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Stable gastric pentadecapeptide BPC 157 in honeybee (*Apis mellifera*) therapy, to control *Nosema ceranae* invasions in apiary conditions

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Nosema ceranae can cause major problems, such as immune suppression, gut epithelial cell degeneration, reduced honeybee lifespan, or suddenly colony collapse. As a novel approach in therapy, we hypothesize the stable gastric pentadecapeptide BPC 157 in honeybee therapy, to control *N. ceranae* invasions in apiary conditions: BPC 157 treated sugar syrup (0.25 L sugar syrup supplemented with 0.1 μg/ml BPC 157), as well as the pure sugar syrup (0.25 L sugar syrup; control), was administered to honeybee colonies in feeders situated under the roof of the hives, during 21 consecutive days, at the end of beekeeping season. The strength of honeybee colonies was increased 20 and 30 days after initial feeding with BPC 157 supplement (Day 1, 36.100 ± 698; Day 20, 64.860 ± 468; Day 30, 53.214 ± 312 estimated number of honeybees), in field conditions. The similar successful outcome occurs with the *N. ceranae* spore loads counted in the homogenates of sampled adult honeybees (Day 1, 6.286 ± 2.336; Day 20, 3.753 ± 1.835; Day 30, 2.005 ± 1.534 million spores/bee). Accordingly, with the noted increased strength of the colonies fed with sugar syrup supplemented with BPC 157, the number of *N. ceranae* spores per honeybee gradually decreased as well. Besides, honeybees infected with *N. ceranae* fed with sugar syrup exhibited severe damage of midgut wall layers and epithelial cells. By contrast, in honeybees infected with *N. ceranae* fed with sugar syrup supplemented with BPC 157, all damages were markedly attenuated, damages of the outer muscular coat, in particular. In conclusion, the results of the first field trial on diseased honeybee colonies with BPC 157 indicate significant therapeutic effects with the used oral therapy with BPC 157 supplementation.

1 | INTRODUCTION

As a novel approach in therapy, we hypothesize the stable gastric pentadecapeptide BPC 157 (Sikiric et al., 2010, 2012, 2014) in honeybee therapy, to control *Nosema ceranae* invasions in apiary conditions. Nosemosis C is parasitic disease of honeybees caused by *N. ceranae* which can cause major colony health problems, such as immune suppression, gut epithelial cell degeneration, and reduced honeybee lifespan (Vejsnæs, Neilsen, & Kryger, 2010). As its emergence as a novel pathogen of *Apis mellifera*, *N. ceranae* has been generally associated with heavily diseased and moribund colonies (Vejsnæs et al., 2010). The *Nosema* species are primary parasites and replicate within epithelium of midgut and consequently impairs digestion and absorption of nutrients (Dussaubat et al., 2012). This tissue degeneration and prevention of gut epithelium renewal may explain early honeybee death.

On the other hand, BPC 157 is an anti-ulcer peptide used in trials for ulcerative colitis and now is in trials for the treatment of multiple sclerosis that largely interacts with NO-system and is thought to be novel mediator of Robert's cytoprotection in rat studies (Sikiric et al., 2010, 2012, 2014). Thereby, BPC 157 exerts endothelium and mucosal protection in stomach, counteracts variously induced

gastrointestinal lesions in different species (Duzel et al., 2017; Sikiric et al., 2010, 2012, 2014, 2017), but has not been tested in social insects. Several molecular pathways are discussed as possible targets of BPC 157 (Cesarec et al., 2012; Chang, Tsai, Hsu, & Pang, 2014; Chang, Tsai, Lin, Hsu, & Pang, 2011; Hsieh et al., 2017; Huang et al., 2015; Tkalcevic et al., 2007). Thereby, BPC 157 was considered to be suitable for the extension of the beneficial effect to the entire gastrointestinal tract (Seiwerth et al., 2014; Sikiric et al., 2010, 2012, 2014, 2017; Sikiric et al., 2011, 2013, 2016; Duzel et al., 2017). Likewise, BPC 157 is effective in different species, shows lethal dose not achieved, and is safe in clinical trials (Sikiric et al., 2010, 2012, 2014, 2017). Accordingly, it seems to be suited for generalization of the therapy to achieve a desirable counteraction in honeybee therapy of the effects of *N. ceranae* invasions.

Because of all the demonstrated properties and wide range of healing effects, especially in tissues of the gastrointestinal tract, we hypothesized that BPC 157 as a food supplement, if added to the honeybee diet, could improve the healing of microdamages in the midgut epithelium of honeybees infected with *N. ceranae* spores, reduce the number of the spores and consequently improve the immunity status and strength of the honeybee colonies. The aim of this study was to determine the impact of multiple supplemental feeding of naturally diseased honeybee colonies with the BPC 157, to investigate the number of *N. ceranae* spores and the histological structure of midgut in honeybees originating from the treated colonies, in apiary conditions. Additionally, the honeybee colonies strength was assessed.

