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RESEARCH PAPER

Effects of Vitamin Supplements in a Pollen Substitute on Some Characteristics of Bee Nucleus Colonies

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Abstract

This study aimed to evaluate the effects of vitamin supplementation in a pollen substitute on the performance of honey bee colonies (*Apis mellifera mellifera*). Twenty nucleus colonies housed in Langstroth-Ruth hives were randomly selected and fed a basic diet consisting of corn gluten, sugar, and brewer's yeast residuals, yielding 21.0% crude protein, 7.1% crude fat, and 2.6% crude ash. This diet was enriched with a vitamin mixture at doses of 1, 2, and 3 grams. Control group I received a sugar-water syrup (1:1, w/v), while control group II received the basic diet without vitamin supplementation. The three experimental groups were given the diet with varying amounts of the vitamin mixture. Results indicated that experimental group II demonstrated colony strength comparable to experimental group I but significantly higher than the control group by 14.6-49.4%, and 34.3% higher than experimental group III ($P<0.05$). Queen bee egg yield increased significantly, with control group II showing a 27.5% increase, experimental group I showing 52.1% increase, experimental group II showing 67.6% increase, and experimental group III showing 28.5% increase on July 28th. Similar trends were observed on August 28th, with respective increases of 30.2%, 32.5%, 37.4%, and 14.7% compared to control group I. Additionally, honey yields for experimental group II were significantly higher by 25.8-57.9% compared to the control groups and 46.8% higher than experimental group III ($P<0.05$). These findings suggest that vitamin-enriched pollen substitutes positively impact colony strength, queen bee egg laying, and honey production, underscoring the potential benefits of such supplementation in beekeeping practices.

Introduction

Mongolia harvested 221.5 tons of honey from 10,800 bee colonies in 2020, providing less than 10 percent of its internal needs. With 592 species of honey plants grown in Mongolia, there's potential to support approximately 7 million bee colonies (Ochirbat & Otgonbileg, 2009).

A key factor in sustainable beekeeping is the presence of high-strength bee colonies. In addition to being more resistant to diseases, a strong bee colony consumes more supplementary feed and exhibits a stronger wintering ability. In addition to the better spring development, strong colonies could raise more forager bees and build many honeycombs (Mongolian Foundation of Science and Technology, 2019).

Honey production in Mongolia faces challenges from Varroa destructor, Nosema ceranae, viral infections, predators, and harsh environmental conditions (Tsevegmid et al., 2016). Furthermore, inadequate management practices, including insufficient supplementary feeding, exacerbate colony losses (Mongolian Foundation of Science and Technology, 2019).

Colony losses are often attributed to poor nutrition and starvation. Diets are called pollen substitutes when they contain no natural pollen (Noordyke & Ellis, 2021).

Pollen substitutes, play a crucial role in enhancing colony health by bolstering wintering ability, increasing survival rates, and promoting brood production (Akyol et al., 2006). While much research on pollen substitutes originates from foreign studies, the applicability of their findings varies due to diverse eco-climatic conditions, floral diversity, ingredient availability, and economic considerations across regions. Hence, there's a need for comprehensive scientific endeavors to improve bee colony survival and bolster the Mongolian economy through beekeeping.

In Mongolia, beekeepers commonly use protein-rich substitutes, like Appilekar and Candida, sourced from Russia and the United States of America. While Mongolian researchers have developed several pollen substitute diets, further enhancements are necessary to fortify these diets with essential vitamin supplements (Mongolian Foundation of Science and Technology, 2019).

The aim of this study is to evaluate the impact of vitamin-enriched pollen substitutes on bee colony strength, queen bee egg production, and honey and pollen production.

Material and Methods

The experiment was carried out in Bulgan soum, of Bulgan province (48°53'17.5"N latitude 103°21'26.2"E longitude) in Mongolia, within natural pastures characterized by dominant plant species such as Chamaenerion angustifolium, Geranium pratense, and Phlomis tuberosa. This study spanned from June 26th to September 10th, 2021.

We conducted the experiment, using 20 randomly selected bee colonies of the European dark bee (*Apis mellifera mellifera*) breed. These colonies were selected to ensure approximate uniformity in strength and size, and were housed in deep Langstroth-Ruth hives. Each experimental colony commenced with four deep Langstroth frames, and all colonies had been treated for *Varroa destructor* in accordance with standard practices.

Diet preparation

The pollen substitute diet was prepared in a cake form that contains 57% powdered sugar, 30% brewers' yeast, 10% corn gluten, 3% soybean oil, and was enriched with vitamin mixture of either 1, 2, or 3 grams. The prepared diet cakes were placed on the top bars of the hive and covered with a perforated plastic sheet to prevent drying out.

