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Evaluation of Pollen Supplementary Diets for Honeybee (*Apis mellifera*) Colonies and Their Effects on Some Biological Activities

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Abstract: The quality of the supplemental diet fed to honeybee colonies during dearth period influences the development and strength of the colony. The experiment was performed at Gedo sub site of Holeta Bee Research Center to examine the consumption rate and the effect of different pulses as pollen supplement for honeybee colonies on brood area, pollen and nectar storage, colony population growth as well as honey production. Selection of feed types was made based on information from home-made diets produced by locally beekeepers and preliminary screening of the flour. Honeybee colonies were provided with pollen supplement of soybean (*Glycine max*), chickpea (*Cicer arietinum*) and Pea (*Pisum sativum*), whereas no pollen supplement was provided to the control group. Pollen supplemented fed consumption mean data showed that soybean feed was maximally consumed (284 gm) by honeybee colonies per day and followed by chickpea (252.27 gm) and pea (223.63 gm). The result indicated that the highest brood area (300.66 cm^2/colony), pollen area (219.93 cm^2/colony), nectar area (258.96 cm^2/colony), number of frames covered with bees (9.4 per colony) as well as honey yield (11.5 kg per colony) was observed in honey bee colonies fed with soybean flour, while the least amount of brood, pollen, and nectar area, number of frames covered with bees and honey yield was detected in the control group. The results clearly demonstrated that supplementary feeding increased honeybee population for the survival of dearth period and better colony performance. Thus, the author recommend the commercial production and large scale utilization of soybean diet for the sustained reproduction and buildup of honeybee colonies during floral dearth period.

Keywords: Pollen, *Apis mellifera*, Supplementary Feed, Pulses, Colony Performance

1. Introduction

The development of a pollen supplementary diet for honey bees has been an area of curiosity to the beekeeping industry. Thus, appropriate nutrition, provided by pollen and nectar, is an essential for honeybee health, development, survival, and honey production [1]. Pollen is the major source of protein to honeybee colonies [2], and used to feed the newly reared larvae and young bees to offer the structural elements of muscles and glands. Particularly, extraordinary pollen ingesting by adult worker bees in the first few days after emergence enables them to develop their mandibular and hypopharyngeal glands which produce royal jelly for feeding the newly reared brood and other colony members [3-5].

Honeybees necessitate nectar as a carbohydrate source and pollen as a protein, amino acid, lipid, sugar, starch, vitamin, and mineral source to sustain hive function and to motivate foraging flights [6, 7]. Worker bees do not have considerable protein reserves in their bodies, thus they need a daily diet of about 3.4-4.3 mg of pollen based on their age to achieve this nutritional deficiency [3]. More or less, all protein and vitamins required by bees are derived from pollen stored as bee bread inside the hive when the pollen is not available in the environment [1, 8].

The quantity of protein that colonies received through

pollen can influence bees physiologically and affect their survivability [9]. Thus, the larvae reared under pollen-limited conditions may illustrate slighter weight gains, shorter lifespans, reduced foraging behaviour, and their capability to communicate effectually about food resources [10]. Moreover, shortage of pollen results in decreasing of brood rearing, developmental abnormalities and poor honey production [11, 12]. So, in deficiency of natural pollen sources, non-natural pollen diets can supplement honeybee colonies [8] which is very important for young bee's growth, brood rearing, reproduction and maintenance of bee colony [13, 14] and honey production [15]. Therefore, the beekeepers should be provided an effective pollen supplement diet, when the natural pollen supplies are insufficient to promote colony development and health [16]. Thus, the aim of this study was to evaluate the effect of three pollen supplement diet through measuring their consumption rate and different biological parameters for the local honeybee colonies.

2. Material and Methods

2.1. Study Area

The experiment was conducted at Gedo apiary site of Holeta Bee Research Center starting from September 2020 to August 2021. The exact location of the apiary site was situated at 9° 01' 504" N, 37° 26' 109" E, with an altitude of 2437 m above sea level. Twenty local honeybee (Apis mellifera) colonies were transferred from traditional hives to Zander hives at the start of active season (September) and let them to establish during the first year. From twenty established honeybee colonies fifteen of them were assigned for supplemental diets. Consecutively, a set of other five colonies was assigned as a control group. For consistency, each colony contained equal sealed and unsealed worker broods, pollen, nectar and number of frames covered with bees. Those selected colonies were randomly assigned to three pollen supplemental diet (five colonies per each diet), and each colony was numbered and labeled. Data collection from each honeybee colonies was started during dearth period of the second year.