2 | MATERIALS AND METHODS

2.1 | Honeybee colonies and assessment

The field part of the experiment was conducted during 30 consecutive days (beginning in July 21, 2014) at the professional apiary situated in continental part of Croatia (45°56′54.71′′N, 16°37′46.06′′E), and according to *National classification space units for statistical needs* NUTS 2—HR04 and NUTS 3—HR045, after main harvesting season. To perform the field assay, 20 approximately homologous honeybee colonies naturally infected with *N. ceranae* and accommodated in standard Langstroth Root (LR) hives acquired from the same beekeeper were randomly selected and divided into the two groups (10 per each group). At the beginning of the study, none of the colonies showed clinical signs of brood diseases and the last treatment against mite *Varroa destructor* invasion was carried out 20 days earlier (CheckMite®, a.m. coumaphos) to avoid the negative effects of mite parasitization on colony health. No insecticides were in use in the area surrounding the apiary during the experiment. During the clinical inspection of honeybee colonies, approximately 60 forager honeybees per colony were collected from the hive entrance for microscopic examination for the presence of *Nosema* spp. spores, as well as multiplex PCR analyses for species estimation (Tlak Gajger, Vugrek, Grilec, & Petrinec, 2010). Clinical signs of disease, the presence of a queen and honeybee mortality were checked on every inspection of the honeybee colonies at experimental apiary. The examiners were blind about the given treatment.

2.2 | Drugs

Pentadecapeptide Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val, M.W. 1419, named BPC 157 which is a part of the sequence of human gastric juice protein, coded BPC, which is freely soluble in water at pH 7.0 and in saline, was prepared (Diagen, Slovenia). The peptide with 99% high pressure liquid chromatography (HPLC) purity with 1-des-Gly peptide as a biologically inactive impurity was used (Sikiric et al., 2010, 2012, 2014, 2017).

2.3 | Meteorological conditions

All data about important weather conditions (average values per month) were obtained from Croatian Hydrometeorological Department, local Climatic-meteorological station Krizevci. All parameters were measured three times per day, at 7:00 a.m., 2:00 p.m., and 9:00 p.m.

2.4 | Field assay procedure, feeding and estimating the strength of honeybee colonies

The first group of honeybee colonies was additionally fed with 0.25 L sugar syrup (1:1 water-sugar; Viro secer, Croatia) supplemented with 0.1 μg/ml BPC157, per day. The second group of honeybee colonies received only 0.25 L sugar syrup prepared and provided in the same way (controls). Treated sugar syrup, as well as the pure sugar syrup, was administered to honeybee colony in feeders situated under the roof of the hives, for 21 consecutive days. The dose was modified according to the doses used in mammals (Sikiric et al., 2010, 2017).

To estimate the strength of honeybee colonies, a Liebefeld method was performed, with visual determination of number of adult bees and brood amount (Imdorf & Gerig, 2001). The estimation of honeybee colonies was conducted at 1st, 22nd, and 43rd day from the beginning of experiment, during morning hours, around 9:00 to 10:00 a.m. before first massive forage flights of bees. Owing to easier assessment with bees or brood covered comb areas, the frame for LR hive was used and prior divided with plastic grid to 1 dm^2 quadrants.

2.5 | Determination of infection level and *Nosema* species

Samples consisted of about 60 foragers—adult honeybees which were taken at the hive entrance on the 1st day as initial sampling performed before the first feeding was provided, then on 10th, 20th, and 30th day after beginning of field experiment. Honeybee samples were collected into clean plastic receptacles by catching bees in front of the hives entrances directly or using long tweezers. Sampling was performed around midday. Honeybees were counted in each sample, their abdomens were separated and were

thoroughly crushed and homogenized in a plastic container which containing 1 ml of water per bee. *Nosema* spp. spores were counted in each sample using a Bürker–Türk haemocytometer, and the infection level was calculated (Anonymous, 2013). Each numbering procedure was replicated three times. The counting equipment was carefully washed after each sample counting to avoid contamination with spores from the previous sample. Extraction of genomic DNA and further molecular analysis were performed as described elsewhere (Tlak Gajger et al., 2010).

2.6 | Histological analyses

In total, 20 bees were collected from each testing colony on day 30 after initial treating, and the intestines of each honeybee were extracted after brief exposure to low temperature (10 min at 4°C). For extraction purposes, a larger pair of forceps was used to hold the head and the thorax of each honeybee, and a smaller pair of forceps to hold the top of the last abdominal segment and carefully pull out the intestines. Extracted midguts were fixed in a 4% formaldehyde solution, dehydrated in 96% ethanol, inserted into paraffin blocks, and sliced into 6–7 μm thick sections with a microtome. Dewaxed sections were stained for general morphological purposes according to the Hemalaon–Eozinic method (HE; Roulet, 1948).

Microscopic examination of histological preparations was performed under a bright field microscope (Olympus Bx41), at 400× magnification, and the photographic records of the preparations were taken using an Olympus DP12 U-TVO camera. Honey bee midgut wall layers and epithelial cells analysis included outer muscular coat (circular and longitudinal outwards); median basement membrane coated with a stratum of soaring cylindrical epithelium; epithelium cells with small regenerator cells between them; rabdorium with perithrophic membrane; nuclei clearly visible inside epithelium cells; cytoplasm was filled with fine and dense homogeneous inclusions with intact and regular cell boundaries; and damages were scored (1–4) as follows: 1—without alterations, 2—moderate alterations, 3—strong alterations, 4—total alteration or not present.

