Scientific Paper

Quantification of free proline in the diet of the stingless bee livestock of the Matanzas and Mayabeque provinces▲

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Abstract

An optimum nutrition through the consumption of a balanced amino acid-rich diet is essential for the good functioning of the immune system of animals. For such reason, the objective of this work was to determine the quantity of free proline, present in the consumed feedstuffs (pollen and honey) by the stingless bee livestock in the Matanzas and Mayabeque provinces. To determine the free proline through spectrophotometry, 60 g of pollen were taken, from sealed amphorae, and 80 mL of honey in each of the ten randomly chosen sampled beehives (five per province). After verifying that the data fulfilled the assumptions of variance homogeneity and normal distribution, a simple variance analysis was performed. The means were compared by Duncan's test, for a significance level of 5 %. For all the analyses the statistical package Infostat®, version 1.1, was used. The results indicated that, from the statistical point of view, the quantity of free proline found in the honeys from both provinces significantly differed. Meanwhile, the free proline values found in the pollen from Matanzas, were in the range from 4,2 to 19,5 mg/g. The one from Mayabeque showed values from 5,9 to 7,1 mg/g. It is concluded that the honeys, like the pollens, which constitute the feeding source of the stingless bee livestock of both provinces, have an adequate proportion of free proline. Thus, the livestock located in both zones has the necessary amino acid support in its diet to develop adequate immune response to any microbial agent.

Keywords: amino acids, honey, pollen

Introduction

In Cuba, the stingless bee livestock is composed by all the managed hives, belonging to the species *Melipona beecheii* Bennett, 1831 (Apidae: Meliponini), registered or not (Lóriga, 2015). As in any other flock, the maintenance of the animals' health by means of the defense mechanisms (collective and individual) provided by the immune system, has vital importance.

In bees, this system has cell and humoral components (Bulet *et al*., 1999). The cell defense mechanisms are mediated by hemocytes, which reside in the hemolymph and participate especially in phagocytosis and encapsulation (Chapman, 2013). The humoral response involves the synthesis of antimicrobial peptides, which act in response to infections caused by bacteria, fungi and parasites (Gätschenberger *et al*., 2013).

According to Gutiérrez and Orduz (2003), among the most studied antimicrobial peptides is abaecin, present in the hemolymph of bees, composed between 34 and 39 amino acids. Proline, which stands out among these amino acids, has marked antimicrobial activity against gram positive and gram negative bacteria.

In order to function, biological systems need nutritional elements that come from a set of foodstuffs supplied through the diet. In bees, they correspond, mainly, to pollen and nectar (Brodschneider and Crailsheim, 2010). They are both highly important for the immunological system of these insects (Di Pasquale *et al.,* 2013).

From the above-explained facts, due to the importance of this contribution of these amino acids in the diet of bees for the good functioning of their immune system, the objective of this work was to

▲Paper presented in the 5th International Convention Agrodesarrollo 2019 celebrated on October 22-26, 2019. Plaza America Convention Center. Varadero, Cuba

determine the quantity of free proline, present in the foodstuffs (pollen and honey) consumed by the stingless bee livestock in the Matanzas and Mayabeque provinces.

Materials and Methods

Location. The study was conducted in two meliponaries. One is located in the Pastorita zone, Matanzas municipality, Matanzas province, and the other is in the zone of the North People's Council of the San Nicolás Municipality, in Mayabeque.

Samples. Ten beehives were randomly selected, five in each site. In Mayabeque the hives 14, 2, 48, 5 and 6 were chosen. In Matanzas, 1, 9, 11, 12 and 13 were selected. In each of the beehives, 60 g of stingless bee pollen and 80 g of honey were taken from sealed amphorae. The sampling was performed in April, 2018.

Calibration curve. It was established through the utilization of proline patterns of different concentration, subject to the same process indicated for the samples. The calibration curve was elaborated with the absorbance data of the patterns from the different proline concentrations and the equation of the calibration straight line.

