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Original Research

Determination of the Effect of Live Weight on Various Quality Parameters of Commercially Reared Queens (*Apis mellifera* L.) in Turkey

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Abstract

The Mediterranean region in Turkey is considered highly suitable for commercial queen bee rearing due to its temperate climate characteristics. Therefore, in the research, the queens to be examined in terms of quality criteria were obtained from commercial queen bee enterprises in Antalya, Mersin, Adana, and Hatay provinces in the Mediterranean region. In April-May, 5 queen bees from each of the 36 enterprises rearing queens were included in the study. Characteristics such as queen weight, spermatheca volume and diameter, and spermatozoa stored in the spermatheca are considered quality criteria, and the factors affecting them were evaluated on a total of 180 queens. Queens taken from the enterprises were divided into three groups according to their weight; light (172.56±1.75 mg), moderate (193.70±0.62 mg), and heavy (214.13±0.95 mg). The average spermatheca diameters in the light, medium, and heavy groups were 0.982±0.006 mm, 1.053±0.002 mm, and 1.215±0.008 mm, respectively. According to the average results obtained regarding the quality criteria in the study, it was determined that 60.55% of the queens evaluated in the study (the heavy group) were at standard quality levels. On the other hand, in the light and medium groups, it was observed that all values were below the quality standard values. These results show that while evaluating the quality criteria in queen bee breeding enterprises, selection can be made according to the weight of the queen bees.

Keywords: honey bee, live weight, reproductive features, quality criteria, queen rearing

Introduction

Turkey is one of the leading countries in beekeeping in the world with its suitable climate, vegetation, over 8 million honey bee colonies, and over 100,000 tons of honey production in 2022 [1]. Although commercial queen breeding was started in the late 1970s and queen production has increased to over 400,000 over the years, queen breeding in Turkey is not sufficient to meet the demand. As a result, hundreds of queen breeders participate in the production chain every year to meet this demand [2].

The reproductive capacity of a colony's queen is important for colony strength and worker bee production

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[3-5]. For this reason, the most important member of a honey bee colony is the queen [5, 6], and queen quality is a critical factor for successful beekeeping [7]. In beekeeping, it is a very important advantage to start with the use of quality breeders and qualified queens for economical beekeeping [8]. The characteristics that will be transferred to future generations through queen bees are of great importance in terms of colony behavior and productivity [9, 10]. Many factors affect queen quality, including genetic and environmental factors. Genetic characteristics are transferred to worker bees through the queen and the drones that mate with her [11, 12]. Therefore, the most important factor affecting the quality of the queen bee is its genetic material. With selection methods such as family, combination, and index applied to this material, some characteristics of bees such as viability, docility, development in spring, hygienic behavior, swarming tendency, honey yield, wintering ability, and colony development are evaluated [10, 13-15].

Quality criteria in reared queen bees: we can evaluate this by looking at features such as queen weight at emergence, oviposition weight, spermatheca volume and diameter, stored spermatozoa in spermatheca, and the number of egg tubes [16-18]. Factors affecting the emergence weight of a queen bee: we can list them as breed (line), cell builder, age of larva grafting, number of queen cells in the grafting frame, and environmental conditions [19-22]. Research conducted by many researchers at different times and places indicates a high correlation between body weight at emergence and reproductive capacity [20-23]. On the other hand, some researchers divided the queen bees into 3 groups according to their body weight: heavy (200 mg and above), medium (between 190-200 mg), and light (190 mg and below), and accepted the queens with 200 mg or more as high quality [24, 25]. Many researchers agree that spermatheca capacity increases due to the decrease in larval age in queen rearing [8, 25, 26]. A queen's productivity and longevity depend on the queen's ability to store spermatozoa and the size of the spermatheca [27]. Researchers report that queen bee sperm sac size may be a quality factor, with more sperm being stored in a larger sperm sac [18, 27, 28].

A good-quality queen should have a high body weight, multiple ovaries, and a large spermatheca. In addition, the queen must be able to successfully mate and store enough live spermatozoa in the spermatheca sac and be resistant to diseases [28-30]. Since it is the source of the hereditary structure, the characteristics of the colony, such as strength, hard work, being cautious or aggressive, being susceptible or resistant to diseases, wintering ability, honey yield, tendency to swarm, and tendency to collect pollen and propolis, depend on the queen bee and the drones mating with her.