2.7 | Statistical analysis

To assess and verify the differences in the spore load data between the groups at different sampling dates, one-way analysis of variance (ANOVA) and Mann–Whitney *U* test were performed using statistical software package Statistica-StatSoft v.7. The number of spores in honeybee samples collected at four sampling dates was compared per sampling date (1st, 10th, 20th, 30th) using one-way ANOVA, while the two-samples comparison was carried out by means of nonparametric Mann–Whitney *U* test to compare the mean values between groups. Additionally, the effect sizes were calculated and expressed in d_{cohen} values, between experimental and control groups, for 20 and 30 days after initial feeding with BPC 157 supplement. Statistical significance testing was conducted using a significance level of $\alpha = .05$ to define statistical differences (0.95 confidence interval).

3 | RESULTS

During the experiment at the apiary, there have been no reports of any toxic or other negative effects on vitality of adult honeybees or development of their brood. The objective of molecular analyses performed using multiplex PCR was *Nosema* species determination, and all analyzed honeybee samples showed infection only with *N. ceranae*.

3.1 | Meteorological parameters and the strength of honeybee colonies

Meteorological parameters of air temperature, relative air moisture, and number of days with rain during active beekeeping season 2014 revealed particular detrimental conditions; more days with rain, very low temperatures, alongside with mild winter, particularly favoring invasions with *N. ceranae* (Table 1). During experimental feeding, the control and experimental honeybee colonies consumed the complete amount of offered sugar syrup. Strength of experiment and control honeybee colonies during feeding with and without BPC 157 supplement in field conditions conducted three times during active beekeeping season 2014 is presented with Figure 1. The strength of honeybee colonies increased during feeding with BPC 157 supplement in field conditions. Calculated effect sizes between experimental and control groups were as follows: $d_{cohen} = 2.306$, confidence interval 1.586–3.026 for 20; and $d_{cohen} = 2.683$, confidence interval 1.844–3.520 for 30 days after initial feeding.

3.2 | Number of *N. ceranae* spores per honeybee

Nosema ceranae spore loads counted in the homogenates of sampled adult honeybees were diminished following BPC 157 treatment (Table 2, Figure 2). Calculated effect sizes between experimental and control groups were as follows: $d_{cohen} = 1.204$, confidence

TABLE 1 Overview of the meteorological circumstances (air temperature, relative air moisture, and number of days with rain) during active beekeeping season 2014, meteorological station Krizevci, Croatia

Meteorological circumstances (average values per month)			
Month 2014	Air temperature (°C)	Relative air moisture (%)	Number of days with rain
March	10.0	67	5
April	12.7	75	12
May	15.0	63	19
June	19.8	68	9
July	21.2	76	7
August	19.5	79	11
September	15.8	88	19
October	13.0	85	11
November	8.2	89	10

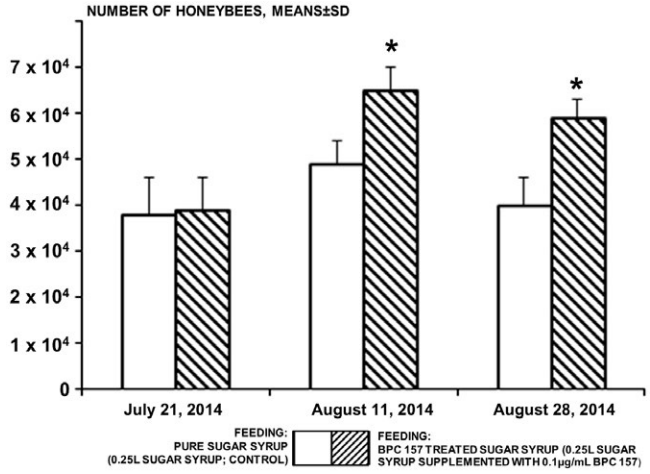

FIGURE 1 Strength of the honeybee colonies—estimated number of the honeybees during additional feeding in field conditions. Mean ± *SD*; **p* < .05 vs. control

interval 0.251–2.156 for 20; and d_{cohen} = 1.93, confidence interval 0.869–2.992 for 30 days after initial feeding. Accordingly, with the noted increased strength of the colonies fed with sugar supplemented with BPC 157 (Figure 1), the number of *N. ceranae* spores per honeybee gradually decreased as well.

3.3 | Microscopic examination

Honeybees infected with *N. ceranae* fed with sugar syrup exhibited severe damage of midgut wall layers and epithelial cells (Table 3). The outer muscular coat was partly present or is visibly damaged. The epithelium cell membranes were burst and destroyed. The epithelium cell boundaries were not clearly delineated and most cell membranes had been degraded; some cells had no invisible nuclei, while the nuclei of other cells appear to be scattered; degenerative and lytic processes were present within the cells. Cytoplasm of epithelium cells was densely granulated with vacuoles of various sizes. By contrast, in honeybees infected with *N. ceranae* fed with the sugar syrup supplemented with BPC 157, all of aforementioned damages were markedly attenuated, in particular damages of the outer muscular coat (Table 3, Figure 3). Analyses of midgut histological preparations showed homogenous microscopy assessment for each experimental group, respectively, and because of those, results were interpreted as the same values for Minimum/Median/Max (Table 3).

