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Number of brood combs and development of a new hive of honey bees (*Apis mellifera* L.)

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ABSTRACT: In Peru, the increase in agro-export crops has generated a greater demand for beehives for pollination, which needs to be satisfied. Therefore it is necessary to improve beekeeping technology. The study's objective was to evaluate the effect of the number of brood combs on the development of new bee hives (*Apis mellifera* L.). The treatments were: an adult hive with six brood combs and a six-month-old queen (control), a new hive with one brood comb and a newly fertilized queen, and a new hive with two brood combs and a freshly fertilized queen. All hives received one litre of sugar syrup every seven days. Data were submitted to analysis of variance and Tukey test form means separation. New hives formed with one brood comb had similar performances to those formed with two brood combs in daily bee weight gain (5 vs 7 g), the number of bees (46 vs 64), pollen reserve (3.3 vs 5.9 cm^2) and honey reserve (60 vs 59 cm^2); but two brood combs had a higher brood area about single brood combs (74 vs 45 cm^2) (p = 0.002). Developing new hives with a single brood comb could be an alternative when a rapid multiplication of hives is required.

Key words: beekeeping; breeding; honey; Peru; production

Número de favos de criação e desenvolvimento de uma nova colmeia de abelhas melíferas (*Apis mellifera* L.)

RESUMO: No Peru, o aumento das culturas agro-exportadoras gerou uma maior demanda de colmeias para polinização, que precisa ser atendida e, por conseguinte, é necessário melhorar a tecnologia apícola. O objetivo com essa pesquisa foi avaliar o efeito do número de favos de criação no desenvolvimento de novas colmeias de abelhas (*Apis mellifera* L.). Os tratamentos foram: uma colmeia adulta com seis favos de criação e uma rainha com seis meses de idade (controlo), uma colmeia nova com um favo de criação e uma rainha recém-fertilizada, e uma colmeia nova com dois favos de criação e uma rainha recém-fertilizada. Todas as colmeias receberam um litro de xarope de açúcar de sete em sete dias. Os dados foram submetidos a uma análise de variância e ao teste de Tukey para a separação das médias. As novas colmeias formadas com um favo de cria tiveram desempenhos semelhantes às formadas com dois favos de cria no ganho de peso diário das abelhas (5 vs 7 g), no número de abelhas (46 vs 64), na reserva de pólen (3.3 vs 5.9 cm^2) e reserva de mel (60 vs 59 cm^2); mas os favos de duas crias tinham uma área de criação superior à dos favos de uma só cria (74 vs 45 cm^2) (p = 0,002). O desenvolvimento de novas colmeias com um único favo de criação pode ser uma alternativa a considerar quando é necessária uma rápida multiplicação de colmeias.

Palavras-chave: apicultura; criação; mel; Peru; produção

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Introduction

Until two decades ago, beekeeping in the central Peruvian coast was characterized as a complementary activity to agriculture, where the sale of honey represented a secondary source for the farmer. However, due to the increase in agro-export crops, beekeeping is now an independent activity that relies on crop pollination services and is complemented by the sale of hive products.

Peruvian beekeepers face many challenges in maintaining a sufficient number of hives to enable them to subsist independently. The increase in monoculture and the massive application of pesticides is a constant threat that endangers the existence of apiaries (Maucourt et al., 2018; Vides-Borrell et al., 2019). Moreover, during the transhumance season, when hives are transferred from the coast to the highlands, the beekeeper must contend with additional threats like hive burning and theft.

Queen fecundity is a critical factor for the productive success of hives and is related to age; the younger they are, the higher oviposition (Amiri et al., 2017). The queen is the only reproductive female in the hive; the presence of a healthy and high-quality queen is essential to ensure the laying of a large number of female and male eggs (Tarpy et al., 2013), to reach a maximum population of highly productive bees (Hoover & Ovinge, 2018; Khan et al., 2022), and to maintain the social coherence of the hive through her pheromones (Tarpy et al., 2013). Queen posture and brood rearing begin when the pollen pool is adequate and is independent of nectar flow (Usha et al., 2014). To stimulate queen laying, beekeepers provide sugar syrup as a natural availability of pollen.