Queen bees obtained from commercial queen bee enterprises were grouped according to their live weights and examined in terms of quality criteria. In this study, it was aimed to determine concretely the effect of live weight on other quality parameters in queen bees obtained from commercial queen enterprises. Thus, the issues that both the queen bee producers and the beekeepers who purchase and use them in their colonies should pay attention to in terms of meeting the quality criteria have been determined.

Material and Methods

Fieldwork of Research

When purchasing queens from enterprises that reared queens in the Mediterranean region, at the same time, information about their enterprise's capacity, queen production method, and honey bee breeds they used was also collected. The mated queens to be used in the research were purchased in April-May from enterprises engaged in commercial queen rearing in the provinces of Antalya, Mersin, Adana, and Hatay, which form the coastline of the Mediterraneanregion. Twenty of these enterprises produce in Antalya, eleven in Mersin, one in Adana, and four in Hatay. These enterprises, which are engaged in queen-rearing activities, are enterprises with different production capacities (700-24,000 queens). It has been observed that all queen-rearing enterprises use the larva grafting (Doolittle) method. In this region, which was very suitable for queen bee production in the early period, 180 queen bees purchased from 36 enterprises constitute the main material of the study.

Laboratory Work of Research

After weighing the live weights of the egg-laying queens on a sensitive scale (mg level), three groups were formed based on their weight at the beginning of egg-laying (mg/queen). Queens weighing 185 mg and below were classified as light, those between 185 and 199 mg as moderate, and those weighing 200 mg and above as heavy. After the live weight measurements of the queen bees, the spermatheca of the queen bees were removed, and the tracheal network was cleaned (Fig. 1). Then, preparations were made for microscopic measurement of spermatheca, sperm count, and spermatozoa counting (Fig. 2 and Fig. 3).

Spermatheca of light, medium, and heavy queens were removed, and diameter was measured with a 4.5x10 magnification stereo microscope by means of an ocular micrometer without a tracheal net [8]. The number of spermatozoa and the volume of spermatheca were determined using the method described by [30-32].

To determine the total quantity of spermatozoa within the sperm sac of the queen bee, we calculated the number of spermatozoa in a 1 ml mixture contained within the square section of the Thoma slide. This calculation was based on the volume of the square section, which measures 1 mm x 1 mm x 0.1 mm, resulting in a volume of 0,1 mm^3.

Fig. 1. Removal of spermatheca from queen bee and cleaning of tracheal net.

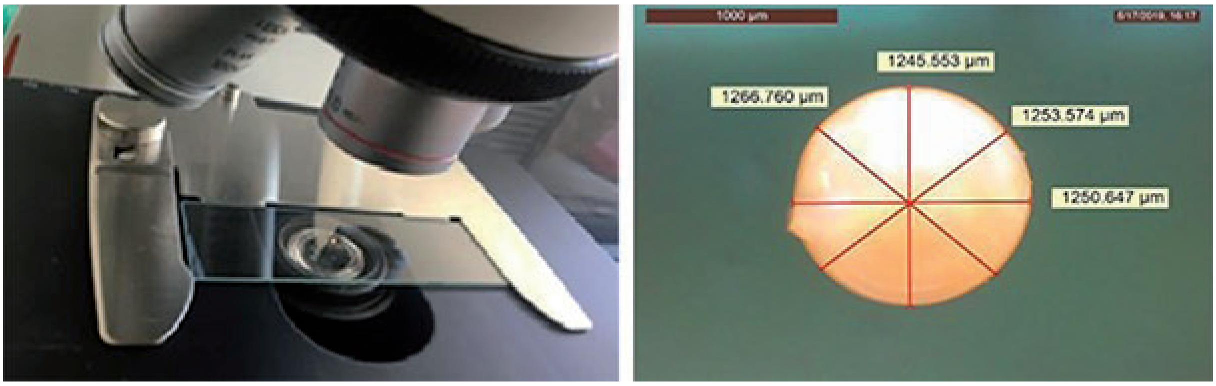

Fig. 2. Measurement of spermatheca with a microscope.