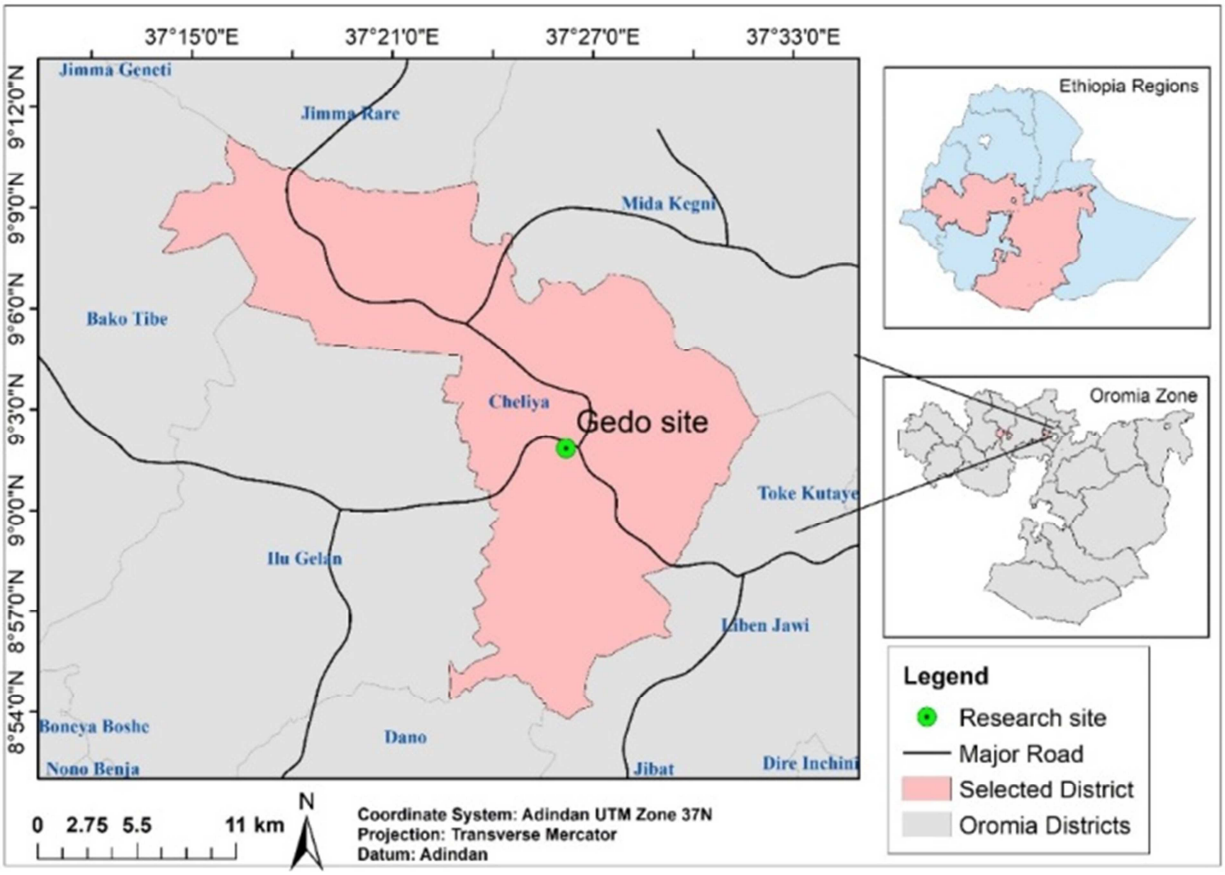

Figure 1. *Map of the study area.*

2.2. Selection and Preparation of Pollen Supplementary Feeds

The selection of feeding materials was made based on information with reference to home-made diets produced locally by beekeepers and keeping in mind the nutritional requirements of honeybees. The selected materials are soybean (*Glycine max*), chickpea (*Cicer arietinum*), and pea (*Pisum sativum*). These three pulses were chosen for this study because they were readily available and reasonably priced in the study area. The pulse flour was prepared as roasted and the skins of pulses came off and milled to the texture and compatibility to be accepted by honeybees.

