Scientific Paper

Relation between the nutritional and immunological status of the stingless bee livestock in two provinces of Cuba

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Abstract

Objective: To determine the relation between the nutritional and immunological status of the stingless bee livestock in two provinces of Cuba.

Materials and Methods: Sampling was carried out in July and December, 2019, in the 24 randomly selected beehives, 12 in each locality, in the Matanzas and Mayabeque provinces. From each of the beehives new rearing boards were extracted, from which six third-instar larvae were collected, as well as the corresponding larval food, in order to determine the nutritional and immunological status of the stingless bee livestock from both localities. The analysis and interpretation of the biological and climate variables was done depending on the purpose of the research through variance analysis, analysis of principal components and cluster analysis.

Results: The best results in the studied variables were obtained in December for the stingless bee apiaries located in both provinces. The existence of two components was identified, which explained 69,49 % of the variability of the data set. The first one of these components (PC1) explained in itself 50,72 %. In the clustering process three groups were formed. The analysis allowed to identify group III as the one with the best performance.

Conclusions: The relation between the nutritional and immunological status of the stingless bee livestock of Matanzas and Mayabeque was proven through the variables selected for this study. In addition, the evaluation period was the indicator that marked the behavior adopted by these animals and not the locality.

Keywords: *Melipona beecheii*, beehives, larvae

Introduction

The rearing of stingless bees (meliponiculture) is practiced throughout Meso-America, and although its most commercialized product worldwide is honey, it offers valuable resources, especially for family agriculture, constituting a sustainable low-cost activity. In addition, it provides benefits to the environment, due to the direct effect exerted by the pollinating action of bees on the improvement of biodiversity, acting upon a large variety of wild and agricultural plants (Quezada-Euán, 2018).

The Cuban stingless bee rearing activity has incipient development, which requires increasing the knowledge of its nature, biology, behavior, management and epidemiological situation (Martínez-Machado *et al.,* 2019). Because of the growing interest generated by the rearing of these bees in diverse population sectors, with possibilities of insertion in the animal husbandry sector of the country, since 2015 this activity has been progressively organized in Cuba. This is due to the fact that besides the added value this important ecosystem regulation service represents (Garibaldi *et al*., 2015), these bees have the particularity of showing an additional mechanism of buzz pollination, which makes them particularly effective in certain economically important cultivars.

This excellent pollinator has a highly developed social organization, which allows it to live in permanent colonies and adapt to management systems established by man, as a particular type of livestock with specialized productions (Lóriga, 2015). Like the other animal husbandry activities, the adequate management of this species that allows to reach optimum nutritional and immunological statuses is necessary, so that healthy stock is guaranteed.

Bee feeding depends on the available nutritional resources, such as pollen and nectar (Vaudo *et al*., 2015). The diet protein they obtain from pollen, provides the organism with essential amino acids for the synthesis of antimicrobial peptides, which are transported by the hemolymph. Thus, balanced feeding can influence positively as prevention tool against diseases, by stimulating

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the immunological system for the synthesis of immunoproteins (Sattler, 2001; Ponton *et al.,* 2013). Meanwhile, the carbohydrates present in the nectar constitute the energy source to develop the metabolic processes associated to the humoral and cellular immune system (Erler, 2014). According to Cotter *et al.* (2011), the connection between nutrition and immunity is close, because it has been observed in different animal species that the caloric restriction affects the immune function.

In Cuba studies have been conducted about the clinical and health status of beehives (Lóriga, 2015), as well as of the characterization of the feeding potential through the palynological analysis of the collected pollen (Leal-Ramos and Sánchez, 2013; Pérez, 2016). Yet, these results have been precise and independent, without investigating yet the relation between each of them. That is why the objective of this work was to evaluate the relation between the nutritional and immunological status of the stingless bee livestock of Matanzas and Mayabeque.

