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Abstract

The main objective of this study was to investigate the effects of pollen patty with supplementation of different concentrations of curcumin-steviol glycoside complex (CSG) in *Apis mellifera* (*A. mellifera*). Twelve colonies of *A. mellifera* were conducted from July 10th to August 21st for 42 days. *A. mellifera* were assigned to four dietary treatments with 3 replicates of equal size as follows: (NC, no supplementation of pollen patty; PC, supplementation of basal pollen patty; T1, supplementation of basal pollen diets + 0.04% of CSG; T2, supplementation of basal pollen diets + 0.08% of CSG). The percentage of CSG was calculated based on the total weight of pollen patties. Thorax weight was significantly increased ($p < 0.05$) in the T2 diet compared with the NC and PC diet. There was no significant difference ($p > 0.05$) in pollen patties consumption among the PC, T1, and T2 diets. The T1 and T2 diets showed significantly higher ($p < 0.05$) honey production than the PC and NC diets. Also, the PC diet showed significantly higher ($p < 0.05$) honey production than the NC diet. The T2 showed significantly higher ($p < 0.05$) brood area than the PC and NC diets at 28 and 42 days. In addition, the PC and T1 diets showed significantly higher ($p < 0.05$) brood areas than the NC diet. The T1 and T2 diets showed significantly higher ($p < 0.05$) catalase and superoxide dismutase (SOD) 1 gene expression than the PC and NC diets. The expression of the thioredoxin reductase (Trxr) 1 gene was significantly higher ($p < 0.05$) in the T1 diet, and decreased in the order of the PC, T2, and NC diets. The expression of the SOD2 gene was significantly higher ($p < 0.05$) in the T1 diet than the PC and T2 diets and was significantly lower ($p < 0.05$) in the NC diet. Therefore, supplementation of CSG to pollen patty might be the ideal strategy to improve *A. mellifera* performances.

Keywords (3 to 6): *Apis mellifera*, Curcumin-steviol glycoside complex, Pollen patty

Introduction

Pollen-supplementary diets play a major role in honeybee health and honey production. Supply of artificial pollen diets to honeybee colonies is necessary for the development of young bee brood rearing, reproduction and maintenance of bee colonies, and honeybee production [1-3]. In cases of insufficient pollen supply, the immune system of bees and their strength weaken, which directly increases their mortality rate from attacks by various bee pests and pathogens [4-6]. Thus, most beekeepers feed honeybee colonies with pollen supplements such as defatted soybean, maize, and gram flour, especially when the natural pollen is not sufficient to maintain colony health and immunity in June-July [3, 7, 8]. Also, beekeepers supply artificially synthesized food known as pollen patties to increase food storage and nutrition in the winter season [9]. Therefore, several researchers have formulated and tested various artificial pollen diets to supply sufficient nutrients to maintain bee colonies [10-12].

Pollen patties, which contain bee-collected pollen, are mixed with different ingredients to meet the desired nutrient requirement [13]. Supplements contain bee-collected pollen mixed with other ingredients, such as soybean flour and honey, to form the desired patty consistency [14]. Therefore, numerous studies have evaluated the effects of supplying pollen patties and identifying new materials for improving honeybee performance and honey production [4, 12].

Curcumin, which is produced by *Curcuma longa L.*, is a natural phenol that promotes therapeutic properties such as anti-inflammatory, anticarcinogenic, and antioxidant activities [15-17]. Also, curcumin has been shown to be a bifunctional antioxidant that scavenges reactive oxygen species and triggers an antioxidant response to exert antioxidant activity both directly and indirectly [18, 19]. However, curcumin possesses low absorption due to its impaired water solubility, unstable chemical structure, and rapid metabolism in the body [20, 21]. To improve the bioavailability of curcumin, steviol glycosides have been used to increase the solubility by

utilizing the solubilizing properties [22]. Steviol glycosides are substances extracted from stevia (*Stevia rebaudiana* Bertoni) leaves that have been reported to improve solubility by dissolving soluble substances [23, 24]. Thus, the supplementation of pollen patties with a curcumin-steviol glycoside complex (CSG) could be an ideal strategy to increase immune systems and alleviate the adverse effects of bacteria and pathogens.

Therefore, the main objective of this study was to investigate the effects of pollen patty with supplementation of different concentrations of CSG on body weight, diet consumption, honey production, brood area measurement, and antioxidant gene expression.

Materials and Methods

Experimental colonies with pollen patty diets

Twelve colonies of *A. mellifera* were conducted from July 10th to August 21st for 42 days at Chungbuk National University (36˚37’48" N, 12727’5” E) in Cheongju-si, Republic of Korea. The formulation of pollen patties is shown in Table 1. The CSG used in this experiment was obtained from a commercial company (BIOTEN, Jeongeup, Korea). *A. mellifera* were assigned to four dietary treatments with 3 replicates of equal size as follows: (NC, no supplementation of pollen patty; PC, supplementation of basal pollen patty; T1, supplementation of basal pollen diets + 0.04% of CSG; T2, supplementation of basal pollen diets + 0.08% of CSG). The percentage of CSG was calculated based on the total weight of pollen patties. Each of the four groups consisted of 1 populated frame and 3 brood frames. Pollen patty diets were directly placed over the brood nests of bee colonies and covered with plastic sheets to prevent drying. They were freely and easily available to the *A. mellifera* colonies. The consumption of pollen patties was checked every day, and new pollen patties (300 g) were supplied every week.

Chemical compositions of pollen patties

Compositions of moisture content, crude protein, ether extract, crude ash, crude fiber, and nitrogen free extract (NFE) were analyzed according to the standard recommended by the Association of Official Analytical Chemists (AOAC) [25].

Moisture content was calculated by drying the sample in an oven at 100℃ for 2 h. The dried sample was placed into desiccators, cooled down and then reweighed. This process was repeated until a constant weight was obtained. Crude protein was analyzed by the Dumas method (Rapid MAX N-Exceed, Elementar, Langenselbold, Germany) [26]. The ether extract was analyzed by using a Soxhlet extractor (EAM model, Misung Scientific Co. Ltd, Seoul, Korea) [25]. Crude ash was analyzed according to the method of AOAC by using dry oven circulation (550℃) [25]. The

89 percentage of crude fiber was determined according to the method of AOAC [25]. Calculating
90 the NFE used the following formula: 100 - (Crude protein + Ether extract + Crude fiber + Crude
91 ash+ H_2O). All the analyzed data were expressed as mean ± standard deviation.