2.3. Supplementary Feeding

Feeding of the flour was performed externally to provide bees' easy access to collect as it did for natural pollen. Honeybees by nature need to hum to collect powdery substances, which is an easy method and consumes minimum time and labor [15]. Supplemental feeding was administered to the experimental colonies from March 1, 2021 to August 25, 2021. In the study area, March to May is identified as the dry season, while July to August is known as the rainy season. From each diet, 500 g was poured onto canvas of (1 m x 1 m) and placed in the middle of the experimental colonies for three days in a week during the experimental period at various sites in the same apiary to avoid robbing. The consumption rate of each diet was measured every day in the late afternoon by calculating the differences in diet weight before and after feeding in grams per the experimental colonies.

2.4. Measurement of Different Attributes of the Experimental Colonies

The comb areas occupied by capped and uncapped brood in colonies were measured every 21 days by overlaying a grid premarked 5 cm by 5 cm on each side of every brood comb, and the area covered by the brood was visually summed [17]. The total brood population was calculated from the total area occupied by the brood comb. In addition to this, the comb areas occupied by pollen and nectar stores were also measured in the same way and the hoarding capacity was estimated. Moreover, the mean honeybee population was measured by the number of frames covered with bees [7]. The adult honeybee population was estimated every 21 days by measuring the total number of frames entirely covered with bees.

2.5. Honey Production

Data on honey production in kg/colony were collected at the end of the nectar flow season to compare honey yield in colonies fed pollen supplemental diets with control colonies in order to investigate the impact of pollen supplemental diets used in this study [18].

2.6. Data Analysis

Treatment effects on brood, pollen, and nectar area attributes were evaluated using one-way ANOVA, and means were separated using the Tukey Honest Significant Difference test (TSD test), and food consumption rate, bee population strength, and honey production were assessed using descriptive statistics.

3. Result and Discussion

3.1. Diet Consumption Rate

The efficacy of three pollen supplement diets was investigated in honey bee, *A. mellifera* colonies to determine diet consumption rate, as well as their effect on worker brood area, pollen area, nectar area, adult bee population, and honey production. The mean consumption rate of each feeding type used during both dearth periods were indicated in Figure 3. During the first three months of the dry season (March-May), the consumption rate of each feed type was observed to be slightly increasing. Similar trends were detected during the rainy season (July-August) as they were during the dry season (Figure 3). However, because of the availability of natural pollen in the field from the end of May to the end of June, pollen supplement diet consumption was gradually decreased. In both dearth periods, honeybee colonies were attracted and preferred to ingest soybean over chickpea and pea. Whereas throughout the study period, the consumption rate of diet pea was recorded as the last ranking order in the present study. The current finding was consistent with those of Ofijan [19], who reported that soybean was more palatable and consumed by honeybees than other feedings, particularly when natural pollen supplies were scarce or not available in the field. Thus, honeybees preferred diet that supply the right ratio of nutrients required for maximum survival and homeostasis through assessing the nutritional composition of the feeds offered to them [20]. Furthermore, DeGrandi-Hoffman et al. [7] showed that the differences in nutritional content of diets, as well as digestibility and accessibility of nutrients to worker bees, influence the amount of brood that can be produced even when consumption rates are comparable.

Figure 2. *Feeding of pollen supplement diet for bee colonies (A. worker bees feeding on pollen supplement diet of chickpea, B. packed pollen on the hind legs of worker bee during fed on supplement diet, C. bee collected pollen).*