4 | DISCUSSION

The efficacy of BPC157 (Sikiric et al., 2010, 2012, 2014, 2017; Sikiric et al., 2011) in reducing *N. ceranae* spores number in the honeybee midgut over a short period of time and suggestion that it may limit the mortality rate in honeybees and be beneficial in nosemosis type C treatment should be validated respecting particular detrimental conditions in 2014 active honeybee season (Table 1), particularly favoring invasion with *N. ceranae*. There were more days with rain, very low average year air temperatures, alongside with mild winter, very early honeybee brood development, increasing of *V. destructor* mite population (Zorat, 2015), decreasing the immunological status of the colonies and stress caused by hunger (lack of natural food in environment). Obviously, these harms encountered may indicate that this therapy effect was obtained versus especially detrimental environmental circumstances.

In particular, BPC157 treated colonies had significantly more honeybees during the second and third clinical inspection (estimation of the number of bees in mid and late August) which is the physiological period of establishing long-living winter honeybees. It seems that BPC 157 treated colonies growing at a larger scale and consequently had better possibility of successful overwintering. According to beekeeper report, all treated honeybee colonies survived while four of the control colonies died during late October, before winter period. In support, Tsagkarakis, Rokkas, and Katsimpoulas (2015) reported that their nontreated control colonies had collapsed before the end of the experiment.

All analyzed honeybee samples showed infection only with *N. ceranae* and that is not surprising taking into account the previously reported global geographical outspread (Higes et al., 2008; Klee et al., 2007) and huge prevalence in Croatian apiaries (Tlak Gajger et al., 2010).

Results of this field study demonstrated that BPC 157 administrated via sugar syrup as a food supplement significantly reduced (Table 2; Figure 3) the number of *N. ceranae* spores in the midgut of honeybees originating from the treated group. It should be emphasized that the honeybee colonies fed with sugar syrup supplemented with BPC 157 had a reduced number of spores compared to the initial spore count (average reduction of 40.3% on 20th day and 68.1% on 30th day). Control honeybee colonies showed lower average reduction of *N. ceranae* spores.

Finally, the conclusive prospect (i.e., BPC 157 application as an innate treatment to successfully oppose invasion with *N. ceranae*) is supported by a variety of beneficial effects of BPC 157 noted

TABLE 2 Mean number of estimated *Nosema ceranae* spores per bee ± standard deviation (*SD*) collected in honeybee colonies fed with sugar syrup supplemented with BPC 157, on four sampling days

Sampling day	Mean value (MV)	Min	Max	Standard deviation (*SD*)
Initial sampling - 1st	6,286,458.00	3,791,667.00	9,828,125.00	2,336,209.00
10th	7,891,667.00	6,625,000.00	8,583,333.00	789,861.00
20th	3,753,125.00	744,792.00	6,208,333.00	1,835,803.00
30th	2,005,208.00	93,750.00	4,703,125.00	1,534,232.00

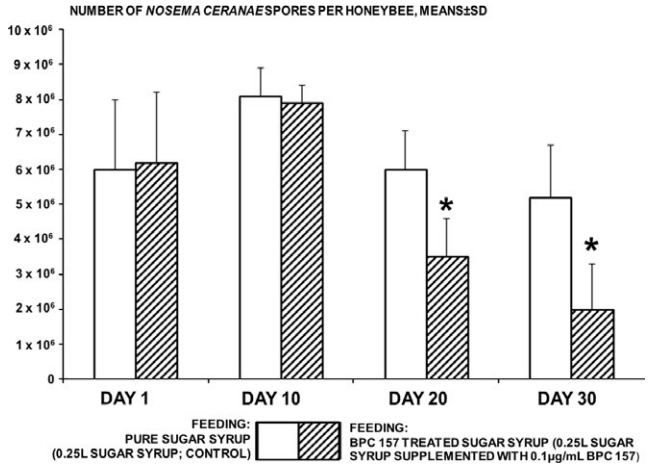

FIGURE 2 Number of *Nosema ceranae* spores per honeybee during additional feeding in field conditions. Mean ± *SD*; *p < .05 vs. control

in colitis lesion studies (Klicek et al., 2013; Lojo et al., 2016; Sikiric et al., 2001; Veljaca et al., 1995). As indicated previously, BPC 157 has been tested in clinical trials as an agent against ulcerative colitis, but studies have shown its efficacy throughout complete gastrointestinal tract (Sikiric et al., 2017). BPC 157 reduces high myeloperoxidase (MPO) activity in colonic tissue (Veljaca et al., 1995), and in particular, heals fistulas (Baric et al., 2016; Grgic et al., 2016; Klicek et al., 2008), rescues failed anastomosis healing (Klicek et al., 2013; Sever et al., 2009; Vuksic et al., 2007), and markedly improves intestinal adaptation following massive bowel resection (Sever et al., 2009). Studies have shown different molecular mechanisms involved in BPC 157 beneficiary effects (Chang et al., 2011, 2014; Hsieh et al., 2017; Huang et al., 2015). Thus, recently the increased expression and internalization of VEGFR2, and the activation of the VEGFR2-Akt-eNOS signaling pathway have been implicated (Hsieh et al., 2017). However, further studies will be needed to designate these effects to the affected honeybee midgut.