92 **Body weight**

93 *A. mellifera* were divided into three body parts to determine the effects of CSG. Total body
94 weight, thorax weight, head weight, and abdomen weight were measured by dehydrating to a
95 persistent temperature (60°C for a period of 48 h) [27].

96 **Diet consumption**

97 The amount of pollen patty consumed was calculated by subtracting the weight of pollen
98 patties and the weight of 1-day-old pollen patties after being placed in the colony (Patty
99 consumption = beginning patty weight-ending patty weight). The weight of pollen patties was
100 measured every day. The data were obtained by recording each formulated diet. The total
101 consumption for each diet during the experimental period (42 days) was also calculated.

102 **Honey production**

103 At the end of the experiment, the production of honey was measured in g by harvesting with
104 an extracting machine (Manual honey harvester) to compare honey production for each colony.

105 **Brood area measurement**

106 Sealed worker brood area was calculated after 14, 28 and 42 days by using measuring a frame
107 wire grid with divisions giving an area of one square inch each [28-30] and then converted in to
108 cm^2 by multiplying with 2.54. Sealed brood was used as a criterion for evaluating the
109 development of colonies.

110 **Reverse transcription and quantitative polymerase chain reaction**

111 *A. mellifera* were collected at 42 days, and the head, wings, and legs were removed to obtain
112 the thorax and abdomen. The RNA was extracted from the obtained thorax and abdomen using

the total RNA extraction kit (iNtRON Biotechnology, Seongnam, Korea). The mRNA was converted to cDNA using high-capacity cDNA Reverse transcription kit (Applied Biosystems, Waltham, MA, USA). The mixed solution was heat treated at 25°C for 10 min, at 37°C for 2 h, and at 85°C for 5 min. Gene amplification was performed using the Fast qPCR 2×SYBR Green Master Mix (Applied Biosystems). Gene amplification was performed for 40 cycles as followed cycle: 50°C for 2 min and 95°C for 10 min; 15 secs at 95°C; 1 min at 53°C; 15 secs at 95°C; 1 min at 53°C. The target genes were catalase, thioredoxin reductase 1 (Trxr1), superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2) and glyceraldehyde-3-phosphate dehydrogenase 2 (GAPDH). Primers used in the amplification are shown in Table 2 below. Normalization was performed using the reference gene GAPDH. Relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method [31].

Statistical analysis

All data were statistically processed using the one-way ANOVA using JMP Pro 16 (JMP® Pro version 16.0.0, SAS Institute, Cary, NC, USA), using each pen as the experimental unit. Differences among all treatment means were determined using the Tukey multiple-range test. The level of significance was established at $p < 0.05$.

Results

Body weight

As shown in Table 3, thorax weight was significantly increased ($p < 0.05$) in the T2 diet (9.80 g) compared with the NC (8.90 g) and PC diet (9.00 g) at 42 days. There was no significant difference ($p > 0.05$) in head, abdomen, and total BW at 0, 14, 28, and 42 days.

Diet consumption

As shown in Table 4, there was no significant difference ($p > 0.05$) in pollen patties consumption among the PC, T1, and T2 diet.

Honey production

As shown in Figure 1, the T1 and T2 diets showed significantly higher ($p < 0.05$) honey production than the PC and NC diets. Also, the PC diet showed significantly higher ($p < 0.05$) honey production than the NC diet.

Brood area

As shown in Figure 2, the T2 diet showed significantly higher ($p < 0.05$) brood area than the PC and NC diets at 28 and 42 days. Also, the PC and T1 diets showed significantly higher ($p < 0.05$) brood areas than the NC diet. There was no significant difference ($p > 0.05$) at 0 and 14 days.

Gene expression

As shown in Figure 3, the T1 and T2 diets showed significantly higher ($p < 0.05$) Catalase and SOD1 gene expression than the PC and NC diets. The expression level of the Trxr1 gene was significantly higher ($p < 0.05$) in the T1 diet, and decreased in the order of the PC, T2, and NC diets. The expression level of the SOD2 gene was significantly higher ($p < 0.05$) in the T1 diet than in other diets and was lower in the NC diet.

Discussion

Total body, thorax, head, and abdomen weight

A higher thorax weight in *A. mellifera* has been suggested to induce stronger and more agile flight, which improves their foraging activities [32]. Numerous studies have demonstrated the positive correlation between thorax weight and flight performance [33, 34]. Therefore, higher thorax weight is considered an index of higher flight performance in *A. mellifera* [35, 36].

During the flight, *A. mellifera* significantly increases its metabolic rate, which, in turn, increases its flight foraging activity times in collecting pollen [34, 35]. Carbohydrate catabolism plays a major role in producing an adequate metabolic rate to improve flight in *A. mellifera* [39]. Also, Teulier et al. [40] have demonstrated that *A. mellifera* utilizes carbohydrates as a metabolic fuel for flight. Moreover, Brodschneider et al. [35] have reported that when insufficient nutrition is provided, delayed maturation of the enzymes of carbohydrate metabolism induces impaired flight performance, which decreases the thorax weight in *A. mellifera*.

In this study, we observed a higher thorax weight and amount of NFE in supplementation of CSG. According to Ghosh and Jung [9], the NFE represents the soluble carbohydrates in pollen patties. This result indicates that supplementation of CSG increases the content of the carbohydrate in the pollen patty. Also, a previous study has reported that supplementation of curcumin could increase the digestibility of carbohydrates by improving intestinal enzymes [41]. Therefore, increased thorax weight might be reasonable due to the increase of carbohydrate and enhanced utilization of carbohydrates by supplementing CSG in this study.