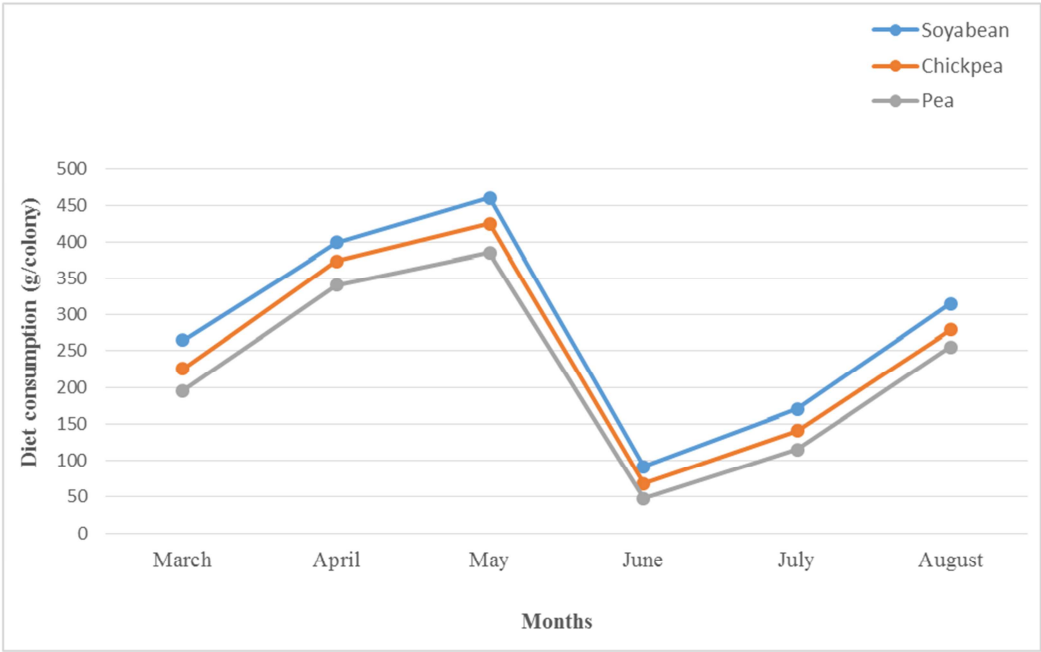

Figure 3. *Pollen supplement diets consumption rate in gram.*

3.2. Effect of Pollen Supplement Diets on Sealed Brood Areas

The results in Table 1 demonstrated that there were extremely significant variations in brood rearing activities between all treated colonies with evaluated pollen supplements and control colonies. The results clearly showed that colonies fed diet soybean produced the highest rate of sealed worker brood, with a mean of 300.66±15.14 cm^2/colony, followed by diet chickpea and pea, with a mean of 266.93±12.63 and 243.63±10.69 cm^2/colony, respectively. The control group had the lowest brood rearing rate, with a mean of 78.76 ± 5.06 cm^2/colony. The current finding was consistent with Sabir et al. [21], who reported that soybean products were a good substitute as pollen supplement and had positive effect on brood rearing activities. In addition the results obtained by Abusabbah et al. [22] from feeding soybean flour alone or in combination with other flour indicate that it has a great potential for enhancing colony maintenance and b]rood rearing during a pollen shortage. Furthermore, Sihag and Gupta [23], Lamontagne-Drolet et al. [24] and Islam et al. [25] also reported that honeybees increased the surface of the sealed brood area after consuming various pollen supplemental diets. Similarly, Abd El-Wahab et al. [6] found that sealed brood area increased in supplementary fed bee colonies compared to unfed bee colonies.

3.3. Effect of Pollen Supplement Diets on Storage of Bee Bread and Nectar

The data tabulated in Table 1 showed highly significant differences in pollen storage between all experimental colonies with tested diets and control colonies. Accordingly, colonies fed by diet soybean significantly produced the highest rate of pollen storage with a mean of 219.93±11.18 cm^2/colony, followed by diet chickpea and pea with a mean of 202.63±10.16 and 179.70±8.07 cm^2/colony, respectively. The lowest pollen storage rate was recorded for unfed control colonies with a mean of 57.20±6.07 cm^2/colony. The average nectar area on frame were indicated in Table 1. The maximum nectar storage (258.96±25.53 cm^2) was found on the frame that fed the soybean flour, but control colonies stored less nectar with a mean of (61.03±9.12 cm^2). This finding revealed that, there were significant differences in the average amount of pollen and nectar stored across all fed and unfed control colonies. The current finding was consistent with Prakash et al. [26], who reported that feeding soy flour continuously increased pollen storage from the start of the experiment to the end and stayed much greater than the control group. Sihag and Gupta [23] and Pătruică et al. [27] also reported that after feeding pollen supplement, bee colonies pollen hoarding capacity was better than the control. These findings imply that the colonies are constantly producing and rearing more brood, as well as foraging for extra pollen and nectar. Furthermore, Somerville and Collins [28] suggested that colonies maintained with pollen supplements during times of scarcity of natural pollen would be better able to rear more brood and collect surplus nectar early.