Histological analysis performed in this study shows regeneration of the epithelium layer in midgut of honeybees, and consequently mostly regenerated epithelium layer with new enterocytes in midgut of honeybees originated from colonies treated with BPC 157. Epithelium has functions of secretion and resorption in honey bees, and it can be hypothesized that the levels of both functions were increased. The function of the secreted mucous layer is very important

TABLE 3 Midgut wall layers and epithelial cells microscopy assessment

Midgut wall layers and epithelial cells assessment	Damages of midgut wall layers and epithelial cells scored (1–4), Min/Med/Max during experimental feeding, *p < .05 vs. control	
	Honeybees from colonies fed with sugar syrup	Honeybees from colonies fed with sugar syrup supplemented with BPC 157
Outer muscular coat (circular and longitudinal outwards)	4/4/4	1/1/1*
Median basement membrane coated with a stratum of soaring cylindrical epithelium	3/3/3	2/2/2*
Epithelium cells with small regenerator cells between them	4/4/4	2/2/2*
Rabdorium with perithrophic membrane	4/4/4	3/3/3*
Nuclei clearly visible inside epithelium cells	4/4/4	2/2/2*
Cytoplasm filled with fine and dense homogeneous inclusions with intact and regular cell boundaries	3/3/3	2/2/2*

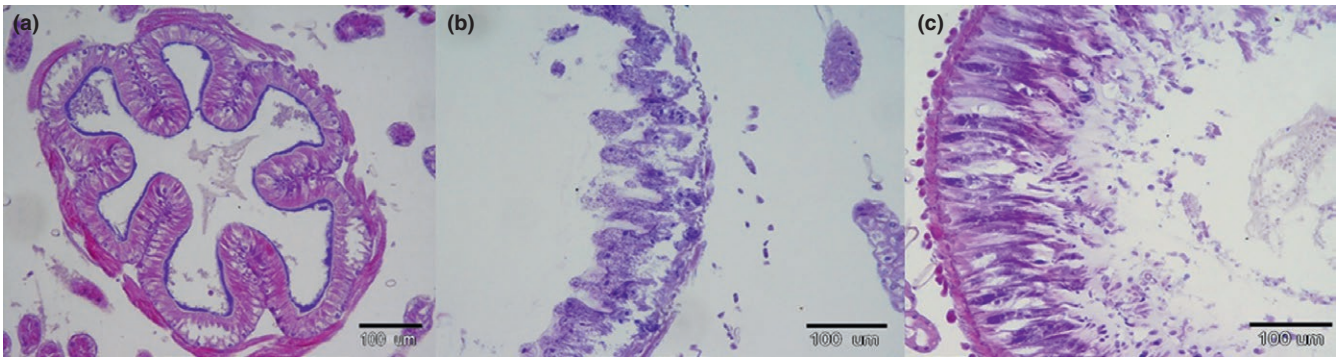

FIGURE 3 Honeybee midgut illustrative presentation. All layers of the midgut wall in young worker honeybee in which *Nosema* spp. spores were not coprologically found (a); Due to the presence of a large number of spores and consequent high osmotic pressure, destroyed epithelial cells in honeybee infected with *N. ceranae* fed with sugar syrup (b); Mostly regenerated epithelium layer with new enterocytes in midgut of honeybees infected with *N. ceranae* fed with sugar syrup supplemented with BPC 157 (c)

for lubricating undigested food and also plays a significant role in osmoregulation and the transfer of proteins or amino acids, fluids, and ions (Vegetti, Rowlerson, Radaelli, Arrighi, & Domeneghini, 1999). In previously examined appliance of herbal preparation Nozevit to diseased honeybee colonies, the mechanic protection was observed as the lumen of treated bees was coated with a firm layer (Tlak Gajger, Kozaric, Berta, Nejedli, & Petrinec, 2011).

However, it was shown that *N. ceranae* induces the most perilous damage, extensive oxidative stress along with impairment of cell signaling, and tissue integrity in the midgut (Dussaubat et al., 2012). As residual ROS can cause inflammatory disease, a balance between synthesis and elimination of ROS via antioxidants is necessary to protect the host gut (Dussaubat et al., 2012). Therefore, the antioxidant system may play an essential role during midgut invasion, which is however, largely disabled in invaded honeybees (Dussaubat et al., 2012). Thus, although this was not specifically investigated in insect midgut, it should be noted that various free radicals-induced lesions in other organs were also counteracted by BPC 157 administration (Ilic et al., 2010; Luetic et al., 2017; Sikiric, Seiwerth, et al., 1993), and in particular, ischemia/reperfusion injuries in rat colitis (Duzel et al., 2017). Thus, it was suggested that BPC 157 may serve as a possible antioxidant (i.e., BPC 157 contains four carboxylic groups, all of them could be active in scavenger process and if reactivated [e.g., by glutathione or by enzymes], the overall beneficial activity could be very high; Duzel et al., 2017). Additionally, as suggested (Duzel et al., 2017), BPC 157 is present in the most body tissues where can catch reactive free radicals and inactivate them on crucial positions where other antioxidants cannot reach (Sikiric, Petek, et al., 1993).