In contrast, no significant differences were observed in total body, head, and abdomen weight in this study. Previous studies demonstrated that supplementation of dietary protein increases the size of the hypopharyngeal gland, which results in a higher head weight in *A. mellifera* [42, 43]. Also, Ullah et al. [44] reported that the highest body weight was observed when sufficient

protein (30 g of soybean flour) was available. However, there were no sufficient differences in the crude protein content of pollen patties (0.06-0.08%) between the cases of supplementation or non-supplementation of CSG in this study. Although the recommended amount of protein in pollen patty has not been identified, it demonstrates that the amount of protein in pollen patty may be insufficient to increase the weight of honeybees. Therefore, a higher amount of protein in the pollen patty might be required to increase the body weight of *A. mellifera*.

Diet consumption

Dietary curcumin consumption implicates the prevention of oxidative stress, which results in enhanced longevity in *A. mellifera* [45]. In addition, Avni et al. [46] have demonstrated that greater consumption of supplements (such as protein and carbohydrates) led to enhanced brood production and tended toward higher honey yields as well. Regarding diet consumption, several studies have indicated that diets with additional nutrition supplements were consumed at higher rates relative to diets without the additional nutrient supplementation [1, 10, 47]. Also, Anvi et al. [46] have reported that pollen patties consisting only of carbohydrates were more consumed than those consisting of protein and lipid sources. Similarly, Scheiner et al. [48] have demonstrated that high sucrose concentrations increase the phagostimulating effects to induce the consumption of pollen patties. Therefore, we guessed that diet consumption might be increased due to the supplementation of pollen patty with CSG. However, no significant differences were noted in the total diet consumption between the supplementation of pollen patties with CSG and those without it. These results indicate that the NFE (differences among the PC, T1, and the T2 diets: 0.69-1.50%) was insufficient to trigger the phagostimulating effects of increasing the consumption of pollen patties containing the CSG.

Honey production

The amount of honey production is correlated with pollen collection and consumption in honeybees [10]. Insufficient nutrient supplementation causes impaired strength and health in *A. mellifera*, which accounts for the decreased foraging activity in terms of collecting pollen into their colonies [1, 2, 49]. The present results confirmed that the supplementation of pollen patties with CSG yielded higher honey production compared to that without the supplementation. As shown in Table 1, pollen patties with the CSG showed relatively higher NFE levels (0.69-1.50%) to the non-supplementation of CSG. Carbohydrates are considered a major source of fuel for foraging flights, which refers to the activity of collecting pollen in the honey colonies [47]. Thus, carbohydrate supplements could provide sufficient nutrients to the colonies and increase honey production by improving their strength and health. Numerous studies have reported that the supplementation of pollen patties enriched with carbohydrates increased honey production when compared to the case of non-supplementation of pollen patties to the colonies [4, 51-53]. Therefore, increased honey production might be reasonable due to the supplementation of pollen patty with CSG in *A. mellifera*.

Brood area

In this study, the supplementation of pollen patties with CSG resulted in improved brood area. The brood area at day 42 was approximately 10% higher in the T2 supplemented with pollen patty than in NC without pollen patty supplementation. In addition, the T2 supplemented with the CSG showed a significantly higher area than the PC. Supplementing *A. mellifera* with additives possessing antioxidant properties has been shown to improve their health and functionality [54-56]. Curcumin, when used in feeding, can reduce oxidative stress through its antioxidant function [18, 19, 57]. Tawfik et al. [58] have reported that reducing oxidative stress improves the colony strength and health of honeybees. The size of the brood area is highly correlated with the number of colonies and populations as it can predict the number of new bee

larvae born [59]. As a result, improving the brood area could improve the colony strength and, thus, increase the honey production [40]. Based on the above results, we suggest that supplementing CSG when feeding pollen supplements to bees can improve their brood area.

Gene expression

In this study, the expression of genes related to antioxidants, Catalase, and SOD1 was significantly higher in the T1 and T2 supplemented with the CSG. In addition, the treatment group fed with pollen patties showed significantly higher values than the NC treatment for Trxr1 and SOD2. It shows a similar trend to the results of Alaux's study [60] analyzing gene expression after feeding pollen patties to *A. mellifera*. Feeding pollen patty appears to increase the expression of antioxidant genes and adding 4% of the CSG appears to further improve it. Bees can fly up to 7km a day to collect pollen or nectar in nature [61, 62]. Flight requires a lot of energy, which increases metabolism. Additionally, it triggers the production and accumulation of reactive oxygen species (ROS) in the body, causing faster aging [63, 64]. ROS causes significant oxidative stress in *A. mellifera* [65-67]. A decrease in the health and lifespan of bees can lead to weakened colony strength and decreased productivity [68]. Rueppell et al. [68] have reported that delaying nurse-to-forager can increase lifespan by up to 8-fold. In other words, the lifespan of *A. mellifera* improves when ROS production decreases due to the absence of flight for pollen or nectar collection. Catalase, SOD1, SOD2, and Trxr1 measured in this study are considered powerful enzymes that can remove ROS [69, 70]. Feeding pollen patty and supplementing with CSG is expected to reduce oxidative stress by increasing the expression of antioxidant enzymes and improving the health of bees.

Conclusion

In this study, supplementation of pollen patties with CSG showed improved thorax weight, honey production, brood area, and antioxidant gene expression. This result indicates that supplementing pollen patties with a CSG enhanced the performance of *A. mellifera*. Therefore, CSG as supplement to pollen patty might be the ideal strategy to improve *A. mellifera* performances.

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References

1. Mattila HR, Otis GW. Influence of pollen diet in spring on development of honeybee (Hymenoptera: Apidae) colonies. J Econ Entomol. 2006; 99(3): 604-13. 10.1093/jee/99.3.604

2. Manning R. Fatty acids in pollen: a review of their importance for honeybees. Bee world. 2001; 82(2): 60-75. doi: 10.1080/0005772X.2001.11099504

3. Saffari A, Kevan PG, Atkinson J. Consumption of three dry pollen substitutes in commercial apiaries. J Apic Sci. 2010; 54: 13-20

4. Islam N, Mahmood R, Sarwar G, Ahmad S, Abid S. Development of pollen substitute diets for *Apis mellifera* ligustica colonies and their impact on brood development and honey production. Pak J Agric Res. 2020; 33(2): 381-8. doi: 10.17582/journal.pjar/2020/33.2.381.388