The increase in monocultures has generated an economic opportunity for beekeepers. Crops such as avocado (*Persea americana*) and blueberry (*Vaccinium corymbosum*) require the participation of pollinating insects to make fruit production profitable (Sagwe et al., 2021; Martin et al., 2022). To meet this demand, beekeepers have to increase the number of hives. There are several methods for hive multiplication, the most common of which is to form a new hive from two brood combs, two food racks, and the introduction of a newly fertilized queen, although when necessary, only one brood comb is used. It is unknown which of the two methods is the most effective and how long it takes for the brood chamber to reach the same surface area as an adult hive. The objective of this research was to evaluate the effect of the number of brood frames on the development of new honeybee colonies (*Apis mellifera* L.).

Materials and Methods

Location

The research was conducted in an apiary located in the agricultural area of San Jose, Santa María District, Huaura Province, Lima Region, Peru (Figure 1); latitude 11° 08' 05" S, longitude 77° 11' 32" W; altitude of 668 m a.s.l.; average temperature 22 °C and average relative humidity 81% (SENAMHI, 2023); between February and March 2022. The study area has multiple floral sources for natural food for bees, such as "chilco" (*Baccharis latifolia*), "pájaro bobo" (*Tessaria absinthioides*), "willow" (*Salix babylonica*) and "brittlebush" (*Encelia farinosa*), among others.

Figure 1. Apiary showing the adult hives used to form the new nuclei, located on the banks of the Huaura River.

Treatments

The control treatment consisted of four adult hives, each containing six brood combs and a queen that was six months old. Treatment one: Six new hives, each hive with one brood comb and newly fertilized queen. Treatment two: Five new hives, each hive with two combs of brood and a newly fertilized queen.

Variables evaluated

Weight of bees in the hive: The complete hive (box, frames with bees, floor, and lid) was weighed using a platform scale with a 60 kg maximum capacity (Kambor ®) and a 5 g approximation. Then, the combs were carefully shaken so that the bees could fall into a new hive of known weight, and the empty hive was reweighed. The weight of the bees resulted from the difference between the two weights. We determined the weights at the beginning and end of the experiment.

Number of bees: The number of bees was obtained by estimation. The total weight of the bees was divided by the average weight of the worker bee. Five Benton transporters were used to determine the weight of the worker bees. Five worker bees were captured, introduced into each transporter, and weighed. The average weight of the worker bees was 110 ± 16 mg.

Brood area: The area occupied by immature worker bees (larva and pupa) was measured with the help of a grid system (wire mesh), based on the methodology of Delaplane et al. (2013). The area of each grid was 1.61 cm^2 (1.27 cm x 1.27 cm). The grid was placed over the brood comb, and photos were taken for later evaluation. In the new hives (nuclei) it

was not necessary to measure the brood area, because all the nymphs were hatched (those coming from the mother hive) and the queen introduced to the nuclei had just started laying.

Food reserve: The grid system (wire mesh) was used for the evaluation, based on the methodology of Delaplane et al. (2013). The area of honey and pollen stored in the hives of control was evaluated at the beginning and 28 days later, while in the hives of treatments 1 and 2, the final evaluation was carried out at 44 days.

Experimental design

A completely randomized design with three treatments was used. The number of replicates for each treatment is shown in Figure 2.

Procedures

From an apiary of 10 hives, 11 nuclei or new hives were formed, of which six were with only one brood comb (two of the queens did not fertilize), and five were with two brood combs (one of the queens did not fertilize). All evaluations in the hives were carried out between 7 and 10 am.

Two days after the nucs were formed, the queens to be hatched were placed, 10 days after the hatching, or 15 days after the queen laid the egg. Evaluation of these hives began at the onset of queen laying. From day 7 onwards, inter-daily monitoring was carried out to determine the onset of laying (Figure 3).

Four adult hives (complete brood chamber and 6-month-old queen) were monitored for 28 days. Similarly, new hives formed with one or two brood combs were evaluated until one of the treatments reached brood chamber fill, similar to that of the adult (control) hives, which in the study was day 44.

All hives in the experimental treatments received one liter of sugar syrup (sugar-to-water ratio of 2:1.5) every seven days after the onset of laying, while the hives in the control group received it from the beginning of the experiment.

Data analysis

The data evaluated complied with the assumptions of normality and homogeneity of variances, which were verified with the Shapiro-Wilk test and Levene's test, respectively.

