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Determination of some biochemical parameters of worker honeybees (*Apis mellifera* L.) belonging to different age groups

Ethem AKYOL[1,*], Zeliha SELAMOĞLU[1], Mehmet Fuat GÜLHAN[2]

[1]Department of Biology, Faculty of Arts and Science, Niğde University, Niğde, Turkey

[2]Department of Medicinal and Aromatic Plants, Vocational School of Technical Sciences, Aksaray University, Aksaray, Turkey

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Abstract: The catalase activity, total protein, and total RNA levels of worker bees of different age groups (4, 9, 15, 20, and 24 days old) within the same honeybee colony were studied. The catalase activities of group I (4 days), group II (9 days old), group III (15 days old), group IV (20 days old), and group V (24 days old) were 4.10 ± 0.59, 4.32 ± 0.57, 4.32 ± 0.57, 4.41 ± 0.49, and 4.47 ± 0.48 kU/g, respectively. There were significant differences in total protein and total RNA levels among the different age groups of workers. The calculated total protein levels of group I, group II, group III, group IV, and group V were 22.28 ± 0.77, 21.53 ± 0.59, 20.95 ± 1.05, 18.73 ± 0.93, and 18.24 ± 1.83 g/dL, respectively. The total RNA levels of group I, group II, group III, group IV, and group V were 22.42 ± 0.16, 21.26 ± 0.12, 19.30 ± 0.08, 16.22 ± 0.08, and 11.37 ± 0.07 µg/µL, respectively. The results show that there was no significant difference among the average values of all age groups in catalase activity ($P > 0.05$), but significant differences were calculated among the total protein values of all age groups ($P < 0.05$).

Key words: *Apis mellifera*, total RNA, total protein, catalase, age, worker bee

1. Introduction

The honeybee is a social insect that lives in a colony that includes thousands of associates that work together with various duties: e.g., queens, workers, and drones (Zhang et al., 2007). A typical honeybee colony includes between 10,000 and 60,000 worker bees depending on the season of the year and a unique queen (Wegener et al., 2009). Workers generally live for 5–6 weeks during efficient times (spring and summer) and for about 4–5 months during inefficient times (fall and winter).

Workers usually begin foraging behavior when they are older than 2–3 weeks. The age at which workers begin looking for food is a powerful and decisive period in their lifetime. Tasks of workers may be classified into two broad subgroups: foraging bees that gather food outside, and nonforaging bees that directly nurture the offspring (Peters et al., 2010). This behavioral classification is regulated by the age of the worker honeybees, as well as hormones. Taylor et al. (1992) stated that low titers of juvenile hormone were reported in nursing workers, while the titers were high in 3-week-old workers just prior to foraging. Therefore, comparing the total protein values of worker bees at different ages helps in understanding their structural, metabolic, and physiological distinctions, and the social structure and environmental interactions of honeybees.

Although various studies have been carried out on honeybees and other insects, there are only a limited number of studies on the catalase (CAT) activities of worker bees at different ages (Weirich et al., 2002; Strachecka et al., 2014a, 2014b). The aims of this study were to determine CAT activity, total RNA level, and total protein, which play a significant role in the honeybee life cycle in different age groups of worker honeybees.

2. Materials and methods

2.1. Samples and experimental design

The Italian hybrid (*Apis mellifera* L.) bee genotype was obtained from a beekeeping farm in Niğde Province. The queen was caged within a queen-excluder comb-cage containing one empty comb for egg-laying. Twenty days after egg-laying, the comb was transferred into incubators. After worker bees emerged, they were marked with different numbers and then released into the hive. Group I workers were removed from the hive when they were 4 days old. Group II, III, IV, and V workers were collected when they were 9, 15, 20, and 24 days old, respectively. Around 130–150 workers from each age group were stored at –80 °C in deep freeze as stock cultures.