Control group I received 150 mL of sugar-water (1:1, w/v) syrup five times every two days. Control group II was fed a basic diet of 100 grams. Meanwhile, the three experimental groups were provided with 100 grams of the basic diet enriched with vitamin mixture (refer to Table 1) every 14 days from June 26th to August 1st, 2021. Subsequently, we assessed the impact of these different diets on colony strength, queen bee egg-laying ability, and honey yield.

Table 1. Pollen substitute diet options, in gramms

Name of ingredients	Basic Diet	Diet+Vit 1	Diet+Vit 2	Diet+Vit 3
Brewers' yeast	30	30	30	30
Corn gluten	10	10	10	10
Sugar	57	57	57	57
Soybean oil	3	3	3	3
Vitamin mixture*	-	1	2	3

*Vitamin mixture contains Vitamin A 180 IU, C 10 mg, D 20 IU, B_1 0.2 mg, B_2 0.2 mg, B_5 0.4 mg, B_6 0.12 mg, B_9 1 mg, B_{12} 0.2 µg per gram

Feed intake

The intake was determined by subtracting the weight of the feed 14 days after providing it to the colony from the fresh weight of the diet (measured in grams per colony). Subsequently, the diet consumption rate during the experimental period for each group was calculated by dividing the amount of intake by the total feed amount, and then multiplying that result by 100.

Measurement of total bee strength

The strength of all experimental colonies was assessed at 21-day intervals from June 26th to September 10th, 2021. This assessment involved recording the total number of frames completely covered by honey bees and estimating the bee population concurrently. To determine bee population, the deep Langstroth brood frame, densely covered by bees on both sides and marked as A and B, was utilized. Each of these frames was calculated to contain 880 bees, with the density calculated by multiplying this number by a coefficient of 1.38 to obtain the total number of bees (Delaplane et al., 2013).

Measurement of brood, pollen and honey stores

The number of squares containing total brood was assessed at 21-day intervals using a grid with a standard frame size of 435:230 mm. These frames were further divided into small cells using a ratio of a 5 cm horizontal and a 5 cm vertical transect intersecting. Each frame was placed on each side of a comb to ensure comprehensive assessment. The size of the larvae, pupa, and pollen area was determined by capturing a photo of the measuring frame using a high-resolution camera (3648 x 2736, 10 megapixels), following the method described by Delaplane et al. (2013). Subsequently, measurements of all frames with brood populations were summed for each colony, referencing the methodology outlined by Jeffree (1951). Honey production per colony in each group was calculated based on the total honey harvested.

Determination of the chemical composition of feed ingredients and diet

The chemical composition analysis of feed ingredients and the diet enriched with vitamin supplements was conducted for contents of CP, EE, Ash by Official Methods of Analysis (AOAC, 1990) at the Feed Evaluation Laboratory of the School of Animal Science and Biotechnology under the Mongolian University of Life Sciences.

Statistical analyses

Experimental data were processed using the IBM SPSS Statistics Subscription program for descriptive statistics and One-way analysis of variance (ANOVA). The P-value <0.05 was considered statistically significant.

Results

Chemical composition of pollen substitute and its intake by nucleus colonies

The fundamental principle of a pollen substitute is that it must comprise all the necessary ingredients with nutritional value, appropriate texture, consistency, and palatability for honey bees. The chemical composition of pollen substitutes utilizing brewers' yeast and corn gluten is illustrated in Table 2.

According to Table 1, the CP of brewers' yeast is 43.7%, corn gluten is 76.1%, EE is consequently 0.1, 1.9%. Meanwhile CP for basic diet in experiments is 21.0, EE is 7.1%, and ash is 2.6%.

Table 2. Chemical composition of ingredients and pollen substitute, %

Characteristics	Brewers' yeast	Corn gluten	Basic diet
CP	43.7	76.1	21.0
EE	0.1	1.9	7.1
Ash	7.1	4.8	2.6

According to Table 3, feed consumption rate was 89.3% for control group II, 96.0% for experimental group I, 100% for experimental group II, and 44.8% for experimental group III (*P*<0.05). The consumption rate for the control II, experimental I, and experimental II groups was similar. But it was for experimental group III was approximately 44.5-55.2 percent lower than the control group II and experimental groups I and II (*P*<0.05).

Table 3. Intake and consumption rate of pollen substitute by bees

Difference of experimental groups	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P>value*
Feeding amount, g	-	300	300	300	300	n.s.
Intake, g	-	268^{a}	288^{a}	300^{a}	89.7^{b}	*
Consumption rate, %	-	89.3^{a}	96.0^{a}	100^{a}	44.8^{b}	*

n.s.: not significance, *: *P*<0.05

Chemical composition of pollen substitute and its intake by nucleus colonies

A nucleus colony essentially constitutes a small hive comprising bees in all stages of development, along with an egg-bearing queen, and enough workers to cover four combs. The strength of nucleus colonies are shown in the table 4.

