids required by honey bees were present. However, six of the 10 required amino acids were present in levels slightly lower than required for honey bee growth and development (De Groot 1953). As long as the amino acids are available in the pollen diet, however, honey bees may compensate to some degree from lower protein levels and lower levels of required amino acids by consuming more pollen (Pernal and Currie 2001). There is typically an abundance of diverse pollen available for honey bees to collect in late summer in the Maritimes which may also improve bee immunity (Alaux et al. 2010; Di Pasquale et al. 2013). Although our study was conducted in Nova Scotia, due to regional geographical similarities (including similar foraging resources, weather conditions, and management practices), we feel confident in extrapolating our findings to the Maritime Provinces. Further study on natural pollen collection is warranted, however, due to our small sample size for collected pollen.

The nutritional profile found in late summer-collected pollen may explain why there was no benefit to feeding pollen substitute to colonies in late summer in our study. If the colonies were able to obtain protein and required amino acids from late summer pollen, then the addition of pollen substitute would not be necessary for colony growth and function. Furthermore, in 2019 we noticed a disadvantage to feeding colonies pollen substitute with respect to colony growth. From an economic perspective, applying pollen substitute cost $3.50 per pollen patty. This meant colonies that received 3 lbs of pollen substitute during the trial cost the beekeeper an additional $10.50 per colony for 3 lbs of pollen substitute, yet no economic benefit was observed.

Under typical Maritime beekeeping conditions when there is an abundance of pollen available in late summer, there does not seem to be an advantage to feeding colonies pollen substitute at this time. Recent dry seasons have been documented, however, and under these circumstances (limited late summer or early fall pollen availability), feeding pollen substitute may be advantageous.

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