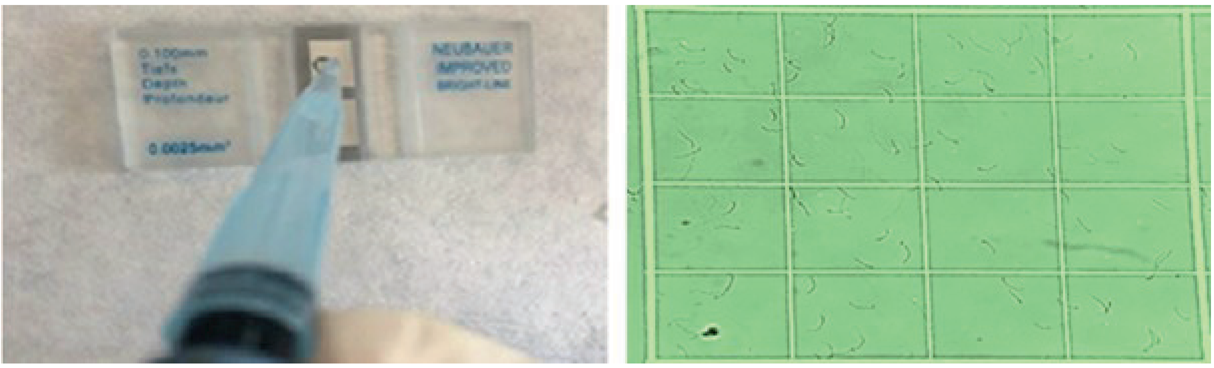

Fig. 3. Preparation for sperm count and spermatozoa count.

$$\text{The amount of spermatozoa in 1 ml of mixture} = \frac{\text{number of spermatozoa observed}}{\text{Number of square observed}} \text{x}10.000$$

As indicated in the provided formula, once the sperm quantity in 1 ml of the mixture is determined, the obtained result is then multiplied by a factor of 10 to calculate the total sperm quantity present in the sperm sacs of the queens [8].

Statistical Analysis

The normality of the data distribution was assessed through the Kolmogorov-Smirnov test. Subsequently, a one-way ANOVA analysis was conducted using the SPSS statistical program to examine the data, and comparisons of the means of significant features were carried out using Duncan's Multiple Range Test. In addition, PCA (Principal Component Analysis) was employed to elucidate the relationship between the study samples and the resulting analysis findings, as well as to uncover the distribution patterns within the data.

Table 1. The average values (mean ± standard error) of the parameters of the reproductive characteristics of the examined queen bees

Class ofqueens	N	%	Average weight of queens (mg)	Average diameter of spermatheca (mm)	Average spermathecavolume (mm^3)	Average number of spermatozoa ($\times 10^6$)
Light	25	13.88	172.56±1.75^c	0.982±0.006^c	0.497±0.009^c	1.658±0.770^c
Moderate	46	25.55	193.70±0.62^b	1.053±0.002^b	0.613±0.005^b	2.751±0.531^b
Heavy	109	60.55	214.13±0.95^a	1.215±0.008^a	0.954±0.020^a	5.087±0.132^a
Total	180	100	203.01±1.29	1.114±0.008	0.803±0.018	4.014±0.131

^{a,b,c} averages denoted by different letters different from each other, Duncan, (P<0.01), N:Number, mg: Milligram, mm: millimeter, mm^3: Cubic millimeter

Results and Discussion

Evaluation in Terms of Queen Weight

The effects of queen bee weights examined in the research based on quality criteria are summarized in Table 1.

It was determined that the live weight of the commercially produced queens, which were grouped as light, medium, and heavy, ranged from 145 to 248 mg, and the average was 203.01±1.29 mg (Table 1). The difference between queens in the light (172.56±1.75 mg), medium (193.70±0.62 mg), and heavy (214.13±0.95) groups was very significant (P<0.01).

The first two Principal Components (PC) explained 100.00% of the variance (PC1: 98.78%; PC2: 1.22%). It was determined that the heavy group of queen bees had a positive effect on the criteria considered as quality criteria. When Fig. 4. is examined closely, it is understood that when purchasing queen bees from commercial queen bee enterprises, preference should be made considering the characteristics of the heavy group (200 mg and overweight) in the research. It is seen that the heavy group meets the quality criteria accepted as 98.78%. (Fig. 4).

The average queen weight in the examined enterprises was determined to be 203.01±1.29 mg, and it was seen that 60.55 % of the total (heavy group) were within quality standards in terms of live weight (Table 1). High live weight in queen bees is preferred and considered a quality factor [20, 33, 34].

In light of these data, we can argue that one of the most important queen bee quality criteria is the live weight of the queen bee. However, the results of studies investigating the relationship between the queen bee's reproductive organs and external measurements such as the queen bee's thorax width, head width, and wing lengths are not compatible with each other. For example, while the number of stored sperm and mating frequency had a positive effect on thorax width [35], no effect was found on the number of ovaries, ovarian weight, or the number of matings [23, 34].