Materials and Methods

Characterization of the experimental areas. The study was conducted in Cuba, in two stingless bee apiaries constituted by rational beehives (with sizes adjusted to the biological requirements of the species), belonging to beekeepers with more than 10 years of experience in the activity. One apiary is located in the Pastorita zone, Matanzas municipality, Matanzas province. This facility is located at los 23°02'25'' North latitude and 81°30'58'' West longitude, at an altitude of 14 m.a.s.l. The other apiary is in the zone of the North People's Council of the Sa Nicolás municipality, in Mayabeque, at 22°47'13'' North latitude and 80°55'05'' West longitude, at an altitude of 34 m.a.s.l.

Experimental procedure. The sampling was carried out in July and December, 2019, on the 24 randomly selected beehives, 12 in each locality, according to Morales-Vallejo (2012). From each one of the beehives new rearing boards were extracted, which were placed inside previously identified nylon bags (name of the stingless bee apiary from which it came and beehive number). They were immediately transferred to the Biotechnology Laboratory of the Pastures and Forages Research Station Indio Hatuey to be processed. Then, the cells were uncapped from the boards to collect the larval food and six third-instar larvae, corresponding to each sampled beehive, for a total of 144 individuals, according to Pinto (2010), in order to determine the nutritional and immunological status of the stingless bee livestock of both localities.

Meteorological information. It was obtained from the provincial meteorological centers of Matanzas and Mayabeque (table 1). The recorded meteorological variables were compared with the historical mean of the last 15 years for each month.

Experimental measurements. For the nutritional status the biological variables total protein of the larval food (TPLF Bradford (1976); total body protein

Table 1. Climate indicators during the evaluation period.

Period	Minimum temperature, °C	Mean temperature, °C	Min RH, %	Mean RH, %	Max RH, %	Rainfall, mm
Matanzas						
July	26,5	29,4	61,9	73,8	83,8	4,9
Historical average	25,7	28,8	65,0	76,0	86,0	105,4
December	22,2	24,9	67,2	78,6	87,9	2,1
Historical average	21,7	23,9	67,0	77,0	87,0	49
Mayabeque						
July	23,2	27,2	53,5	79,9	95,6	8,5
Historical average	22,3	26,8	53,0	80,0	96,0	196,3
December	17,2	22,9	48,2	79,0	96,7	0,4
Historical average	16,9	22,0	53,0	80,0	96,0	30,0

Min RH: minimum relative humidity; Mean RH: mean relative humidity; Max RH: maximum relative humidity

(TBP) by Bradford (1976) with modifications, total body lipids (TBL) according to Barnes and Blackstock (1973) and weight, which was determined independently in a Sartorius scale, with accuracy of 0,1 mg, were taken into consideration. For the immunological status, the total protein present in the hemolymph (TPH) was quantified through the technique proposed by Bradford (1976). And finally, the climate variables (table 1) associated to these two statuses were minimum temperature (Min T), mean temperature (Mean T), minimum relative humidity (Min RH), mean relative humidity (Mean RH), maximum relative humidity (Max RH) and rainfall (R).

Mathematical analysis. An analysis that contemplated two studied factors was carried out, locality (Matanzas and Mayabeque) and period (abundance and scarcity of meliferous flora, determined by July and December, respectively), was conducted. For the biological variables variance analysis was used, according to simple classification model. The Student Newmal Keuls (SNK) multiple comparison test was used and the differences were declared significant at values of $p \leq 0{,}05$. Meanwhile, to explain the relation among the indicators that denote the nutritional and immunological status of the stingless bee livestock, a factorial analysis was done through the principal component analysis (PCA) according to the methodology proposed by Morrison (1990). In order to identify the variables (biological and climate) that had the highest influence on the variability extracted by each component, it was taken into consideration that the sum or preponderance factors reached a value higher than 0,70.

To arrive at more integral and objective considerations from all the study, a cluster analysis was performed, which allowed to group the beehives with similar characteristics, depending on the evaluated variables. As grouping criterion, the Euclidian Distance and Ward method were used, as a form of ascending hierarchical aggregation (Torres *et al.,* 2006). To identify the beehives, codes were established composed by roman numerals, which define the combination between locality and period (table 2), followed by the real label of the sampled beehive (table 5). In all cases, the program SPSS Statistics® version 22.0 for Microsoft Windows (IBM Corp., 2013), was used.