* Correspondence: eakyol@nigde.edu.tr

2.2. Preparation of biochemical analyses

For biochemical analysis, 1 g of workers (10–11 individuals) for each age group was weighed and then homogenized in 100 mL of 2 mM phosphate buffer at pH 7.4. After that, the patterns were sonicated for 1.5 min (30 s of sonication interrupted with 30 s of pauses on ice). The sonicated patterns were centrifuged at 12,000 × *g* for 15 min at 4 °C, and supernatants were held at –80 °C until analysis. The preparation of samples for biochemical analysis of each age group was repeated 7 times.

2.3. Protein assay

In the protein analysis, 1 mL of supernatants was used. Total protein was evaluated by the colorimetric assay of Lowry et al. (1951) using bovine serum albumin as the standard. The entire procedure was repeated 7 times for each age group.

2.4. Measurement of total RNA levels

In the measurement of total RNA levels, 75 µL of supernatants was used. Three grades of the supernatants of the bee samples were extracted and then predicated and washed with ethanol and finally redissolved in double-distilled water. This procedure produced 3 grades of supernatants. After all steps of the procedure were followed, total RNA amounts were measured spectrophotometrically at 280 nm (Chomzynski and Sacchi, 1987). The experiment was replicated 7 times for each age group.

2.5. Determination of CAT activity

The CAT activity in the supernatant samples was measured by assaying H_2O_2 at 240 nm, according to Aebi (1984). For each measurement, 20 µL of supernatant was used. It is presented as kU/g P, where the first-order ratio is permanent (Aebi, 1984). The CAT activity test for each age group was repeated 7 times.

2.6. Statistical analysis

The data for each biochemical analysis consisted of 7 replicates. The data for each test were analyzed separately with SPSS 16.0 by using one-way analysis of variance (ANOVA). Differences between means were determined using Duncan's multiple range test, with 0.5% as the significance level ($P < 0.05$).

3. Results

The total protein and RNA levels decreased as the workers aged, but there was a negative correlation between CAT activities and the aging of the workers (Table). The total protein and RNA values were statistically significantly different ($P < 0.05$) among the different age groups of workers, but CAT values were not ($P > 0.05$) (Table).

4. Discussion

The effects of age on the antioxidant defense systems and prooxidant generation were analyzed by comparing the activity of catalase and rates of H_2O_2 generation in the bees. Peiren et al. (2008) sought evidence of the presence of SOD, CAT, and GST in the venom glands of bees; Weirich et al. (2002) in postmitochondrial fractions of tissue homogenates (spermathecae, muscle, and ventriculi), in hemolymph plasma, and in semen of honey bees; and Strachecka et al. (2014b) in the hemolymph of workers. The antioxidant capacities of workers differ according to age and feeding/diet type of bees. Overexpression of CAT appeared to have no or only slightly negative effects on the lifespan of organisms and to elevate resistance to H_2O_2 (Orr and Sohal, 1992). It seems that CAT overexpression has no effect on normal living conditions, but it may have a role in aging. In our study, CAT activities only slightly increased

Table. The catalase activity, total protein, and total RNA levels in different age groups of worker bees (*Apis mellifera* L.).

Age group	Parameters			
	n	Total protein (g/dL)	Total RNA(µg/µL)	Catalase (kU/g P)
		Mean ± SD	Mean ± SD	Mean ± SD
Group I (4 days)	7	22.28 ^{a} ± 0.79	22.42 ^{a} ± 0.16	4.10 ± 0.59
Group II (9 days)	7	21.53 ^{ab} ± 0.59	21.26 ^{b} ± 0.12	4.32 ± 0.57
Group III (15 days)	7	20.95 ^{ab} ± 1.05	19.30 ^{c} ± 0.08	4.34 ± 0.55
Group IV (20 days)	7	18.73 ^{b} ± 0.93	16.22 ^{d} ± 0.08	4.41 ± 0.49
Group V (24 days)	7	18.24 ^{b} ± 1.83	11.37 ± 0.07 ^{e}	4.47 ± 0.48

Means in the columns followed by different letters indicate statistically significant differences ($P < 0.05$).

with increasing age in the workers. These increases in the CAT activities were not statistically important.