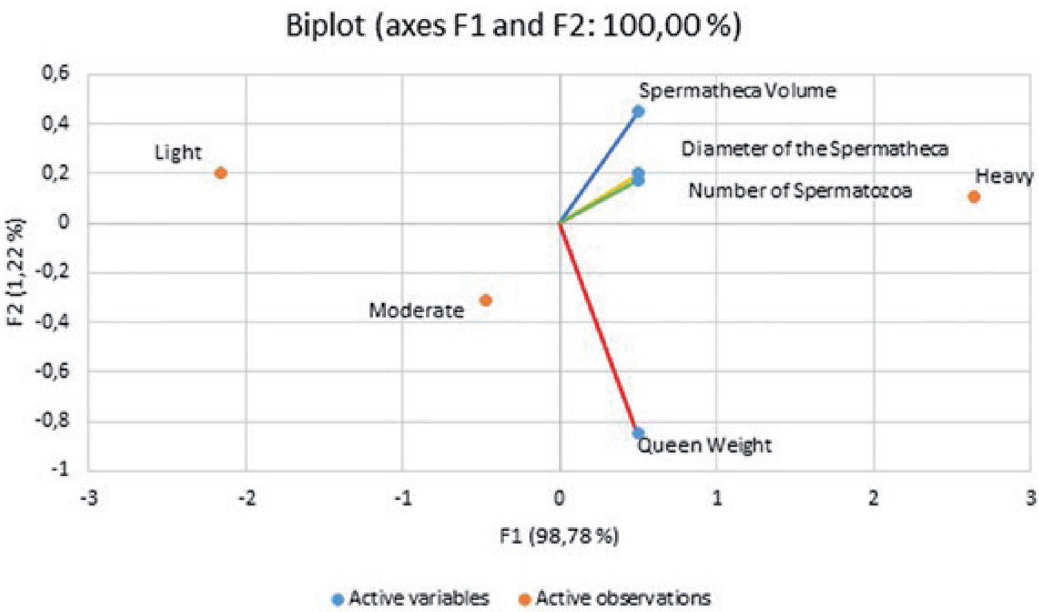

Fig. 4. Scatter plot showing the charge distribution of reproductive traits on the first two principal components.

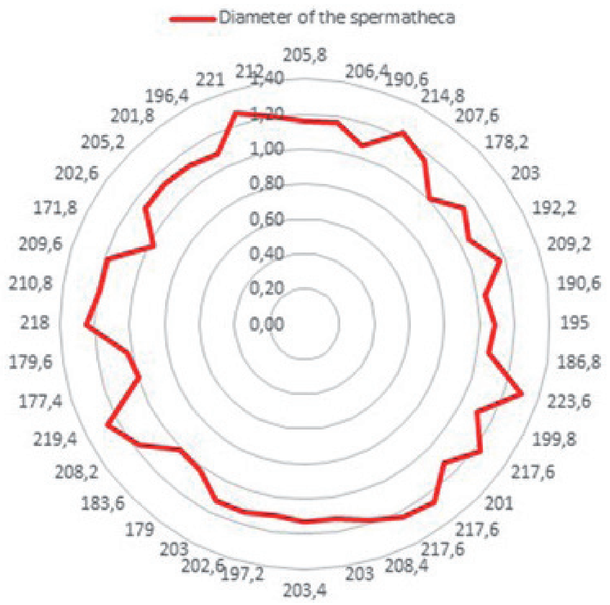

Fig. 5. Variation in spermathecae diameters according to queen weight.

Evaluation in Terms of Spermatheca Diameters

When Fig. 5 is examined in detail, as the live weight of the queen increases, spermatheca diameters also increase. It was found that spermatheca diameters ranged between 0.95 and 1.47 mm in queen bees obtained from commercial enterprises, and the mean spermatheca diameter was 1.114±0.008 mm (Table 1). The difference between queens in the light (0.982±0.006 mm), moderate (1.053±0.002 mm), and heavy (1.215±0.008 mm) groups was very significant ($P<0.01$). On the other hand, a $R2 = 0.877$ positive and linear relationship was determined between queen weight and spermatheca diameter (Fig. 6). According to the results obtained, it is seen that only the heavy group meets the standard values in terms of the examined feature.