Results and Discussion

Table 3 shows the studied variables. In the two sampled localities, the total protein of the larval food and total protein of the hemolymph reached the highest values in December, without significant differences between them. The same did not occur in July, when significant differences were observed between these variables. This result can be explained from the fact that the total protein of the

Table 2. Combinations used to design the beehives belonging to each group formed in the cluster analysis.

Code	Locality	Period
I	Matanzas	July
II	Matanzas	December
III	Mayabeque	July
IV	Mayabeque	December

Table 3. Effect of the studied locality and period on the nutritional and immunological status of the stingless bee livestock.

Variable		TPLF, g/100g	Weight, mg	TBP, g/100g	TBL, g/100g	TPH, μg/mL
Locality	Period					
Matanzas	July	15,19[b]	76,30[b]	30,47[c]	6,53[d]	524,10[b]
	December	19,89[a]	81,27[b]	32,50[b]	12,42[b]	3961,37[a]
Mayabeque	July	14,32[b]	76,13[b]	31,33[c]	8,43[c]	911,28[b]
	December	20,70[a]	89,11[a]	35,60[a]	14,32[a]	3517,99[a]
SE ±		0,911	0,080	0,322	0,571	193,553

TPLF: Total protein of the larval food; TBP: total body protein; TBL: Total body lipids; TPH: total protein in the hemolymph.
Means followed by different letters in the column indicate significant differences among treatments, according to Student Newman Keuls test ($p \leq 0{,}05$).

hemolymph increases, as the total protein present in the larval food increases. According to Cremonez *et al.* (1998), there is a direct relation between the protein contained in the hemolymph of bees and the protein contribution of the food they consume from the pollen they collect.

In larval feeding, pollen plays a fundamental function (Corona Apicultores, 2012). According to Vollet-Neto *et al.,* (2010), the larval food of stingless bees is constituted by large quantities of pollen, which in the case of *Melipona marginata* reaches 50 % of its total volume (Rensi, 2006). Subsequently, larvae are extremely dependent on the protein present in the larval food that comes, to a large extent, from pollen. The protein content affects adult as well as immature individuals, and a low quantity of protein can weaken the colony (Brodschneider and Crailsheim, 2010).

With regards to the variable weight (table 3), the highest value was reached in December, in the Mayabeque locality, and differed significantly from the record in July. Nevertheless, Mayabeque did not show significant differences in both evaluated periods. Meanwhile, the variables that determine the degree of body reserves (table 3) of the larvae (TBP and TBL), in the case of the total body protein in July, for both localities, did not show significant differences between them, but it did with regards to December, month in which the highest value was reached in Mayabeque. Regarding the total body lipids, the best result was also visualized in the Mayabeque locality in December, which differed from the statistical point of view with regards to the other studied periods. According to Moret and Schmid-Hempel (2000), these reserves are very important for bees, because their capacity of immunological response to any pathogen agent or stress situation depends, to a large extent, on their nutritional status.

To understand the relation among the biological variables that have incidence on the nutritional status of the stingless bee livestock, it is necessary to know that insects are not capable of synthesizing sterols. Thus, they obtain them from the food they consume. In the case of bees, they incorporate them through the pollen that contains sterols (less than 0,5 %), which are essential for metabolism, because they act as precursors of cholesterol to generate fat reserves in the body of the animal, acting at times of food scarcity and being eventually transformed into glucose. These lipids should be absorbed by the intestine and become others, according to the needs and diets of each species (Clark and Block, 1959). It should be emphasized that the lipid constituents and the spectrum of fatty acids can make up to 20 % of the weight of the adult bee (Corona Apicultores, 2012).

According to Kleinschimdt (1990), besides lipids, proteins are necessary for the growth, development and maintenance of the body structures of all living beings, because they are present as tissue constituents, and fulfill functions as biological catalysts in numerous metabolic pathways. Proteins are essential for feeding the larvae and the whole development of young bees, as well as for repairing the cells and organs of older bees.

