NATURAL POLLEN DIETS AND THEIR EFFECTS ON HEMOLYMPH PROTEIN LEVELS IN HONEY BEES (HYMENOPTERA: APIDAE)

Vanagas, L[1]; Basualdo M[2]; Oliva M, Rodríguez EM[2]; Solana H[2] and Bedascarrasbure, E[1,2].

1. INTA.

2. Fac. Cs. Veterinarias-UNCPBA. Campus Universitario. 7000 Tandil, Bs.As. Argentina.

Nutrition plays a fundamental role in the prevention of diseases by maintaining the physiological balance and favouring the expression of defence mechanisms against pathogens (1). The protein status of a bee seems to be the major determinant of its longevity. This protein status is mainly determined by the quantity of protein in its fat body and hemolymph, and the most important component protein is the vitellogenin (2).

The availability and quality of pollen sources is very important for colony development. In this work we used pollen obtained with pollen traps in an area where agricultural practices have led to an impoverishment of the flora (3). Using natural pollen with different protein concentration as diets we were able to compare the protein status in individual bees by determining the quantity of protein in hemolymph and the vitellogenin presence.

OBJECTIVE

To compare the protein levels and vitellogenin presence in hemolymph in bees of controlled age fed with natural pollen of different protein concentrations in laboratory conditions.

MATERIALS AND METHODS

Honey bees from an experimental apiary in Buenos Aires Province were forced to controlled birth in a special cage designed to keep the frame inside the colonies. Newly born bees were placed in wooden cages and transferred to in incubator with controlled temperature (33-34°C). These were fed with sucrose solution and water ad-libitum. Treatments were: three cages with a mixture of pollen of high protein concentration (HPC 25%), three cages with a mixture of pollen of low protein concentration (LPC 10%) and two cages with non-protein diet (control). Total crude protein of each diet was previously quantified by Kjeldahl (4).

Around 30 bees were sampled at different time intervals (0 – 24 hs; 2; 6; 9; 13; 16; 21; 25 days). Hemolymph was taken from bees with Drummond microcapilars (previously washed with 0.1% phenylthiourea). Samples were kept at -20°C until used.

Total protein concentration was determined using Coomasie Brilliant Blue G 250 (5) using bovine seroalbumine (BSA) as standard. SDS-PAGE was performed in 7% gels and then coloured with Coomasie Brilliant Blue R-250, decoloured and scanned. Western blots were performed with a primary antibody anti-vitellogenin diluted 1:2500 in PBS. The reaction was revealed with 3, 3`-diaminobenzidine.

Data were analyzed using a mixed model with repetitive measurements with PROC MIXED of Statistical Analysis Systems, Version 9.1.3 (SAS, Institute Inc., Cary, NC, USA).

RESULTS

Consumption was evaluated at the moment of taking samples by weighing food, and was similar (around 1.45 g/day) for three treatments.

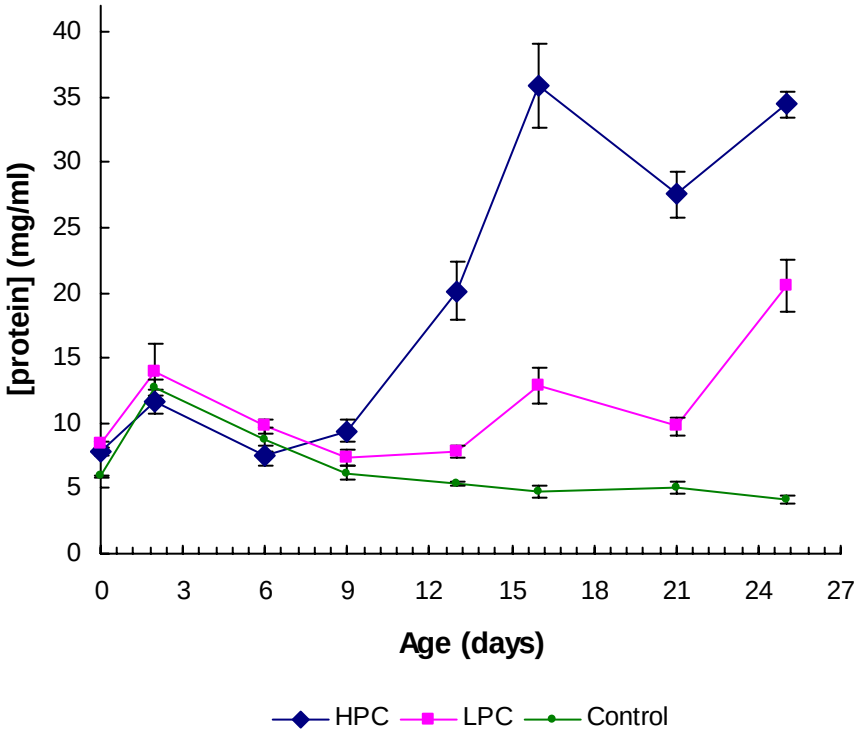

Figure Nº1: Total protein concentration in hemolymph at different ages. HPC: pollen with 24% protein; LPC: pollen with 10% protein; control: sugar syrup.