Evaluation in Terms of Spermatheca Volume

The average spermatheca volume of queens obtained from commercial enterprises was calculated as 0.803±0.018 mm^3. This value was determined as 0.497±0.009 mm^3 in the light group, 0.613±0.005 mm^3 in the moderate group, and 0.954±0.020 mm^3 in the heavy group. The difference observed between the groups was very significant ($P<0.01$). According to the results obtained, it is seen that only the heavy group meets the standard values in terms of the examined feature (Table 1).

Evaluation in Terms of the Number of Sperm Stored

During this study, the average quantity of spermatozoa stored within the spermatheca of queen bees was determined to be 4.014±0.131 million. The number of sperm stored in the light, medium, and heavy groups was 1.658±0.770, 2.751±0.531, and 5.087±0.132 million, respectively, and the difference between the weight groups was very significant ($P<0.01$). In terms of the examined feature, it was determined that the heavy

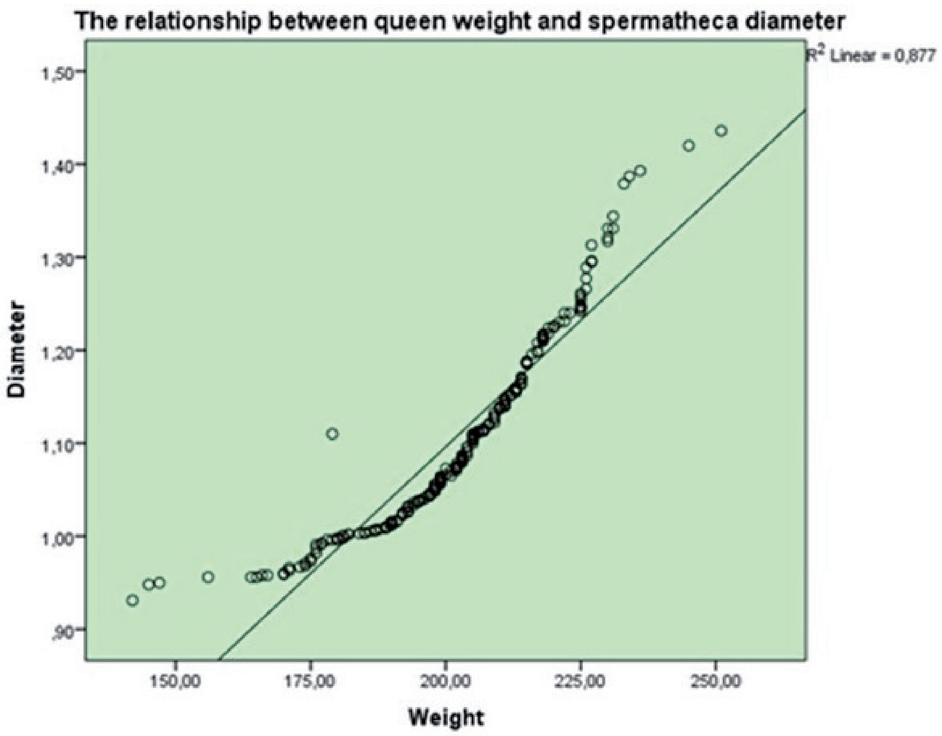

Fig. 6. The relationship between weight and spermathecae diameter.

group met the standards, and the other two groups were below the standard values (Table 1).

This study, which primarily concentrated on evaluating the quality parameters of queens reared by commercial breeders in the Mediterranean region of Turkey, has yielded a few noteworthy findings. The Mediterranean region's temperate climate, known for its suitability for commercial queen rearing, played a central role in this research.

When evaluated in terms of queen weight, according to the results of the study, it is seen that the heavy group meets the standard values for queen weight. The other two groups are below the standard value. Live weight (mg/queen), which is a primary quality characteristic of queen bees, is evaluated in three groups; light (less than 185 mg), medium (between 185-199 mg), and heavy (200 mg and above) (Table 1). Queen bees weighing 200 mg or more are of high quality [24, 29].

As a matter of fact, it has been determined that heavy queen bees can generally control the colony better, store more sperm, produce more egg protein, and lay more eggs [4, 22, 29].

The study revealed that the average live weight exceeded the previously determined average of 167.8 mg for various bee breeds in the Mediterranean Region, as reported in Arslan et al. (2015) [36]. It was determined that it was compatible with the average (206,23 mg and 191,04 mg) values of the queens raised in the spring period in Antalya and in the same enterprises [8].