5. Higes M, Martín-Hernández R, Garrido-Bailón E, González-Porto AV, García-Palencia P, Meana A, Bernal JL. Honeybee colony collapse due to Nosema ceranae in professional apiaries. Environ Microbiol Rep. 2009; 1(2): 110-3. doi:10.1111/j.1758-2229.2009. 00014.x

6. Aizen MA, Harder LD. The global stock of domesticated honeybees is growing slower than agricultural demand for pollination. Curr Biol. 2009; 19(11): 915-8. doi: 10.1016/j.cub.2009.03.071

7. Al-Ghamdi AA, Al-Khaibari AM, Omar MO. Consumption rate of some proteinic diets affecting hypopharyngeal glands development in honeybee workers. Saudi J Biol Sci 2011; 18(1): 73-7. doi: 10.1016/j.sjbs.2010.10.001

8. Mahmood R, Wagchoure ES, Sarwar G. Influence of supplemental diets on *Apis mellifera L*. colonies for honey production. Pak J Agric Res. 2016; 26(4)

9. Ghosh S, Jung C. Nutritional evaluation of four commercially available pollen patties in Korea. J Apic. 2015; 30(3): 155-60. doi:10.17519/apiculture.2015.09.30.3.155

10. Safari AM, Kevan PG, Atkinson JL, Guzman-Novoa E. Feed-Bee: A new bee feed is added to the menu. Bee Culture. 2006; 134(1): 47-8

11. DeGrandi-Hoffman G, Wardell G, Ahumada-Segura F, Rinderer T, Danka R, Pettis J. Comparisons of pollen substitute diets for honeybees: consumption rates by colonies and

effects on brood and adult populations. J Apic Res. 2008; 47(4): 265-70. doi: p01/ I0.3896/iBRA.47406

12. Sihag RC, Gupta M. Development of an artificial pollen substitute/supplement diet to help tide the colonies of honeybee (*Apis mellifera L*.) over the dearth season. J Apic Res. 2011; 55(2)

13. Kalev H, Dag A, Shafir S. Feeding pollen supplements to honeybee colonies during pollination of sweet pepper in enclosures. Ame Bee J. 2022; 142(9): 675-9

14. Keller I, Fluri P, Imdorf A. Pollen nutrition and colony development in honeybees-Part II. Bee World. 2005; 86(2): 27-34. doi: 10.1080/0005772x.2005.11099650

15. Ak T, Gülçin I. Antioxidant, and radical scavenging properties of curcumin-steviol glycoside complex. Chem Biol Interact. 2008; 174(1): 27-37. doi: 10.1016/j.cbi.2008.05.003

16. Wilken R, Veena MS, Wang MB, Srivatsan ES. Curcumin-steviol glycoside complex: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Mol cancer. 2011; 10(1): 1-19. doi: 10.1186/1476-4598-10-12

17. Bland SD, Venable EB, McPherson JL, Atkinson RL. Effects of liposomal-curcumin on five opportunistic bacterial strains found in the equine hindgut-preliminary study. J Anim Sci Technol. 2017; 59(1): 1-5.

18. Yan E, Zhang J, Han H, Wu J, Gan Z, Wei C, Wang T. Curcumin alleviates IUGR jejunum damage by increasing antioxidant capacity through Nrf2/Keap1 pathway in growing pigs. Animals. 2019; 10(1): 41.

19. Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox biol. 2013; 1(1): 448-56.

20. Recharla N, Balasubramanian B, Song M, Puligundla P, Kim SK, Jeong JY, Park S. Dietary turmeric (Curcuma longa L.) supplementation improves growth performance, short-chain fatty acid production, and modulates bacterial composition of weaned piglets. J Anim Sci Technol. 2021; 63(3): 575.

21. Kharat M, McClements DJ. Recent advances in colloidal delivery systems for nutraceuticals: A case study–Delivery by Design of curcumin. J Colloid Interface Sci. 2019; 557: 506-18.

22. Zhang F, Koh GY, Jeansonne DP, Hollingsworth J, Russo PS, Vicente G, Liu Z. A novel solubility-enhanced curcumin formulation showing stability and maintenance of anticancer activity. J Pharm Sci. 2011;100(7): 2778-89.

23. Ju DL. The Efficacy and Safety of Non-Nutritive Sweeteners. Korean Diabetes J. 2015; 16(4): 281-6. doi: 10.4093/jkd.2015.16.4.281

24. Zhang J, Hu Z, Lu C, Bai K, Zhang L, Wang T. Effect of various levels of dietary curcumin on meat quality and antioxidant profile of breast muscle in broilers. J Agric food chem. 2015; 63(15): 3880-6. doi: 10.1021/jf505889b

25. AOAC. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Washington, DC, USA. 2007

26. Jung S, Rickert DA, Deak NA, Aldin ED, Recknor J, Johnson LA, Murphy PA Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. J Am Oil Chem Soc. 2003; 80: 1169-73. doi: 10.1007/s11746-003-0837-3

27. Sagili RR, Pankiw T, Zhu-Salzman K. Effects of soybean trypsin inhibitor on hypopharyngeal gland protein content, total midgut protease activity and survival of the honeybee (*Apis mellifera L*). J Insect Physiol. 2005; 51(9): 953-7. doi: 10.1016/j.jinsphys.2005.04.003

28. Sabir AM, Suhail A, Akram W, Sarwar G, Saleem M. Effect of some pollen substitute diets on the development of *Apis mellifera L*. colonies. Pak. J. Biol. Sci. 2000; 3(5): 890-1

29. Seeley TD, Mikheyev AS. Reproductive decisions by honeybee colonies: tuning investment in male production in relation to success in energy acquisition. Insectes Soc. 2003; 50: 134-8. doi: 10.1007/s00040-003-0638-8

30. Amir OG, Peveling R. Effect of triflumuron on brood development and colony survival of free-flying honeybee, *Apis mellifera L*. J Appl Entomol. 2004; 128(4): 242-9. doi: 10.1111/j.1439-0418.2004.00782

31. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001; 25(4): 402-8. doi: 10.1006/meth.2001.1262

32. Hendriksma HP, Pachow CD, Nieh JC. Effects of essential amino acid supplementation to promote honeybee gland and muscle development in cages and colonies. J Insect Physiol. 2019; 117: 103906. doi: 10.1016/j.jinsphys.2019.103906