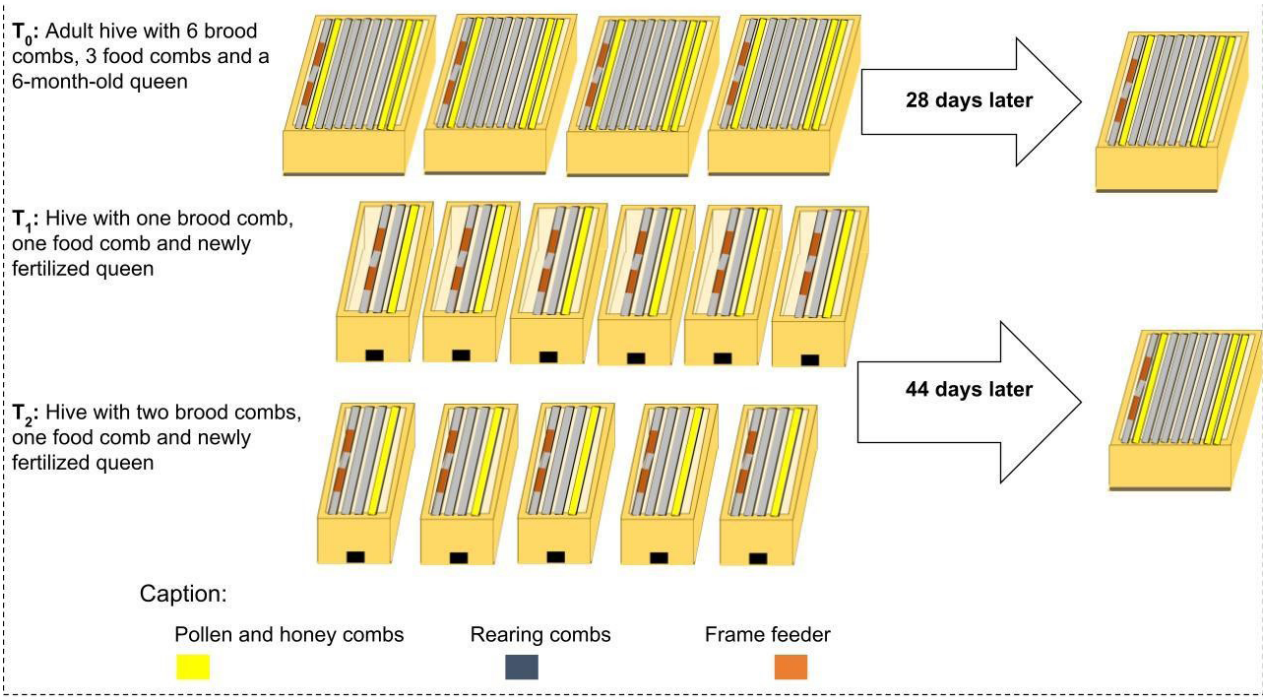

Figure 2. Distribution of the hives in the treatments and their respective replicates.

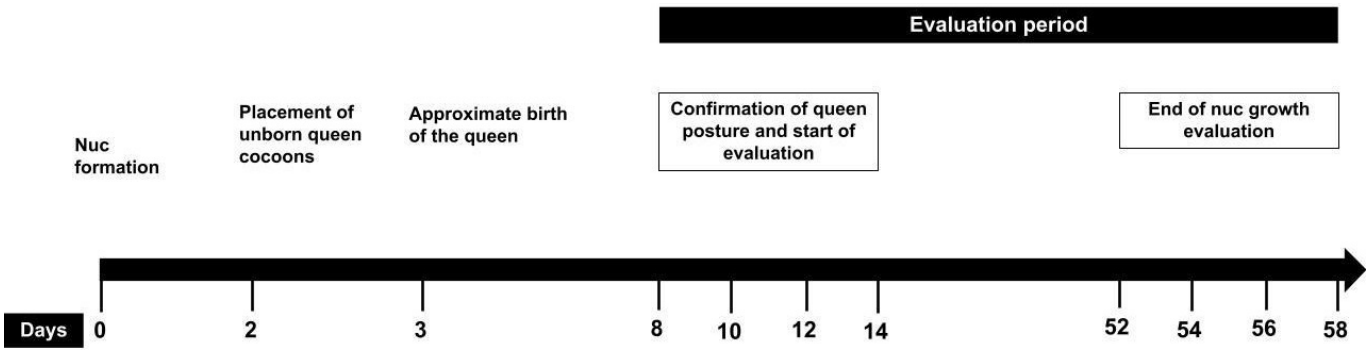

Figure 3. Timeline of new hive growth from nuc formation (day 0) to brood chamber filling (day 56).

