

# Effect of supplemental feeding at various pH on the levels of Vitellogenin expression and proteins in fat bodies of *Apis mellifera*

Sarlo, E.<sup>1</sup>; Quintana, S.<sup>3</sup>; Medici, S.<sup>1,2,3</sup>; Eguaras, M.<sup>1,2</sup>

<sup>1</sup> Laboratorio de Atropodos, Universidad Nacional de Mar del Plata

<sup>2</sup> CONICET

<sup>3</sup> Fares Taie Instituto de Análisis

## Introduction

Vitellogenin (Vg) is the most abundant protein in hemolymph of *A. mellifera* L. and is synthesized only by bees during their first 7 to 10 days of emerged (1). Vg expression in the first four days of life determines the age to begin foraging and whether it preferentially forages for nectar or pollen (2). Honey bee longevity, brood rearing, honey production and resist diseases is reduced when protein availability is inadequate (3;4). In Argentina, the demand for intensive beekeeping submit the colonies to a premature development in an environment of little or no availability of pollen and honey. In view of this situation and others where nutrients are temporarily available, it is essential to supplement with sugar syrup and aminovitaminics compounds. The aim of this work was to determine if the supply of a amino-vitaminic supplement Apipromotor® in sugar syrup at two different pH values affects Vg expression and protein content in honeybees fat bodies.

## Methodology

Six groups were studied: Group 1: bee bread + syrup pH 7,48, Group 2: bee bread +syrup pH 5.86, Group 3: syrup pH 7,48; Group 4: syrup pH 5.86; Group 5: Apipromotor® + syrup pH 7,48; Group 6: Apipromotor® + syrup pH 5,86. At each time of study (0 (T0), 5 (T1), 10 (T2) and 21 (T3) days), 6 bees were taken from each group. RNA was isolated from the abdomen and Vg expression level was quantified by Real Time RT-PCR method, using EvaGreen® as intercalating dye (Fig. 1). Beta actin served as a housekeeping gene (Fig. 2). Relative quantification analysis was performed using ddCt method. Total protein titer was performed by Bradford method.

## Results

The results showed that the protein supplement a different pH had the same effects that bee bread, having a high Vg expression at the first days of life and then declining progressively. Control groups (3 and 4) had an increment in Vg levels at day 10 and then declines together with the other groups (Fig. 3). The same situation was observed when the total protein content was quantified. Total protein content was significantly superior in groups treated with bee bread and Apipromotor® compared to controls (Fig. 4). The acidification didn't produce significant differences

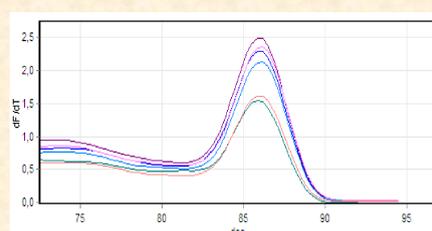


Fig. 1: Vitellogenin melting curve (Tm 86°C)

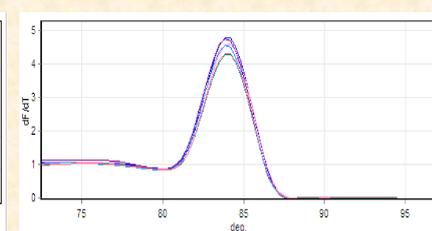


Fig. 2: Beta Actin melting curve (Tm 84°C)

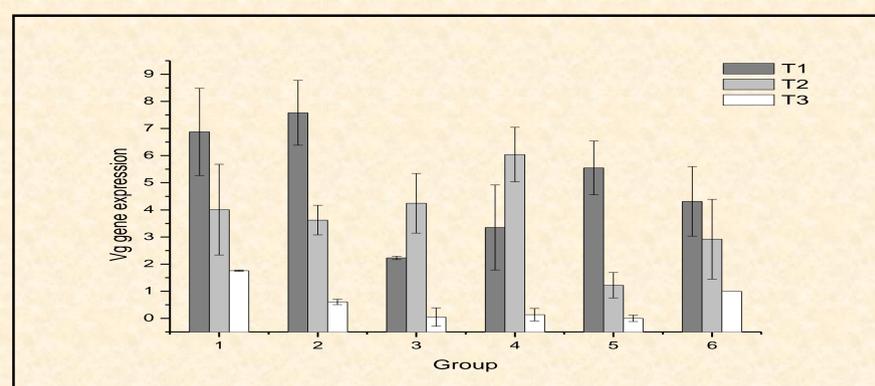


Fig. 3: Vg gene expression for each group at the different times (T) studied

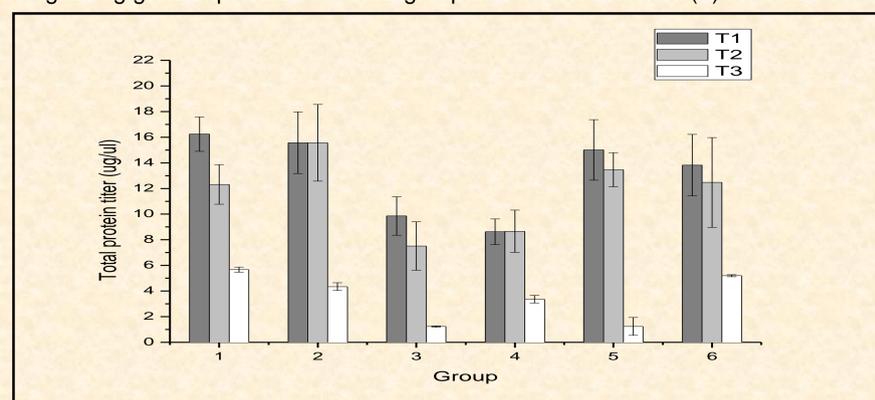


Fig. 4: Total protein content for each group at the different times (T) studied

## Conclusion

In the absence of a protein or aminoacidic source the organism responds lately to the need for a synthesis of Vg by increasing levels of messenger. This increase is not reflected in total protein titers.

These results suggest that the use of a protein supplement complete in free aminoacids (Apipromotor®) has a similar effect to the bee bread in *A. mellifera*, and it could be used as a substitute when there is a shortage of it. Vg gene expression proved to be an objective method to compare the effectiveness of protein diets and pollen quality, being also a faster and less expensive method that could easily be used for routine analysis.

## References

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