33. Ahmed ZH, Tawfik AI, Abdel-Rahman MF, Moustafa AM. Nutritional value, and physiological effects of some proteinaceous diets on honeybee workers (*Apis mellifera L.*). Bee World. 2020; 97(1): 26-31. doi: 10.1080/0005772X.2019.1672983

34. Scofield HN, Mattila HR. Honeybee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. PLoS One. 2015; 10(4): e0121731. doi: 10.1371/journal.pone.0121731

35. Brodschneider R, Riessberger-Gallé U, Crailsheim K. Flight performance of artificially reared honeybees (*Apis mellifera*). Apidologie. 2009; 40(4): 441-9. doi: 10.1051/apido/2009006

36. Jang H, Ghosh S, Sun S, Cheon KJ, Mohamadzade Namin S, Jung C. Chlorella-supplemented diet improves the health of honeybee (*Apis mellifera*). Front Ecol Evol. 2022; 10: 922741. doi: 10.3389/fevo.2022.922741

37. Nachtigall W, Hanauer-Thieser U, Mörz M. Flight of the honeybee VII: metabolic power versus flight speed relation. J Comp Physiol B. 1995; 165: 484-9

38. Harrison JF, Fewell JH. Environmental and genetic influences on flight metabolic rate in the honeybee, *Apis mellifera*. Comp Biochem Physiol A Mol Integr Physiol. 2002; 133(2): 323-33. doi: 10.1016/S1095-6433(02)00163-0

39. Suarez RK. Energy metabolism during insect flight: biochemical design and physiological performance. Physiol Biochem Zool. 2000; 73(6): 765-71. doi: 10.1086/318112

40. Teulier L, Weber JM, Crevier J, Darveau CA. Proline as a fuel for insect flight: enhancing carbohydrate oxidation in hymenopterans. Proc R Soc B Biol Sci. 2016; 283(1834): 20160333. doi: 10.1098/rspb.2016.0333

41. Jiménez-Osorio AS, Monroy A, Alavez S. Curcumin, and insulin resistance molecular targets and clinical evidence. Biofactors. 2016; 42(6): 561-580. doi: 10.1002/biof.1302

42. Hrassnigg N, Crailsheim K. Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera L.*) colonies. J Insect Physiol. 1998; 44(10): 929-39. doi: 10.1016/S0022-1910(98)00058-4

43. Rinkevich FD, Margotta JW, Pittman JM, Ottea JA, Healy KB. Pteridine levels and head weights are correlated with age and colony task in the honeybee, *Apis mellifera*. PeerJ. 2016; 4: e2155. doi: 10.7717/peerj.2155

44. Ullah A, Shahzad MF, Iqbal J, Baloch MS. Nutritional effects of supplementary diets on brood development, biological activities, and honey production of *Apis mellifera L*. Saudi. J Bioll Sci. 2021; 28(12): 6861-8. doi: 10.1016/j.sjbs.2021.07.067

45. Strachecka AJ, Olszewski K, Paleolog J. Curcumin stimulates biochemical mechanisms of *Apis mellifera* resistance and extends the apian lifespan. J Apic Sci.2015; 59(1): 129-41. doi: 10.1515/jas-2015-0014

46. Avni D, Dag A, Shafir S. The effect of surface area of pollen patties fed to honeybee (*Apis mellifera*) colonies on their consumption, brood production and honey yields. J Apic Res. 2009; 48(1): 23-8. doi: 10.3896/IBRA.1.48.1.06

47. Saffari AM, Kevan PG, Atkinson JL. A promising pollen substitute for honeybees. Am Bee J. 2004; 144(3): 230-1

48. Scheiner R, Page RE, Erber J. Sucrose responsiveness and behavioral plasticity in honeybees (*Apis mellifera*). Apidologie. 2004; 35(2): 133-42. doi: 10.1051/apido:2004001

49. Lamontagne-Drolet M, Samson-Robert O, Giovenazzo P, Fournier V. The impacts of two protein supplements on commercial honeybee (*Apis mellifera L*.) colonies. J Apic Res. 2019; 58(5): 800-13. doi: 10.1080/00218839.2019.1644938

50. Diana S, Milagra W. Comparison of Productivity of Colonies of Honeybees, *Apis mellifera*, Supplemented with Sucrose or High Fructose Corn Syrup. J Insect Sci. 2013; doi: 10.1673/031.013.1901

51. Shehata IAA. Evaluation of Carniolan and Italian honeybee colonies fed on artificial diets in dearth and flowering periods under Nasr city conditions. Int J Environ. 2016; 5(2): 19-25

52. Abd El-Wahab TE, Ghania AMM, Zidan EW. Assessment a new pollen supplement diet for honeybee colonies and their effects on some biological activities. Int J Agric. 2016; 12(1), 55-62

53. Ahmad S, Khan KA, Khan SA, Ghramh HA, Gul A. Comparative assessment of various supplementary diets on commercial honeybee (*Apis mellifera*) health and colony performance. PLoS One. 2021; 16(10): e0258430. doi: 10.1371/journal.pone.0258430

54. Hu X, Wang H, Lei C, Zhao X, Zhang W, Liu Z, Xu B. Effect of supplemental pantothenic acid on lipid metabolism and antioxidant function of *Apis mellifera* worker bees. J Apic Res. 2022; 1-11. doi: 10.1080/00218839.2022.2047262

55. Farjan M, Dmitryjuk M, Lipiński Z, Biernat-Łopieńska E, Żółtowska K. Supplementation of the honeybee diet with vitamin C: The effect on the antioxidative system of *Apis mellifera* carnica brood at different stages. J Apic Res. 2012; 51(3): 263-70. doi: 10.3896/IBRA.1.51.3.07

56. Skowronek P, Wójcik Ł, Strachecka A. CBD Supplementation Has a Positive Effect on the Activity of the Proteolytic System and Biochemical Markers of Honeybees (*Apis mellifera*) in the Apiary. Animals. 2022; 12(18): 2313. doi: 10.3390/ani12182313

57. Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí, J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol. 2013; 1(1): 448-56. doi: 10.1016/j.redox.2013.09.003

58. Tawfik AI, Ahmed ZH, Abdel-Rahman MF, Moustafa AM. Influence of winter feeding on colony development and the antioxidant system of the honeybee, *Apis mellifera*. J Apic Res. 2020; 59(5): 752-63. doi: 10.1080/00218839.2020.1752456

59. Eckert CD, Winston ML, Ydenberg RC. The relationship between population size, amount of brood, and individual foraging behaviour in the honeybee, *Apis mellifera L*. Oecologia. 1994; 97: 248-55

60. Alaux C, Dantec C, Parrinello H, Le Conte Y. Nutrigenomics in honeybees: digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. BMC genomics, 2011; 12(1): 1-14. doi: 10.1186/1471-2164-12-496

61. Rissato SR, Galhiane MS, De Almeida MV, Gerenutti M, Apon BM. Multiresidue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination. Food Chem. 2007; 101(4): 1719-26. doi: 10.1016/j.foodchem.2005.10.034

62. Pahl M, Zhu H, Tautz J, Zhang S. Large scale homing in honeybees. PLoS one. 2011; 6(5), e19669. doi: 10.1371/journal.pone.0019669

63. Margotta JW, Roberts SP, Elekonich MM. Effects of flight activity and age on oxidative damage in the honeybee, *Apis mellifera*. J Exp Biol. 2018; 221(14): jeb183228. doi: 10.1242/jeb.183228

64. Corona M, Hughes KA, Weaver DB, Robinson GE. Gene expression patterns associated with queen honey bee longevity. Mech of Ageing Dev. 2007; 126(11): 1230-1238. doi: 10.1016/j.mad.2005.07.004

439 65. He B, Liu Z, Wang Y, Cheng L, Qing Q, Duan J, Li Z. Imidacloprid activates ROS and
440 causes mortality in honeybees (*Apis mellifera*) by inducing iron overload. Ecotoxicol
441 Environ Saf. 2021; 228: 112709. doi: 10.1016/j.ecoenv.2021.112709

442 66. Korayem AM, Khodairy MM, Abdel-Aal AA, El-Sonbaty AA. The protective strategy of
443 antioxidant enzymes against hydrogen peroxide in honeybee, *Apis mellifera* during two
444 different seasons. J Biol Earth Sci. 2012; 2: B93-109

445 67. Olgun T, Dayioğlu M, Özsoy N. Pesticide and pathogen induced oxidative stress in
446 honeybees (*Apis mellifera L*.). *Mellifera*. 2020; 20(2): 32-52

447 68. Rueppell O, Bachelier C, Fondrk MK, Page Jr RE. Regulation of life history determines
448 lifespan of worker honeybees (*Apis mellifera L*.). Exp Gerontol. 2007; 42(10): 1020-32. doi:
449 10.1016/j.exger.2007.06.002

450 69. Li X, Wang Y, Li M, Wang H, Dong X. Metal complexes or chelators with ROS regulation
451 capacity: Promising candidates for cancer treatment. Molecules. 2021; 27(1): 148. doi:
452 10.3390/molecules27010148

453 70. Vives-Bauza C, Starkov A, Garcia-Arumi E. Measurements of the antioxidant enzyme
454 activities of superoxide dismutase, catalase, and glutathione peroxidase. Methods cell Biol.
455 2007; 80, 379-393. doi: 10.1016/S0091-679X(06)80019-1

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Tables

Table 1. Composition and chemical analysis of basal pollen patties with curcumin-steviol glycoside complex (CSG)

Items	PC	T1	T2
Ingredients (g)			
Defatted soy flour	30	30	30
Brewer's Yeast	15	15	15
Pollen	15	15	15
Sugar	40	32	24
CSG	0	8	16
Sugar syrup	100	100	100
Total	200	200	200
Chemical analyzed (%)			
Moisture	12.31 ± 0.27	11.64 ± 0.24	10.85 ± 0.59
Crude Protein	10.39 ± 0.15	10.34 ± 0.02	10.36 ± 0.15
Ether Extract	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
Crude Fiber	3.83 ± 0.11	3.84 ± 0.14	3.80 ± 0.08
Crude Ash	6.08 ± 0.31	6.10 ± 0.28	6.11 ± 0.29
NFE	67.31 ± 0.48	68.00 ± 0.13	68.81 ± 0.72

Abbreviation: PC, supplementation of basal pollen patty; T1, supplementation of basal pollen diets + 0.04% of CSG; T2, supplementation of basal pollen diets + 0.08% of CSG; NFE, nitrogen free extract.

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Table 2. Primer sequences used for the RT-qPCR analysis with the Catalase, Trxr1, SOD1, SOD2 and GAPDH genes

Gene	Primers	Sequence (5'-3')
Glyceraldehyde-3-phosphate dehydrogenase 2 (GAPDH)	Forward	CACATGGAAAATTCAAAGGA
	Reverse	AATGACCAGAAGCTTTTTCC
Thioredoxin reductase 1 (Trxr1)	Forward	TGTGCTGGATTTTTAAATGG
	Reverse	TCCACCCAATGTACAAGAAG
Superoxide dismutase 1 (SOD1)	Forward	CGGCTGAAGTATTCATTACG
	Reverse	ACGCACACTGCTTTAGTCAT
Superoxide dismutase 2 (SOD2)	Forward	GAAAATACCATTGCGATTCA
	Reverse	ATCGGGTCGAACATTTTTAT
Catalase	Forward	CCACTCATTCCTGTTGGTAA
	Reverse	GCATCACCGTAAGTGAACAT

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Table 3. Mean Thorax, head, abdomen, and total body weight of *Apis mellifera* with supplementing different pollen patties with curcumin-steviol glycoside complex (CSG)