Table 1. *Impact of three pollen supplement diet on the biological activities of honeybee colonies.*

Diets	Brood area ± SE	Pollen area ± SE	Nectar area ± SE
Soybean	300.66 ± 15.14^{a}	219.93 ± 11.18^{a}	258.96 ± 25.53^{a}
Pea	243.63 ± 10.69^{b}	179.70 ± 8.07^{b}	214.37 ± 19.72^{a}
Chickpea	266.93 ± 12.63^{ab}	202.63 ± 10.16^{ab}	225.63 ± 21.93^{a}
Control	78.76 ± 5.06^{c}	57.20 ± 6.07^{c}	61.03 ± 9.12^{b}

The different small letter within each column represents statistically significant differences ($p<0.05$).

3.4. Effect of Pollen Supplement Diets on Honey Bee Strength

The effect of pollen supplement feeding on the number of worker bees covered bee frames was observed and recorded. The result showed that, regardless of various feeding period, the colonies fed soybean had the highest number of frames covered by bees (9.4), which was statistically significant from all other treatments. Colonies given diet chickpea and pea had 7.3 and 6.4 number of frames covered by worker bees respectively (Figure 4). There was no significant difference in the number of frames covered by bees for diet chickpea and diet pea, while the control colonies had the lowest value (4.9). The result was consistent with the findings of Moustafa [29], Saffari et al. [30], and Sihag and Gupta [14], who observed that supplement diets produced more honeybee frames in comparison to non-supplemented control colonies. Kumer and Agrawal [31] also reported that, the number of bees covered by frames is positively affected by feeding a protein-rich diet to the bees. Somerville [32] found that, as compared to other bee feeding materials, pea, chickpea and soybean flour are comparatively high in protein and locally available, and are preferred as pollen supplement diets. According to Vollmann [33] soybean has a high amount of protein that accounted for approximately 35-52, followed by mung bean (27.2), and chickpea (22.7). As stated by Saleem et al. [34] the diet prepared from soybean flour supplemented with vitamin B complex and methionine was shown to be the optimum diet for strengthening honeybee colonies. Generally, honeybee colonies fed with supplemental feeding at different periods produced significantly more worker bees than unfed control colonies [25].