The variables described in table 3 are closely related, because the characteristics of protein feeding, in quality as well as quantity, during the larval and juvenile stage, influence directly the body weight and reserves at the level of fatty bodies (Corona Apicultores, 2012).

In general, the best results in all the studied variables were obtained in December, for the stingless bee apiary located in Matanzas as well as for the one in Mayabeque. These results are in agreement with the performance expected for that period, because, according to Köppen classification, the climate of Cuba is considered tropical warm, seasonally humid, with a dry season (DS) that extends from November to April, and a rainy season (RS) from May to October (Academia de Ciencias de Cuba, 1989).

Unlike what occurs with the remaining Cuban livestock, for which the rainy season is more favorable for its feeding, due to the abundance of edible biomass; bees, which obtain their food from flowers, limit their consumption as consequence of the fact that flowers are scarce, precisely in the RS. Rainfall in full flowering is deleterious because the flowers fall to the soil, which affects their availability. This is in addition to the fact that bees cannot go out to forage, for which this phase is considered critical, catalogued as famish in this livestock. On the contrary, December (DS) is characterized by floral abundance. In this season, the flow of nectar and pollen that enters the beehive is higher, and this allows to obtain the necessary food for the maintenance of the colony and thus guarantee a reserve to face the RS (Pérez-Piñeiro, 2017).

Table 4 shows the results of the principal component analysis through which the existence of two components was identified, which explain 69,49 % of the variability of the data set; although it is important to emphasize that the first of these components

Table 4. Matrix of preponderance factors between the principal components (PC) and the climate and biological factors associated to the nutritional and immunological status.

Variable	PC1	PC2
Total protein in the hemolymph (TPH)	0,697	0,481
Total body protein (TBP)	**0,745**	0,065
Total body lipids (TBL)	**0,725**	0,264
Weight	0,459	0,035
Total protein larval food (TPLF)	0,468	0,428
Minimum temperature (Min T)	**-0,974**	0,106
Mean temperature (Mean T)	**-0,965**	-0,082
Minimum relative humidity (Min RH)	-0,537	**0,685**
Mean relative humidity (Mean RH)	0,688	-0,464
Maximum relative humidity (Max RH)	**0,720**	**-0,685**
Rainfall (R)	-0,649	**-0,636**
Proper value	5,58	2,07
Explained variance, %	50,72	18,78
Accumulated variance, %	50,72	69,49

(PC1) explains in itself 50,72 %. PC1 is closely related to the variables total body protein, total body lipids, maximum relative humidity and minimum and mean temperature. Meanwhile PC2 was related to the climate variables, particularly to minimum and maximum relative humidity, as well as to rainfall.

These results are logical, because, during the days following birth, larvae experience consecutive molts that contributes to their growth (larval evolution). The duration of each one of them is subject to the performance of the climate variables, mostly influenced by temperature and relative humidity. The correct development of this process is established for a temperature inside the beehive of 30-35 ºC. However, when the environmental conditions are unfavorable, with lower temperatures than this range, the days between molts can increase and delay the biological cycle of the bee (Milum, 1930). Besides, temperature influences strongly larval growth and respiration (Petz *et al*., 2004).

According to Canaviri and Velasco (2005), environmental factors affect honey production, because they influence the secretion of nectar and the behavior of the colony. In the case of rainfall, the rains in full flowering are deleterious for the bees, because they cannot go out to forage, for which the flow of entrance of pollen and nectar to the beehive decreases, having negative repercussion on larval development.

The biological variables analyzed in the larvae that were sampled have direct repercussion on the survival of the newly-emerged adult bee. And this is explained, according to Vidal and Bedascarrasbure (2002), because when a worker bee emerges, its life expectancy can vary depending on seasonal factors. Among the ones that stand out are, fundamentally, food availability and reserves of body proteins and lipids of storage in the larval period. In addition, the body protein of bee is a good measure of the capacity of beehives to survive and overcome diseases. The higher the level of body protein is, the more capable bees are to produce honey (Kleinschmidt, 1998).