Variables related to hive weight gain, bee population, brood area increase, number of brood combs and honey and pollen reserve were analyzed by analysis of variance and Tukey test for means ($p < 0.05$). All analyses were performed using R statistical software.

Results and Discussion

Bee weight gains

The bees reared under one and two breeding comb treatments had similar weights and were lower than the control ($p = 0.008$). Control hives had the highest daily weight gains (40 g day^{-1}) compared to the gains of one and two breeding comb treatments (5 and 7 g, respectively).

The lower weight gain of newly formed hives with one and two combs is related to the youth of the queen, who is just beginning her reproductive stage. The queen's posture in the hive would be regulated by the availability of nurse bees to feed the larvae. Royal jelly is a viscous secretion from the hypopharyngeal and mandibular glands of the younger nurse bees, used to feed young worker larvae during the first three days (Pasupuleti et al., 2017). Daily weight gains under one and two breeding comb treatments were similar to those obtained by Núñez-Torres et al. (2017) who obtained gains of 7 and 3 g in hives supplemented with soybean meal and pea meal pollen replacer, respectively.

The higher weight gains recorded in the control hives would be explained by the higher laying potential of the queens, which at the beginning of the experiment were six months old (Tarpy et al., 2000; Khan et al., 2022) and by the energetic stimulus of the syrup supplied throughout the evaluation period (Brodschneider & Karl Crailsheim, 2010; Kumar & Agrawal, 2014). These weight gains are within the range reported by Medina-Flores et al. (2018) in jumbo hives with sister queens of the same generation and origin during the season with the least amount of food resources, obtained daily increases of 72, 59 and 33 g in hives supplemented with high fructose corn syrup, sugar syrup plus pollen and invert sugar syrup plus pollen, respectively.

Table 1. Effect of number of brood combs on bee weight gain (g) per hive. Values are expressed as mean ± standard deviation (SD).

Treatments[1]	Weight of bees[1]		Weight gain	
	Beginning	End	Total	Daily
Control	1237 ± 40	2367 ± 451	1130	40 ± 14 [a]
One breeding comb	528 ± 63	753 ± 169	225	8 ± 1 [b]
Two breeding combs	1123 ± 413	1433 ± 275	310	7 ± 4 [b]
p-value				< 0.002

Number of bees

Table 2 shows the effect of the number of brood combs on the bee population at 28 (control) and 44 days (one and two breeding comb treatments); they had a similar increase in bee numbers, but significantly ($p = 0.008$) lower than control, that showed the highest daily increase in bee numbers (233 day^{-1}).

Table 2. The number of bees in adult hives (control group hives with four brood combs) and in new hives with one and two brood combs, were evaluated at 28 days (control) and 44 days (one and two breeding combs).

Treatments	Total bees[1] (n)		Increase in bees[1] (n)	
	Beginning	End	Total	Daily
Control	11242 ± 367	21515 ± 4099	10273	234 ± 85 [a]
One breeding comb	4796 ± 577	6841 ± 1536	2045	47 ± 36 [b]
Two breeding combs	10212 ± 3755	13030 ± 2503	2818	64 ± 47 [b]
p-value				0.008

[a,b] Different letters between rows indicate significant differences ($p < 0.05$).

Adult queens, like the control treatment hives, will have higher laying dynamics (Amirí et al., 2017), and will be supported in their larger population of nurse bees with the capacity to feed brood in the larval stage. The increase in bees from the control hives (233 bees day^{-1}) was higher than those reported by Medina-Flores et al. (2018), who documented increases of 107 bees day^{-1} in adult hives supplemented with syrup from various sugar sources.

Growth of the breeding area

Table 3 shows the effect of the number of brood combs on brood area growth (cm^2). Significant differences were found between treatments (p = 0.02). Two breeding comb treatments achieved a greater increase in brood area per day, compared to one breeding comb and control. Also, the increase in brood area on both sides of the combs was greater in control and the two breeding comb treatments achieved a greater increase than the single breeding comb.

A higher initial bee population allows for a higher brood chamber filling rate (Büchler et al., 2013). In the present study, comparing hives with one and two brood combs, a greater brood area increase was observed in hives with two brood combs, which coincided, although numerically, with the higher initial bee population. Hernandez & Castellanos, (2020) reported daily brood area growths between 11 and 22 cm^2 in the winter season with Jumbo hives, much lower than those of the present study, which grew between 45 and 74 cm^2 of brood area.