Based on the average live weight determined in this study, it is evident that queen bees can be successfully reared to meet desired quality standards during the production period, specifically in April and May, within the Mediterranean region. This observation is in line with prior research, which consistently demonstrates that the spring months of April and May are conducive for producing high-quality queen bees. Conversely, it is established through previous studies that the months of August and September are not favorable for this purpose [8, 31, 32, 36].

When evaluated in terms of spermatheca diameters, in this study, the average diameters of spermatheca for the light, moderate, and heavy groups, respectively, were determined by Akyol et al. [24] and found to be compatible with the values (0.861±0.2 mm, 1.061±0.2 mm, and 1.258±0.2 mm) reported (Fig. 5 and Fig. 6).

On the other hand, for queens reared by the Doolittle method, the average value (0.98±0.1 mm) was found to be lower than the medium and heavy groups, while it was found to be compatible with the light group [37]. According to Hatjina et al. [29], the spermatheca diameter should be 1.2 mm and above in a quality queen. In this study, it is seen that only the values obtained from the heavy group are compatible with this result. According to these results, it was seen that the heavy group (60.55%), which constituted 60.55% of the evaluated queens, was following the quality standards in terms of spermatheca diameter. The diameter of the spermatheca, considered a crucial quality criterion, is reportedly influenced significantly by factors such as the season and the quality and quantity of pollen supplied to the colony. This observation is supported by studies conducted by Chuda-Mickiewicz and Samborski [38].

This study also provides the opportunity to evaluate queen bees in terms of spermatheca volume. While it is desired to have a spermatheca volume of 0.90 mm^3/queen and above in a quality queen [29], the results calculated for the heavy group in this study were found to be above this value. Woyke and Jasinski reported that the size of the spermatheca should not be ignored because the spermatozoa transferred from the oviducts to the spermatheca are related to the volume of the spermatheca rather than the amount of semen removed [18, 39]. On the other hand, it is reported that large queen bees have larger spermatheca volumes and store more sperm, which is also consistent with our study [34]. According to the results of the research, it was seen that only the heavy-group queen bees met the desired quality standards in terms of spermatheca volume. In this study, evaluation was also made according to the number of stored sperm (Table 1). In this study, the values obtained for the light and moderate groups Akyol et al. [24] were found to be lower than the values (4.15±0.1 million, 4.75±0.2 million) reported for the same group, while the value (5.19±0.2 million) obtained for the heavy group was found to be consistent with the mean value reported for the same group.

It has been reported that there are an average of 5.3 million spermatozoa in the spermatheca of naturally mating queen bees [34, 39]. On the other hand, as a quality criterion, the number of stored spermatozoa is required to be 5 million or more [29, 31].

When evaluated in terms of the number of spermatozoa stored in the spermatheca among various queen bee groups, it was observed that the heavy group, which represents 60.55% of the queens supplied from the enterprises, consistently met the quality standards, while the other groups did not reach this threshold.

One of the key findings of this study was the substantial influence of live weight on the quality criteria of queen bees. Queen bees were categorized into three weight groups: light, moderate, and heavy. Queens categorized as part of the "heavy" group, defined by a live weight of 200 mg or more, consistently demonstrated compliance with the established quality standards across various criteria. Specifically, the "heavy" group of queens, representing 60.55% of the total population, exhibited the highest adherence to quality standards, suggesting that selecting queens with a live weight of 200 mg or more can be an effective criterion for improving queen bee quality. This finding underscores the importance of live weight as a practical and reliable measure for both queen bee producers and beekeepers who purchase and utilize queens in their colonies.

Conclusions

In conclusion, this study contributes valuable insights to the field of apiculture, especially in the context of queen bee quality assessment. It highlights the practical utility of live weight as a selection criterion for producing high-quality queens, which is essential for robust and productive honey bee colonies. The results offer guidance to both queen bee producers and beekeepers, encouraging them to consider live weight as a vital factor in their queen selection processes. Furthermore, the study underscores the importance of raising not only high-quality queen bees but also quality drones, as the genetic makeup of both queens and drones significantly impacts colony performance. As the global beekeeping community faces ongoing challenges related to pollinator health and colony sustainability, improving the quality of queen bees becomes increasingly crucial for the industry's future success.

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Conflict of Interest

The authors declare that the research was conducted in an environment in which there was nocommercial or financial relationship with any institution or person that could be construed as a potential conflict of interest.

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