The CAT activities slightly increased in parallel to the age of the workers. This could be the result of high CAT activity associated with increased cell viability and resistance to H_2O_2 in macrophages in the increasing age groups of workers.

Corona et al. (2005) stated that the honeybee is a model organism for surveys of aging and senescence because it has a caste order in which a similar genome reproduces both a long-lived queen and a short-lived worker. They elevated mRNA grades for genes by scrambling 8 of the most important antioxidants and 5 mitochondrial proteins included in respiration. The expression of antioxidant genes usually diminished with age in queens, but not with worker bees. The data of our work pointed out that CAT activities among workers in different age groups slightly increased, but these increases were not statistically significant. Similar findings were reported by Corona et al. (2005).

Our data show a significant change in total protein levels among different age groups. The total RNA levels also decreased depending on the age of the workers. Synthesis of all proteins depends on translation of RNA. Changes in the levels of total protein may be parallel with changes in the levels of total RNA recorded. The total RNA levels may act as an indicator of the total protein amount. Increasing the amount of RNA involved in these structures suggests interaction at the transcriptional level.

As a result, the present study shows that the metabolic processes of enzymes in different age groups cause interactions at the transcriptional and translational levels in bees. The data demonstrate that bees of different ages and labor divisions (depending on diet) may possess different transcriptional and translational expressions that could influence total RNA and total protein levels in bees. These findings will be the first step for new research in the future and contribute to the scientific literature.

In conclusion, protein levels such as enzymes in honeybee workers are related to age and labor division. The total RNA and protein levels in bees are also directly related to diet.

References

Aebi H (1984). Catalase in vitro assay methods. Method Enzymol 105: 121–126.

Chomzynski P, Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanatephenol–chloroform extraction. Anal Bio Chem 162: 156–159.

Corona M, Hughes KA, Weaver DB, Robinson GE (2005). Gene expression patterns associated with queen honey bee longevity. Mech Ageing Dev 126: 1230–1238.

Lowry O, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265–275.

Orr WC, Sohal RS (1992). The effects of catalase gene overexpression on life span and resistance to oxidative stress in transgenic *Drosophila melanogaster*. Arch Biochem Biophys 297: 35–41.

Peiren N, Graaf DC, Vanrobaeys F, Danneels EL, Devreese B, Van Beeumen J, Jacobs FJ (2008). Proteomic analysis of the honey bee worker venom gland focusing on the mechanisms of protection against tissue damage. Toxicon 52: 72–83.

Peters L, Zhu-Salzman K, Pankiw T (2010). Effect of primer pheromones and pollen diet on the food producing glands of worker honey bees (*Apis mellifera* L.). J Insect Phys 56: 132–137.

Strachecka A, Krauze M, Olszewski K, Borsuk G, Paleolog J, Merska M, Chobotow J, Bajda M, Grzywnowicz K (2014a). Unexpectedly strong effect of caffeine on the vitality of western honeybees (*Apis Mellifera*). Biochemistry 79: 1192–1201.

Strachecka A, Olszewski K, Paleolog J, Borsuk G, Bajda M (2014b). Coenzyme Q10 treatments influence the lifespan and key biochemical resistance systems in the honeybee, *Apis mellifera*. Arch Insect Biochem Physiol 86: 165–179.

Taylor DJ, Robinson GE, Logan BJ, Laverty R, Mercer AR (1992). Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. J Comp Physiol A 170:715–721.

Wegener J, Huang ZY, Lorenz MW, Bienefeld K (2009). Regulation of hypopharyngeal gland activity and oogenesis in honey bee (*Apis mellifera*) workers. J Insect Phys 55: 716–725.

Weirich GF, Collins AM, Williams VP (2002). Antioxidant enzymes in the honey bee, *Apis mellifera.* Apidology 33: 3–14.

Zhang L, Li J, Wu (2007). Profile analysis of the proteome of the egg of the high royal jelly producing bees *(Apis mellifera* L.). Agri Sci Chi 6: 1138–1148.