During additional autumn feeding of honeybee colonies, they consumed the complete amount of offered sugar syrup with and without BPC 157 supplement which was ensured by the experimental design based on a low amount of sugar syrup as the supplement carrier. However, the optimal manner of application of this potential dietary supplement for regular use should be adapted to the optimum concentration and treatment replications, actual working conditions of the individual beekeeper (Botias, Martin-Hernandez, Meana, & Higes, 2013), and other zootechnical or environmental factors, before can be used within honeybee colonies disease control management programs. It is, however, noteworthy, that one single initial application may induce a prolonged beneficial effect in ischemia/reperfusion injuries in rat colitis (Duzel et al., 2017). Nevertheless, considering the chances of approval of this drug for apicultural use in the EU, these findings seem to be favorable with respect to several other alternative products that have been evaluated as possible treatments to control nosemosis in field conditions, for example preparations with the herbal basis (Bekesi, Szalai Matray, Harka, Hegedus, & Albert, 2009; Chioveanu, Ionescu, & Mardare, 2004; Maistrello et al., 2008; Nanetti, 2009; Tlak Gajger et al., 2011; Tlak Gajger, Vugrek, Pinter, & Petrinec, 2009; Tlak Gajger, Petrinec, Pinter, & Kozarić, 2009), mineral compounds (Tlak Gajger et al., 2015) or animal lysozymes and essential oils extracted from *Vetiveria zizanioides* (Maistrello et al., 2008), with varying effects which were similar or less effective in comparison with BPC 157.

This kind of therapy is especially applicable in absence of the availability of other authorized veterinary medicine products to beekeepers, when BPC 157 could be used as part of integrated control protocol against nosemosis type C. Also, this is the first appliance of BPC 157 in honeybees as an economically important social insect.

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CONFLICT OF INTEREST

The authors have no conflict of interests.

AUTHOR CONTRIBUTION

Each individual author made the following contribution to the study, and each author has read and approved the final version of manuscript: I.T.G.—planed, designed, and supervised the study, collected, analyzed and interpreted the data, and wrote the article. J.R.—prepared and analyzed honey bee midgut samples in field and laboratory conditions. M.S.Š.—analyzed and interpreted histological preparations. J.V.—interpreted the results and edited manuscript. P.S.—wrote and edited manuscript.

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REFERENCES

Anonymous (2013). Office International des Epizooties (OIE): Chapter 2.2.4., Nosemosis of honey bees. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Retrieved from http://www.oie.int/international-standard-setting/terrestrial-man ual/access-online

Baric, M., Sever, A. Z., Vuletic, L. B., Rasic, Z., Sever, M., Drmic, D., … Sikiric, P. (2016). Stable gastric pentadecapeptide BPC 157 heals rectovaginal fistula in rats. *Life Sciences*, *148*, 63–70. https://doi.org/10.1016/j.lfs.2016.02.029

Bekesi, L., Szalai Matray, E., Harka, L., Hegedus, D., & Albert, A. (2009). *Medication possibilities against new age nosemosis*. In Proc. Workshop "*Nosema* disease: lack of knowledge and work standardization (COST Action FA0803) Guadalajara. Retrieved August 20, 2016, from http://www.coloss.org/news/nosemaworkshop-proceedings-online

Botias, C., Martin-Hernandez, R., Meana, A., & Higes, M. (2013). Screening alternative therapies to control Nosemosis type C in honey bee (*Apis mellifera iberiensis*) colonies. *Research in Veterinary Science*, *95*, 1041–1045. https://doi.org/10.1016/j.rvsc.2013.09.012

Cesarec, V., Becejac, T., Misic, M., Djakovic, Z., Olujic, D., Drmic, D., … Sikiric, P. (2012). Pentadecapeptide BPC 157 and the esophagocutaneous fistula healing therapy. *European Journal of Pharmacology*, *701*, 203–212. https://doi.org/10.1016/j.ejphar.2012.11.055

Chang, C. H., Tsai, W. C., Hsu, Y. H., & Pang, J. H. (2014). Pentadecapeptide BPC 157 enhances the growth hormone receptor expression in tendon fibroblasts. *Molecules*, *19*, 19066–19077. https://doi.org/10.3390/molecules191119066

Chang, C. H., Tsai, W. C., Lin, M. S., Hsu, J. Y., & Pang, J. H. (2011). The promoting effect of pentadecapeptide BPC 157 on tendon healing involves tendon outgrowth, cell survival, and cell migration. *Journal of Applied Physiology*, *110*, 774–780. https://doi.org/10.1152/japplphysiol.00945.2010

Chioveanu, G., Ionescu, D., & Mardare, A. (2004). Control of nosemosis-treatment with protofil. *Apiacta*, *39*, 31–38.