Table 4. The strength of the nucleus colonies

Days	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P<value*
		1. **Strength, in terms of frames actually covered by bees**				
VI/26	2.5	2.8	2.7	2.9	2.9	n.s.
VII/17	4.4^{c}	5.5^{b}	6.6^{a}	7.3^{a}	5.6^{b}	***
VIII/07	6.8^{d}	$8.9^{b,\,c}$	$9.9^{a,\,b}$	10.3^{a}	$7.6^{c,\,d}$	***
VIII/28	8.9^{d}	$11.6^{b,\,c}$	$12.9^{a,\,b}$	13.3^{a}	$9.9^{c,\,d}$	*
IX/10	7.1^{c}	9.3^{b}	$10.4^{a,\,b}$	10.7^{a}	7.9^{c}	*
		2. **Strength, in terms of bee population, numbers/colony**				
VI/26	3051	3340	3233	3522	3537	n.s.
VII/17	5280^{c}	6731^{c}	8033^{b}	8853^{a}	6788^{a}	***
VIII/07	8279^{d}	10778^{d}	$12083^{b,\,c}$	$12448^{a,\,b}$	9229^{a}	***
VIII/28	10763^{d}	14011^{d}	$15708^{b,\,c}$	$16182^{a,\,b}$	11998^{a}	*
IX/10	8610^{c}	11209^{c}	12567^{b}	$12946^{a,\,b}$	9599^{a}	*

n.s.: not significance, *: *P*<0.05, ***: *P*<0.001

In Table 4 it is observed that the strength of both the control and experimental groups ranged approximately from 2.5 to 2.9 frames, with a bee population of 3.05 to 3.5 thousand bees on June 26. Moreover, the strength of the groups improved over time, with the strength of the experimental groups being 11.5% to 50.3% higher than that of control group I (P<0.05) by August 28th. However, the strength of experimental groups I and II was 12.1% to 15.5% higher and 14.4% lower, respectively, than that of experimental group III compared to control group II (P<0.05).

During the experimental period, the eggs laid by the queen were recorded over a period of 21 days, as presented in table 5.

Table 5. Eggs laid by the queen bee, cm^2/colony

	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P<value*
VI/26	1326.6	1452.0	1405.8	1531.2	1537.8	n.s.
VII/17	2678.1^{c}	3414.2^{b}	4074.8^{a}	4490.6^{a}	3443.4^{b}	**
VIII/07	4799.5^{d}	6248.0^{b,c}	7004.8^{a,b}	7216.0^{a}	5350.4^{c,d}	*
VIII/28	5459.5^{b}	7107.1^{a}	7233.6^{a}	7503.8^{a}	6086.1^{b}	***
IX/10	2495.8^{d}	3249.0^{b,c}	3642.5^{a,b}	3752.3^{a}	2782.2^{c,d}	**

n.s.: not significance, *: P<0.05, **: P<0.01, ***: P<0.001

The number of eggs laid by queen bees in the control and experimental groups ranged from 1326.6 to 1537.8 cm^2 in June, 2678.1 cm^2 in the control I, 3414.2 to 3443.4 cm^2 in control II and experimental III, and 4074.8 to 4490.6 cm^2 in experimental I and II groups in the July. However, it was 5459.5 to 6086.1 cm^2 in the control I and experimental III, and 7107.1 to 7503.8 cm^2 in control II, experimental I and II groups in the August.

As of July 28th, the eggs laid by the queen were 27.5 percent higher in control group II, 52.1 percent higher in experimental group I, 67.6 percent higher in experimental group II, and 28.5 percent higher in experimental group III. On August 28th, it was 30.2, 32.5, 37.4, and 14.7 percent more than in control group I.

However, the number of eggs laid by queen bees decreased in September to 2495.8 cm^2 in control group I, 3249.0 cm^2 in control group II, 3642.5 cm^2 in experimental I, 3752.3 cm^2 in II, and 2782.2 cm^2 in III groups.

Table 6. Honey and pollen production of nucleus colonies

	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P<value*
Honey yield, g	7664.3^{c}	9622.5^{b,c}	11259.1^{a,b}	12102.4^{a}	8240.1^{c}	*
Pollen, cm^2	250.4^{d}	320.2^{b,c}	370.3^{a,b}	399.6^{a}	285.4^{c,d}	*

*: P<0.05

During the experimental period, the honey yield was 7.6 kg in the control group I, 9.6 kg in the control group II, 11.2 in the experimental group I, 12.1 in the experimental group II, and 8.2 kg in the group II (Table 6). The honey yield for experimental group II was similar to that of experimental group I, being 25.8-57.9 percent higher than the control groups, and 46.8 percent higher than experimental group III (P>0.05). The amount of collected pollen was 250.4 cm^2 in control group I, 320.2 cm^2 in control group II, 370.3 cm^2 in experimental group I, 399.6 cm^2 in experimental group II, and 285.4 cm^2 in experimental group III (P<0.05).