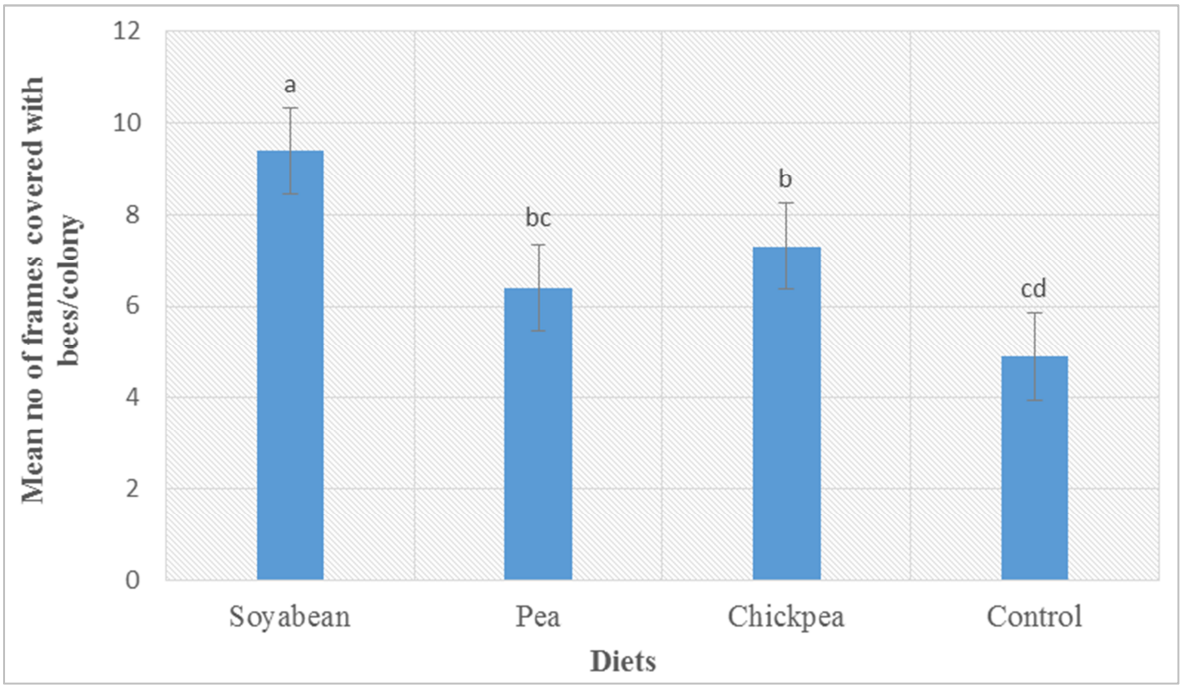

Figure 4. *Average number of frames covered with bees/colony.*

3.5. Effect of Pollen Supplement Diets on Honey Production

The quantity of honey yield per colony after consuming various diets was analyzed. In compared to unfed control colonies (3.5 kg per colony), the colonies fed with soybean diet produced the maximum honey yield (11.5 kg per colony) and followed by chickpea (8.4 kg) and pea (6.9 kg) (Figure 5). The current finding was congruent with those of Ullah et al. [35], who found that colonies fed diet soybean generated considerably more honey than unfed control colonies. According to Silva and Silva [36], DeGrandi-Hoffman et al. [7], and Kumar and Agrawal [37] colonies fed an artificial diet produced more honey yield as compared to unfed control colonies. Abdellatif et al. [38] also reported that providing of pollen supplemental diet for honeybee colonies during a dearth period increased honey yield in the succeeding honey flow season. Singh [39] recommended that, the maintenance of healthy colonies before nectar flow for higher honey production, as the strength of worker bees in a honeybee colony before the honey flow season was considered to be one of the most important factors that influenced honey production. Moreover, Chhuneja et al. [40] reported that, higher consumption of pollen supplemental diet resulted in higher production of brood and more populous colonies produced significantly more honey.

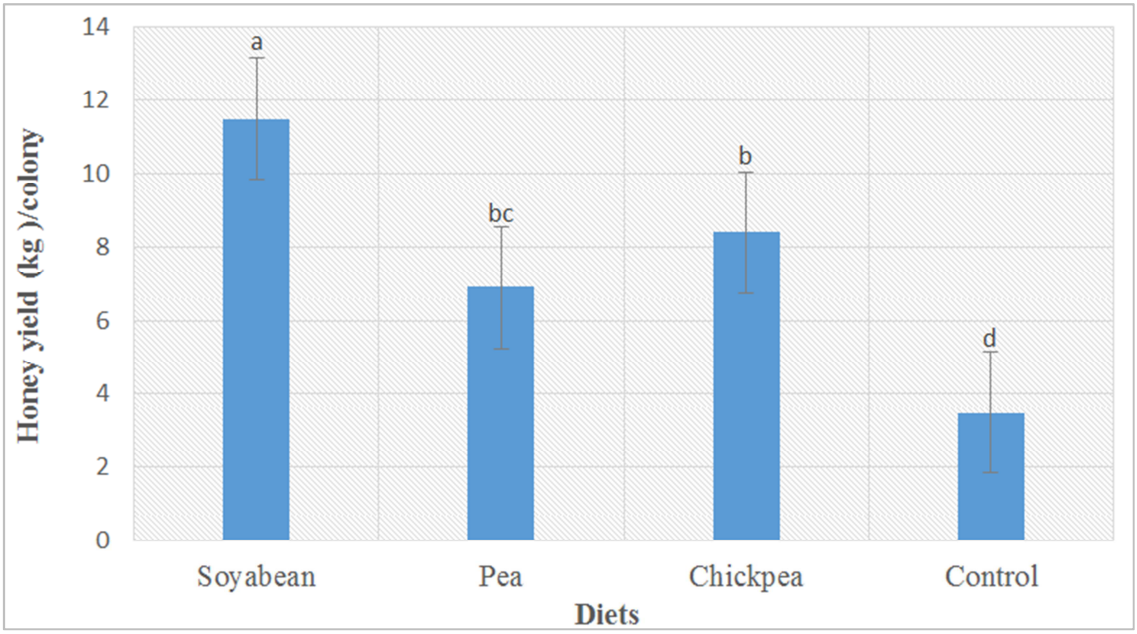

Figure 5. *Average yield of honey (kg/per colony).*

4. Conclusions

It can be concluded that pollen supplement diet should be supplied to bee colonies during dearth periods so that the strength of bee colonies can be maintained for the next honey flow season. Thus, the results obtained suggest that the diet soybean was found highly useful for attaining maximum bee strength and honey production. Therefore, the author recommends the large scale utilization of diet soybean for the sustained reproduction and buildup of honeybee colonies during the dearth periods.

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