Dussaubat, C., Brunet, J.-L., Higes, M., Colbourne, J. K., López, J., Choi, J.-H., … Alaux, C. (2012). Gut pathology and responses to the microsporidium *Nosema ceranae* in the honey bee *Apis mellifera*. *PLoS One*, *7*, e37017. https://doi.org/10.1371/journal.pone.0037017

Duzel, A., Vlainic, J., Antunovic, M., Malekinusic, D., Vrdoljak, B., Samara, M., … Sikiric, P. (2017). Stable gastric pentadecapeptide BPC 157 in the treatment of colitis and ischemia and reperfusion in rats: New insights. *World Journal of Gastroenterology*, *23*, 8465–8488. https://doi.org/10.3748/wjg.v23.i48.8465

Grgic, T., Grgic, D., Drmic, D., Sever, A. Z., Petrovic, I., Sucic, M., … Sikiric, P. (2016). Stable gastric pentadecapeptide BPC 157 heals rat colovesical fistula. *European Journal of Pharmacology*, *5*, 1–7. https://doi.org/10.1016/j.ejphar.2016.02.038

Higes, M., Martín-Hernández, R., Botías, C., Bailon, E. G., Gonzalez-Porto, A. V., Barrios, L., … Meana, A. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental Microbiology*, *10*, 2659–2669. https://doi.org/10.1111/j.1462-2920.2008.01687

Hsieh, M. J., Liu, H. T., Wang, C. N., Huang, H. Y., Lin, Y., Ko, Y. S., … Pang, J. S. (2017). Therapeutic potential of pro-angiogenic BPC157 is associated with VEGFR2 activation and up-regulation. *Journal of Molecular Medicine (Berlin)*, *95*, 323–333. https://doi.org/10.1007/s00109-016-1488-y

Huang, T., Zhang, K., Sun, L., Xue, X., Zhang, C., Shu, Z., … Zhang, W. (2015). Body protective compound-157 enhances alkali-burn wound healing in vivo and promotes proliferation, migration, and angiogenesis in vitro. *Drug Design Development and Therapy*, *30*, 2485–2499. https://doi.org/10.2147/DDDT.S82030

Ilic, S., Drmic, D., Zarkovic, K., Kolenc, D., Coric, M., Brcic, L., … Sikiric, P. (2010). High hepatotoxic dose of paracetamol produces generalized convulsions and brain damage in rats. A counteraction with the stable gastric pentadecapeptide BPC 157 (PL 14736). *Journal of Physiology and Pharmacology*, *61*, 241–250.

Imdorf, A., & Gerig, L. (2001). Course in determination of colony strength. Swiss Federal Dairy Research Institute, Liebefeld CH-3003 Bern Switzerland (after L. Gerig, 1983. Lehrgang zur Erfassung der Volksstärke). *Schweiz Bienen-Zeitung*, *106*, 199–204.

Klee, J., Besana, A. M., Genersch, E., Gisder, S., Nanetti, A., Tam, D. Q., … Paxton, R. J. (2007). Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, *96*, 1–10. https://doi.org/10.1016/j.jip.2007.02.014

Klicek, R., Kolenc, D., Suran, J., Drmic, D., Brcic, L., Aralica, G., … Sikiric, P. (2013). Stable gastric pentadecapeptide BPC 157 heals cysteamine-colitis and colon-colon-anastomosis and counteracts cuprizone brain injuries and motor disability. *Journal of Physiology and Pharmacology*, *64*, 597–612.

Klicek, R., Sever, M., Radic, B., Drmic, D., Kocman, I., Zoricic, I., … Sikiric, P. (2008). Pentadecapeptide BPC 157, in clinical trials as a therapy for inflammatory bowel disease (PL14736), is effective in the healing of colocutaneous fistulas in rats: Role of the nitric oxide-system. *Journal of Pharmacology Science*, *108*, 7–17. https://doi.org/10.1254/jphs.FP0072161

Lojo, N., Rasic, Z., Zenko Sever, A., Kolenc, D., Vukusic, D., Drmic, D., … Sikiric, P. (2016). Effects of diclofenac, L-NAME, L-arginine and pentadecapeptide BPC 157 on gastrointestinal, liver, and brain lesions, failed anastomosis, and intestinal adaptation deterioration in 24-hour-short-bowel rats. *PLoS One*, *14*(11), e0162590. https://doi.org/10.1371/journal.pone.0162590

Luetic, K., Sucic, M., Vlainic, J., Halle, Z. B., Strinic, D., Vidovic, T., … Kokot, A. (2017). Cyclophosphamide induced stomach and duodenal lesions as a NO-system disturbance in rats: L-NAME, L-arginine, stable gastric pentadecapeptide BPC 157. *Inflammopharmacology*, *25*, 255–264. https://doi.org/10.1007/s10787-017-0358-8

Maistrello, L., Lodesani, M., Costa, C., Leonardi, F., Marani, G., Caldon, M., … Granato, A. (2008). Screening of natural compounds for the control of *Nosema* disease in honey bees (*Apis mellifera*). *Apidologie*, *39*, 436–445. https://doi.org/10.1051/apido:2008022