There was a significant age-treatment interaction (P<.0001) (Fig. Nº1). The treatments were analyzed for each sampling day. Differences in total protein concentration between HPC and LPC are significant from day 13 to day 25. On the other hand, total protein concentration is similar for LPC and control until day 13; from then on the quantify is higher for LPC, with a significant difference only at 25 days. (Table 1).

Table Nº1. Total protein concentration in hemolymph (mg/ml) for each treatment at different ages (Mean and standard error)

		HPC		*LPC*		*Control*	
Day	N	Mean	S.E.	Mean	S.E.	Mean	S.E.
0	6	7.81	0.27	8.38	0.25	5.94	0.11
2	12	11.67[a]	0.92	13.9[a]	2.13	12.76[a]	0.63
6	12	7.58[a]	0.77	9.74[a]	0.58	8.68[a]	0.93
9	12	9.42[a]	0.9	7.33[a]	0.6	6.19[a]	0.5
13	12	20.13b[a]	2.24	7.8[b]	0.46	5.37[b]	0.18
16	12	35.85[a]	3.2	12.91[b]	1.38	4.76[b]	0.51
21	12	27.53[a]	1.76	9.76[b]	0.66	5.04[b]	0.42
25	12	34.43[a]	1	20.56[b]	1.95	4.15[c]	0.37

Different letters indicate significant differences between treatments for each ($p<0.05$)

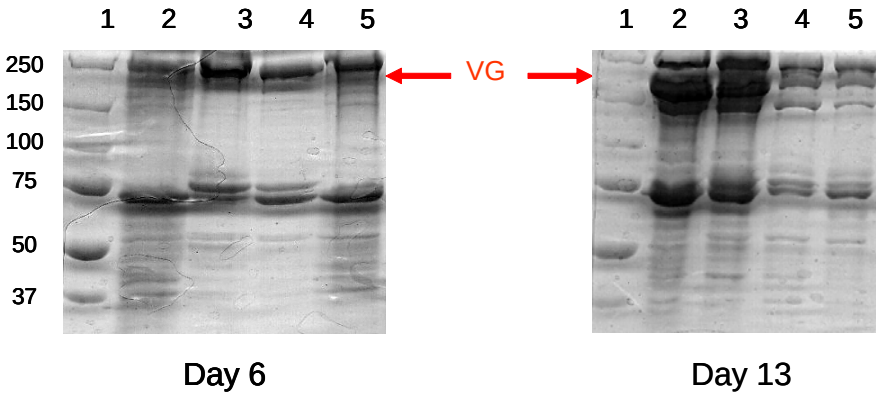

Figure Nº2: SDS-PAGE. Lane 1: molecular weight markers (weight is indicated on the left in kDa); Lanes 2-3: hemolymph samples of treatment HPC, lanes 4-5: LPC.

When comparing ages 6 and 13 days, there is an the increment in total protein concentration in HPC (lanes 2-3), which seems to be due to vitellogenin and hexamerins (around 70 kDa).

Results in figure 3 show a positive mark in the 180 kDa band, ensuring that this corresponds to vitellogenin.

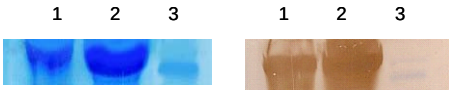

Figure Nº3: SDS-PAGE and its Western-blot using anti-vitellogenin antibody. Lane 1 LPC; lane 2 HPC; lane 3 molecular weight marker corresponding to 180 kDa.

CONCLUSIONS

- At age 2 days there is an increment in total protein concentration regardless of the diet, which could be due to a rapid synthesis of vitellogenin within 0-2 days, as described in literature.

- From age nine days on, total protein concentration in hemolymph in HPC and LPC follows the same pattern, with HPC significant higher levels.

- There is a higher quantity in protein vitellogenin, compared to the rest of the bands.

-There is a notable increment in vitellogenin quantity for HPC but not for LPC at day 13 compared to day 6, in agreement with the results of total protein concentration.

- The results of this work will contribute to generate information on the biochemical variability present in individuals of the colony under different nutritional conditions and supplementation in the field.

References

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