Table 3. Effect of the number of brood combs on brood area increment (cm^2) evaluated at 28 days (control) and 44 days (one and two breeding comb treatments). Values are expressed as mean ± SD.

Treatments	Breeding area		Increase	
	Beginning	End	Total	Daily
Control	1304 ± 62	2684 ± 571	1380	49 ± 2 [b]
One breeding comb	0	2015 ± 429	2015	45 ± 10 [b]
Two breeding combs	0	3186 ± 183	3186	74 ± 4 [a]
p-value				0.002

[a,b] Different letters between rows indicate significant differences ($p < 0.05$).

Figure 4 shows the effect of the number of brood combs on the number of sides of combs with brood, evaluated at the beginning and to the end of 28 days (control) and 44 days (one and two breeding comb treatments). The greater increase in brood area of two breeding comb treatments coincides with the greater number of brood sides in the hive compared to those of single breeding comb and similar to the hives of control.

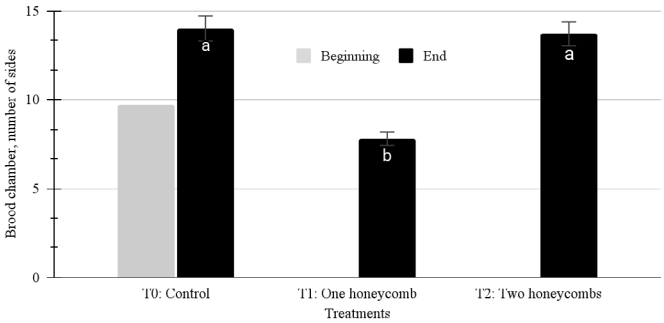

Figure 4. Effect of the number of brood combs on the number of sides of combs with brood evaluated at 28 days (control) and 44 days (one and two breeding comb treatments). Different letters indicate significant differences ($p < 0.05$).

Pollen and honey reserves

Table 4 shows the honey and pollen reserves (cm^2) in the evaluated hives. One and two breeding comb treatments had higher honey and pollen reserve areas ($p < 0.05$) compared to the control.

The one and two-breeding comb hives were characterized by an increase in honey and pollen reserves, while the control hives showed losses in food reserves. The one and two-breeding comb treatment hives increased their honey and pollen reserves because they had a smaller population of bees, which allowed greater storage of these products. Similar results were observed by Hernández & Castellanos (2020) when analyzing the food reserves of honey bees in the winter season, observed that the honey and pollen area in small hives was significantly higher than that of hives with larger capacities, due to the larger population of bees in large hives that consume more quantities of pollen and honey. The situation in the control hives would be explained by the supply of sugar syrup and natural nectar flows being insufficient to meet the food needs of a high bee population.

Table 4. Effect of the number of brood combs on the increase of honey and pollen area (cm^2), evaluated at 28 days (control) and 44 days (one and two breeding comb treatments), values are expressed as mean ± SD.

Treatments	Honey reserve (cm^2)		Pollen reserve (cm^2)	
	Total	Per day	Total	Daily
Control	-399 ± 76	-14 ± 85	-529 ± 148	-18.9 ± 5.3 [b]
One breeding comb	2 660 ± 473	60 ± 11	146 ± 155	3.3 ± 3.5 [a]
Two breeding combs	2 584 ± 533	59 ± 12	261 ± 350	5.9 ± 7.9 [a]
p-value				<0.01

[a,b] Different letters between rows indicate significant differences ($p < 0.05$).

Hoover & Ovinge (2018) observed that honey production was restricted when there was inadequate pollen flow to the hive, without affecting brood production. These results were lower than those obtained by Khan et al. (2022) who reported daily honey and pollen increases of 95 and 43 cm^2 respectively in hives supplemented with sugar syrup.

Conclusions

New hives established from single brood comb gained equivalent daily weight to those formed from two brood combs.

New hives established from single brood comb had a smaller brood area than those formed from two brood combs.

New hives from single brood combs exhibited bee populations comparable to those formed from two brood combs.

New hives from single brood combs showed comparable honey and pollen reserves to those formed from two brood combs.

Compliance with Ethical Standards

Author contributions: Conceptualization: FAB, CVV. Data curation: JRA, SRA, HPA; Formal analysis: FAB, CVV, SCL; Methodology: FAB, JRA, HPA; Project administration: CVV; Resources: FAB, SRA, JRA; Supervision: CVV; Validation: SCL; Writing – original draft: FAB, CVV; Writing – review & editing: SCL, FAB, CVV.

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