The climate and biological variables, associated to the nutritional and immunological status, explained, approximately, 70 % of the total variability in the PC analysis. Nevertheless, to find the groups that presumably exist when combining all the biological variables evaluated in the two localities and in both periods, taking the beehives as basis of the analysis, it is necessary to do a cluster analysis (table 5), which caused the formation of the groups. According to Hair *et al.* (1999), this is a quantitative estimator, which describes the degree of association or similarity between the compared elements.

As shown in table 5, three groups were formed. Group I was constituted by 17 beehives, 10 of them from the Matanzas locality, and seven from Mayabeque, represented 35,42 % of the sampled total. It

Table 5. Groups formed by the cluster analysis.

Dissimilarity coefficient	Formed groups	Quantity	Beehives
1512,90	I	17	I-1, I-11, I-12, I-13, I-3, I-4, I-5, I-10, I-22, I-68, III-14, III-48, III-6, III-5, III-12, III-1, III-16.
	II	19	I-9, I-2, II-12, II-13, II-10, II-68, III-2, III-8, III-9, III-10, III-11, IV-14, IV-48, IV-6, IV-5, IV-11, IV-12, IV-13, IV-16.
	III	12	II-1, II-9, II-11, II-2, II-3, II-4, II-5, II-22, II-2, IV-8, IV-9, IV-10.

should be stressed that all the evaluations that were found in this group corresponded to July. Group II was integrated by 19 hives, 13 from Mayabeque and six from Matanzas, for 39,58 %. This group could be catalogued as mixed, because the December period prevailed with regards to July. On the other hand, III was the smallest one, having 12 beehives, in which the Matanzas provenance (8) prevailed compared with Mayabeque (4), for 25 %. In this last one, the evaluations belonged only to the December period.

The analysis of the results that are shown in table 6 allowed to identify group III as the one that exhibited the best global performance of the studied variables, by showing the highest average values of TPH (5 055,17 µg/mL), TPLF (19,73 g/100g), TBP (33,65 g/100g), TBL (13,43 g/100g) and weight (82,51 mg). This fact was influenced by the climate conditions of December, which in this case are favorable, because it is framed in the DS, characterized by the abundance of meliferous flora, as explained above. This group was followed by II, which was subject to the influence of both periods, but with predominance of the evaluations corresponding to December with regards to those of July. Meanwhile, group I had the worst performance, as it was subject to the influence of the RS and to the unfavorable conditions of July, which bring about floral scarcity in general.

Conclusions

The existing relation between the nutritional and immunological status of the stingless bee livestock of Matanzas and Mayabeque was detected through the variables selected for this study. The evaluation period was the indicator that marked the behavior adopted by these animals and not the locality, due to the prevailing climate conditions during the research stage, which had a marked influence on the data variability.

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Conflict of interests

The authors declare that there is no conflict of interests among them.

Authors' contribution

- Leydi Fonte-Carballo. Carried out the execution of the experiments and wrote the manuscript.
- Marlen Navarro-Boulandier. Developed the statistical processing of the data and participated in the interpretation of the results.
- Maykelis Díaz Solares. Designed the experiments and selected the protocols to be worked in the research.
- Walberto Lóriga-Peña. Supervised the research.
- Jorge Demedio-Lorenzo. Supervised the research.
- Marianny Portal Rodríguez. Participated in the experimental stages**.**

Table 6. Average of the variables and standard deviation of the groups formed in the cluster analysis.

Variable	Group I		Group II		Group III	
		SD		SD		SD
TPH	345,75	234,51	2128,26	744,38	5055,17	810,53
TPLF	13,76	4,22	19,52	5,45	19,73	7,25
TBP	31,10	1,91	32,96	3,06	33,65	2,60
TBL	6,59	3,10	11,96	3,79	13,43	4,12
Weight	76,44	12,98	82,50	9,51	82,51	9,42

PH: total protein in the hemolymph, TPLF: Total protein of the larval food; TBP: total body protein; TBL: Total body lipids.

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