Free proline determination in honey. A quantity of 2,5 mg of homogenized honey was weighed, with accuracy of 0,1 mg approximately. They were dissolved in water through the use of magnetic agitator. Afterwards, they were transferred to a 5-mL gauged balloon flask, and the dissolution was leveled. Later, 0,5 mL were taken and 0,25 mL of 98 % formic acid (H-COOH), from the laboratory material supplier PROLABO, and 1 mL of 3 % ninhydrin solution, were added to it. Likewise, a blank was prepared, in which the work solution was substituted by 0,5 mL of distilled water. Afterwards, the tubes were closed, agitated and maintained for 15 minutes in boiling water bath. Once this process was over, they were taken from the hot water and cooled at ambient temperature during 5 minutes. Five mL of the isopropanol-water solution were added and it was strongly agitated. Finally, the absorbance was measured at 517 nm with regards to the blank, which was treated equally in the 35 minutes that followed cooling.

Calculations and expression of results. If C is the µg/mL of proline, obtained from the calibration curve, and P (g) is the weight of the honey that was used in the sample object of analysis, then:

µg proline= 5C

mg/kg of honey= 5C/P

Determination of proline in pollen. The free amino acids were extracted. For such purpose, 0,25 g of stingless bee pollen were weighed with accuracy of 0,1 mg, approximately, and 25 mL of 80 % ethanol were added. Then, during 30 seconds, the dissolution was disaggregated with the sonicator. Later, they were kept in agitation during 15 minutes before centrifuging (5 min. at 10 000 r.p.m.) and decanting the supernatant. This extraction procedure was performed twice more on the precipitate. The resulting ethanol extract was dried in the rotary evaporator, without exceeding 40 ºC. Afterwards, the residue was re-suspended in ultrapure water and leveled at 25 mL. For its filtration, millipore filters of 0,45 microns were used. Finally, 0,5 mL of the filtrate were taken and the same procedure as for the determination in honey was followed.

Calculation and expression of results. If x is the proline concentration, measured in the calibration line, then:

µg pro/mL= *x*

In the 25 mL, mg pro= *x* x 25/1000 = C

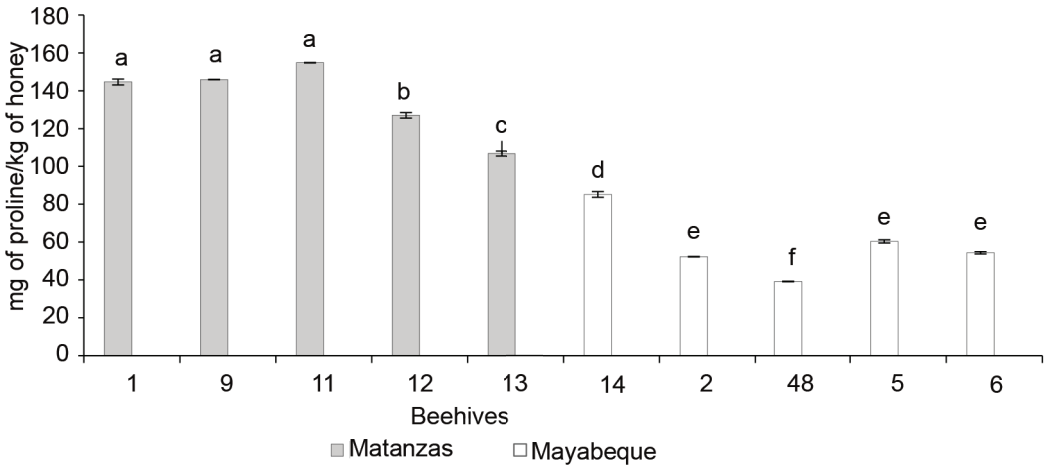
In P (g) of pollen, mg proline/g of pollen = C/P

Statistical analysis. After verifying the fulfillment of the variance homogeneity through Levene's test, and after normal data distribution, according to Sahpiro-Wilk, a simple variance analysis (ANOVA) was performed. The statistical package Infostat®, version 1.1, was used. For mean comparison, Duncan's multiple range test was made for a significance level $p<0,05$.

Results and Discussion

As shown in figure 1, the quantity of proline found in the honeys from both provinces varied considerably, with values between 39,2 mg/kg (beehive 48) and 154,8 mg/kg (beehive 11). From the statistical point of view, there were also significant differences between the honeys from the two provinces, with the exception of hives 1, 9 and 11 from Matanzas, and 2, 5 and 6 from Mayabeque. This could have been influenced by the fact that free amino acids come, mostly, from the floral resources that the bees visit selectively. This criterion coincides with the report by Truzzi *et al.* (2014), who found differences in the proline quantities, according to the botanical provenance.