Items (mg)	NC	PC	T1	T2	SEM	*p*-value
0 days						
Thorax	9.70	9.78	9.39	9.25	0.205	0.244
Head	4.00	3.70	3.98	3.70	0.148	0.460
Abdomen	18.20	23.30	16.60	24.90	4.496	0.510
Total BW	36.05	39.93	33.27	35.05	2.055	0.151
14 days						
Thorax	9.47	9.58	9.76	9.55	0.242	0.856
Head	3.74	3.75	4.17	4.00	0.143	0.117
Abdomen	23.28	24.44	26.20	26.34	2.341	0.758
Total BW	35.04	36.02	34.00	34.78	1.359	0.771
28 days						
Thorax	9.36	9.34	8.95	8.89	0.408	0.772
Head	5.00	5.20	6.00	4.47	0.637	0.406
Abdomen	30.52	30.76	30.41	32.32	2.554	0.947
Total BW	36.68	35.20	38.30	37.30	0.003	0.922
42 days						
Thorax	8.90b	9.00b	9.50ab	9.80a	0.183	0.002
Head	4.05	4.00	4.17	4.05	0.120	0.782
Abdomen	19.76	21.25	21.78	21.85	0.727	0.168
Total BW	36.81	37.91	35.80	35.20	1.547	0.625

Abbreviation: NC, no supplementation of basal pollen patty; PC, supplementation of basal pollen patty; T1, supplementation of basal pollen patty + 0.04% of CSG; T2, supplementation of basal pollen patty + 0.08% of CSG; BW, body weight; SEM, standard error means. [a-b] Means within column with different superscripts differ significantly (n=3, $p < 0.05$).

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Table 4. Diet consumption of *Apis mellifera* with supplementing different pollen patties with curcumin-steviol glycoside complex (CSG)

Items (g)	PC	T1	T2	SEM	*p*-value
Daily consumption	28.27	27.61	28.03	1.493	0.952

Abbreviation: PC, supplementation of basal pollen patty; T1, supplementation of basal pollen patty + 0.04% of CSG; T2, supplementation of basal pollen patty + 0.08% of CSG; SEM, standard error means. Each value is the mean value of 3 replicates.

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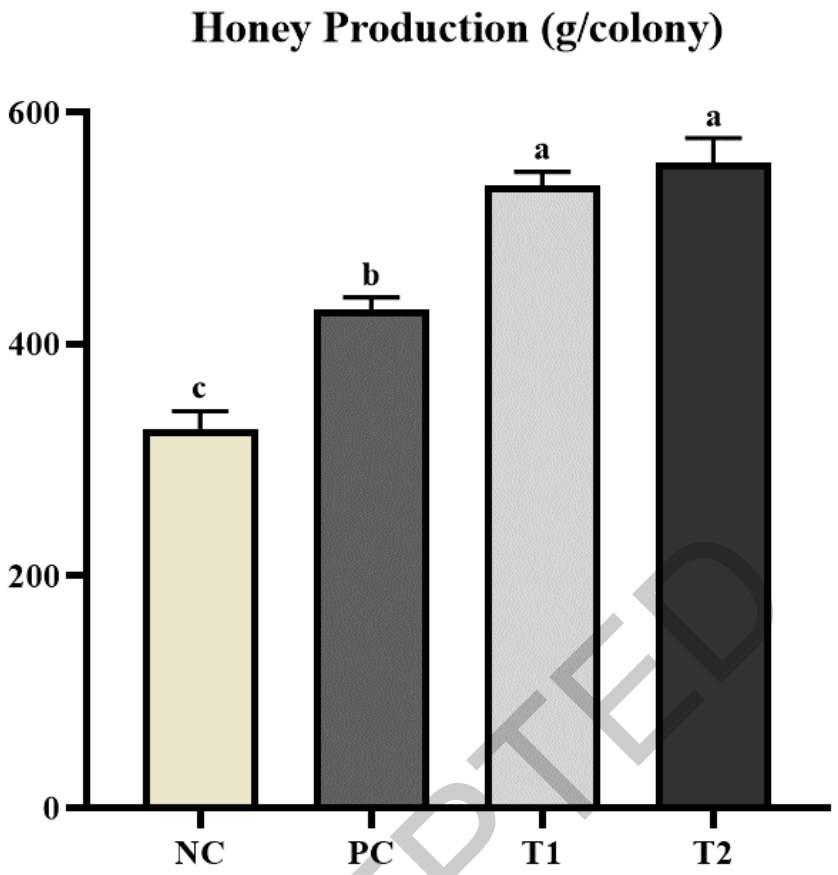

Figure 1. Honey production of *Apis mellifera* with supplementing different pollen patties with curcumin-steviol glycoside complex (CSG). All data are presented as mean ± SEM (n=3). [a-c] Means within column with different superscripts differ significantly ($p < 0.05$). NC, no supplementation of basal pollen patty; PC, supplementation of basal pollen patty; T1, supplementation of basal pollen patty + 0.04% CSG; T2, supplementation of basal pollen diets + 0.08% CSG.