Discussions

Crailsheim et al. (1992) recorded a pollen consumption of 3.4 to 4.3 mg pollen per day per worker. Based on Rortais et al. (2005) a nurse bee consumes an average of 65 mg of pollen, while a worker-larvae consumes 5.40 mg. Consequently, a bee consumes a minimum of 70.4 mg of pollen in her lifetime.

The number of very active foragers in a hive is an important factor. In a very recent study, researchers showed that only 19% of the total forager performed 50% of the colony's total foraging trip (Klein et al., 2019). Thus, these factors could be plausible reasons for different foraging pollen amounts of the honey bee colonies (Ghosh et al., 2020**)**. For this reason, it is necessary to provide nucleus colonies with a plentiful supply of eggs with proper pollen substitutes.

The provision of artificial feeding as pollen substitutes using different protein-rich ingredients such as soy, pea, yeast, casein, egg, and microalgae have been used as a replacement for natural pollen (Ricigliano et al., 2022). This approach has been considered and developed to maintain egg laying, brood rearing, and foraging activities, which may sustain a sufficient bee population in the colony (Paray et al., 2021).

We developed pollen substitute consisting of brewer's yeast and corn gluten that was enriched with vitamins for nucleus colonies. It contains 21% crude protein, 7.1 % crude fat and 2.6 % crude ash.

Pollen substitute must be both palatable for the bees and nutritious. Abd El-Wahab et al. (2016) reported that colonies fed with synthetic diets, consisting of 10 g brewer's yeast, 1 g bee honey, 8 g turmeric and fenugreek powders, 0.5 g A, D and E vitamins, 45 g powdered sugar, 20 mL orange juice, 10 mL mint oil, 30 mL sugar syrup consumed a significantly larger amount of food (100 g.) every 2 weeks interval with no residues of patty.

In our study, the consumption rate was 89.3% for control group II, 96% for experimental group I, 100% for experimental group II, and 44.8% for experimental group III (P<0.05). Consumption rate was also 4-55.2 percent lower in experimental group III than in control group II and experimental groups I and II (P<0.05). Honeybees generally prefer their natural diet over a substitute. However, some researchers have found that bees consume more of the substitute than their natural diets. A potential reason for this preference is that pollen substitutes contain more sugar than natural diets (Noordyke & Ellis, 2021).

It is hypothesized that the reduction of appetite in the experimental group III, when more vitamins were added to the pollen substitute, changed the smell and quality of the feed.

Sihag and Gupta (2011), conducted a feeding experiment using basic recipes processed with soy bean flour+beer yeast residues+honey alone, and four types of combinations enriched with salt, vitamins, minerals separately.

The productivity of bee colonies fed by soy bean flour + beer yeast residues+honey+vitamins+minerals was higher in relation to the control group (Sihag & Gupta, 2011).

Chhuneja et al. (1993) reported that higher consumption of pollen substitute diet resulted in higher production of brood and more populous colonies produced significantly more honey. It is contented that stronger colonies store more honey than the weaker colonies (Kumar et al., 1995).

In our case the strength of nucleus colonies and eggs laid by the queen in experimental groups and control group II were greater than in the control group I.

When pollen substitutes containing beer yeast residue and corn gluten with vitamin supplements were studied, we found that these substitutes had a positive effect on colony strength, queen bee egg production, and honey yield, which aligns with the results of the researchers mentioned above.

Conclusion

In this study, we aimed to evaluate the impact of vitamin-enriched pollen substitutes on the strength of bee colonies, queen bee egg laying, and honey production. We found compelling evidence suggesting that enriching pollen substitutes with vitamins, specifically at a dosage of 2 grams per colony, significantly enhanced various parameters of bee colony productivity.

Our findings revealed that colonies supplemented with vitamin-enriched pollen substitutes exhibited notable improvements in colony strength, as evidenced by increased bee population and coverage of frames. Moreover, queen bees in these colonies demonstrated enhanced egg laying capacity, leading to greater brood production and ultimately stronger colonies. Additionally, the honey yields from colonies fed with vitamin-enriched pollen substitutes were substantially higher compared to those from control groups, indicating improved foraging efficiency and resource utilization.

Ethical Statement

There are no ethical issues with the publication of this article.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Author 1: Investigation, Writing – review & editing, Formal Analysis

Author 2: Investigation, Writing – review & editing; Supervision

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