In Chile, similar results were obtained by Sanhueza Rojas (2016), when analyzing samples of honeys from *Apis mellifera* Linnaeus, 1758. This author reported a large variability in the proline values, even for samples from the same sector and



Different letters indicate significant difference for $p \leq 0,05$..

Figure 1. Quantity of proline in the honey from the Matanzas and Mayabeque beehives.

similar floral origin. This is explained because, in the particular case of honey, an important part of proline is contributed by the bee, and it has its origin in the pollen consumed during the first stages of its life (Crane, 1990).

According to Bosi and Battaglini (1978), the genuine *Apis mellifera* honeys should contain a minimum of 180 mg of proline/kg. However, it is necessary to consider that there are large variations, according to the honey type and the genus of the bee that produces it. In this study, none of the sampled honeys exhibited proline quantities close to that value.

Pollen, according to Brodschneider and Crailsheim (2010), is one of the most important components of the bee diet, and constitutes the main protein/amino acid source. As shown in figure 2, the proline values found in the pollen from Matanzas were in the range from 4,2 to 19,5 mg/g. Beehive 12 stood out, where the highest proline quantity was found, statistically differing from the one found in the other hives. Beehive 9 showed the lowest result, although it did not differ statistically from 11, and from all the beehives belonging to Mayabeque.

On the other hand, no significant differences were found in the proline concentration in the Mayabeque beehives (figure 2). The values were between 5,9 and 7,1 mg/g.

These results are within the parameters reported by Baldi Coronel *et al.* (2004) in bromatological studies of Argentinean pollens, in which proline values were found from 2,30 mg/g to 24,30 mg/g. Generally, proline is the most important free amino acid in mature pollens. The same does not occur in the recently collected ones.

Conclusions

The results confirm that the honeys as well as the pollens, which constitute the feeding sources available for the stingless bee livestock from both provinces, have an adequate proline proportion, although the beehives from Matanzas showed higher production of that substance. Thus, the stingless bee livestock, located in the Matanzas and Mayabeque provinces, has in its diet the necessary amino acid support to develop an adequate immune response to any microbial agent.

Acknowledgements

The authors thank the Swiss Development and Cooperation Agency (SDC) for its contribution to fund this research through the BIOMAS-Cuba project, in its stage III.

They also thank the research group of the Pharmacy School, of the Nutrition and Bromatology area, University of Salamanca.

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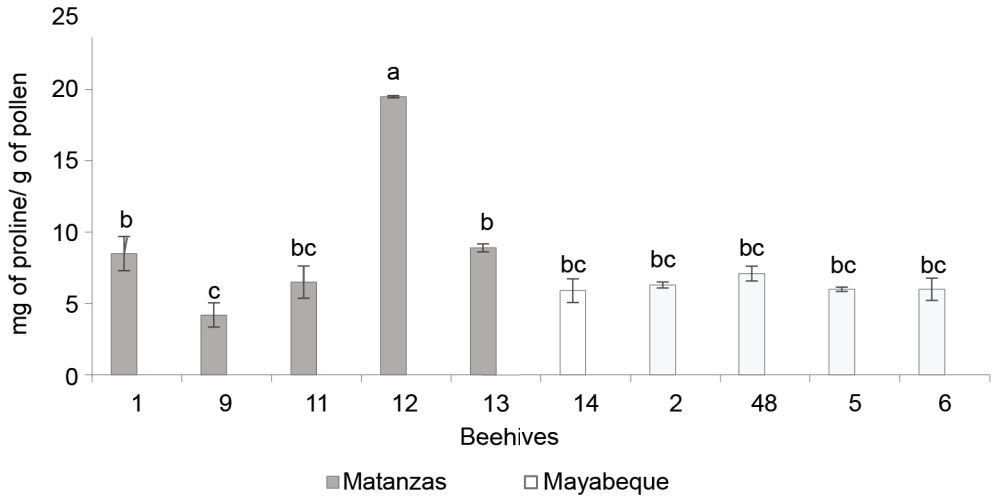

Different letters indicate significant differences for $p \leq 0,05$.

Figure 2. Quantity of proline in pollen on dry basis, from the Matanzas and Mayabeque beehives.

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Received: July 18, 2019
Accepted: August 26, 2019