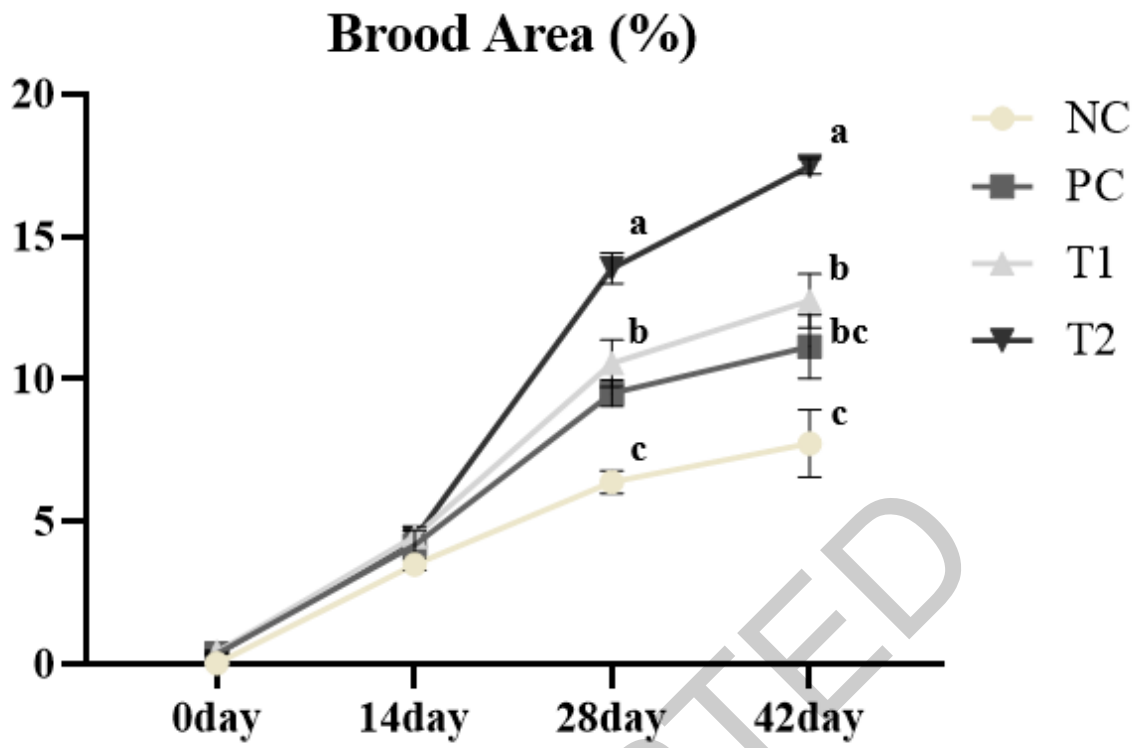

Figure 2. Brood area of *Apis mellifera* with supplementing different pollen patties with curcumin-steviol glycoside complex (CSG). All data are presented as mean ± SEM (n=3). [a-c] Means within column with different superscripts differ significantly ($p < 0.05$). NC, no supplementation of basal pollen patty; PC, supplementation of basal pollen patty; T1, supplementation of basal pollen patty + 0.04% CSG; T2, supplementation of basal pollen patty + 0.08% CSG.

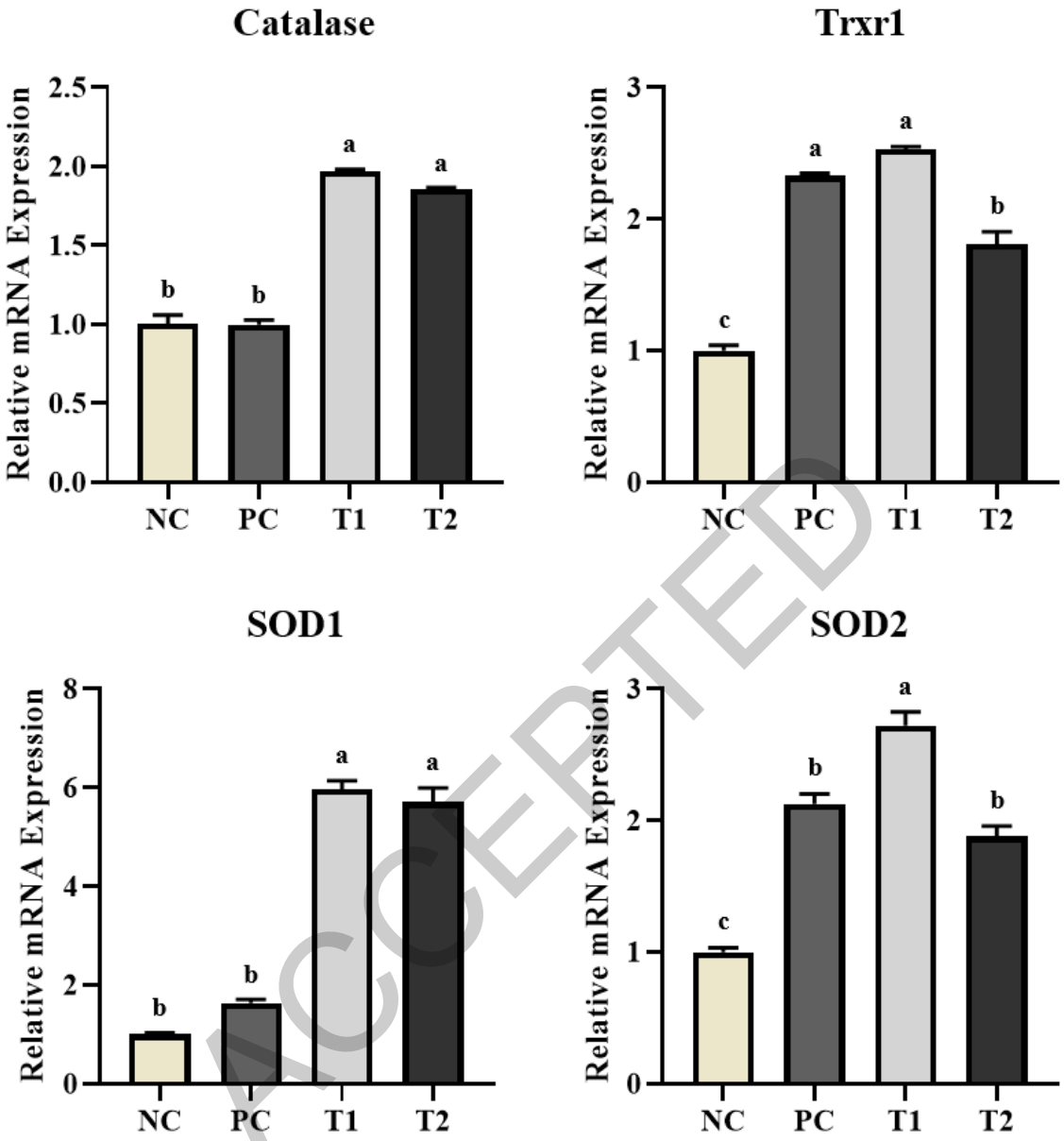

Figure 3. Relative gene expression of *Apis mellifera* with supplementing different pollen patties with curcumin-steviol glycoside complex (CSG). All data are presented as mean ± SEM (n=3). [a-c] Means within column with different superscripts differ significantly ($p < 0.05$). NC, no supplementation of basal pollen patty; PC, supplementation of basal pollen patty; T1, supplementation of basal pollen patty + 0.04% CSG; T2, supplementation of basal pollen patty + 0.08% CSG; Trxr 1, Thioredoxin reductase 1; SOD 1, Superoxide dismutase 1; SOD 2, Superoxide dismutase 2.