Nanetti, A. (2009). *ApiHerb as an alternative product to treat Nosema infection*. In Proc. Workshop "*Nosema* Disease: Lack of Knowledge and Work Standardization" (COST Action FA0803), Guadalajara. Retrieved August 20, 2016, from http://www.coloss.org/news/nosemaworkshop-proceedings-online

Roulet, F. (1948). *Methoden der pathologischen histologie* (567 pp.). Wien, Austria: Springer-Verlag. https://doi.org/10.1007/978-3-7091-3933-2

Seiwerth, S., Brcic, L., Vuletic, L. B., Kolenc, D., Aralica, G., Misic, M., … Sikiric, P. (2014). BPC 157 and blood vessels. *Current Pharmaceutical Design*, *20*, 1121–1125. https://doi.org/10.2174/13816128113199990421

Sever, M., Klicek, R., Radic, B., Brcic, L., Zoricic, I., Drmic, D., … Sikiric, P. (2009). Gastric pentadecapeptide BPC 157 and short bowel syndrome in rats. *Digestive Diseases and Sciences*, *54*, 2070–2083. https://doi.org/10.3748/wjg.v22.i41.9127

Sikiric, P., Petek, M., Rucman, R., Seiwerth, S., Grabarević, Z., Rotkvić, I., … Duvnjak, M. (1993). A new gastric juice peptide, BPC. An overview of the stomach-stress-organoprotection hypothesis and beneficial effects of BPC. *Journal of Physiology (Paris)*, *87*, 313–327. https://doi.org/10.1016/0928-4257(93)90038-U

Sikiric, P., Seiwerth, S., Aralica, G., Perovic, D., Staresinic, M., Anic, T., … Lovric-Bencic, M. (2001). Therapy effect of antiulcer agents on new chronic cysteamine colon lesion in rat. *Journal of Physiology (Paris)*, *95*, 283–288. https://doi.org/10.1016/S0928-4257(01)00039-0

Sikiric, P., Seiwerth, S., Brcic, L., Sever, M., Klicek, R., Radic, B., … Kolenc, D. (2010). Revised Robert's cytoprotection and adaptive cytoprotection and stable gastric pentadecapeptide BPC 157. Possible significance and implications for novel mediator. *Current Pharmaceutical Design*, *16*, 1224–1234. https://doi.org/10.2174/138161210790945977

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Rotkvic, I., … Duvnjak, M. (1993). Hepatoprotective effect of BPC 157, a 15-amino acid peptide, on liver lesions induced by either restraint stress or bile duct and hepatic artery ligation or CCl4 administration. A comparative study with dopamine agonists and somatostatin. *Life Sciences*, *53*(18), PL291–PL296.

Sikiric, P., Seiwerth, S., Rucman, R., Drmic, D., Stupnisek, M., Kokot, A., … Bencic, M. L. (2017). Stress in gastrointestinal tract and stable gastric pentadecapeptide BPC 157. Finally, do we have a solution?. *Current Pharmaceutical Design*, *23*(27), 4012–4028. https://doi.org/10.2174/1381612823666170220163219

Sikiric, P., Seiwerth, S., Rucman, R., Kolenc, D., Batelja Vuletic, L., Drmic, D., … Vlainic, J. (2016). Brain-gut axis and Pentadecapeptide BPC 157: theoretical and practical implications. *Current Neuropharmacology*, *14*, 857–865.

Sikiric, P., Seiwerth, S., Rucman, R., Turkovic, B., Rokotov, D. S., Brcic, L., … Sebecic, B. (2011). Stable gastric pentadecapeptide BPC 157: novel therapy in gastrointestinal tract. *Current Pharmaceutical Design*, *17*, 1612–1632.

Sikiric, P., Seiwerth, S., Rucman, R., Turkovic, B., Rokotov, D. S., Brcic, L., … Sebecic, B. (2012). Focus on ulcerative colitis: Stable gastric pentadecapeptide BPC 157. *Current Medicinal Chemistry*, *19*, 126–132. https://doi.org/10.2174/092986712803414015

Sikiric, P., Seiwerth, S., Rucman, R., Turkovic, B., Stancic Rokotov, D., Brcic, L., … Sebecic, B. (2013). Toxicity by NSAIDs. Counteraction by stable gastric pentadecapeptide BPC 157. *Current Pharmaceutical Design*, *19*, 76–83.

Sikiric, P., Seiwerth, S., Rucman, R., Turkovic, B., Stancic Rokotov, D., Brcic, L., … Sebacic, B. (2014). Stable gastric pentadecapeptide BPC 157-NO-system relation. *Current Pharmaceutical Design*, *20*, 1126–1135. https://doi.org/10.2174/13816128113190990411

Tkalcevic, V. I., Cuzic, S., Brajsa, K., Mildner, B., Bokulić, A., Situm, K., … Parnham, M. J. (2007). Enhancement by PL 14736 of granulation and collagen organization in healing wounds and the potential role of egr-1 expression. *European Journal of Pharmacology*, *10*, 212–221. https://doi.org/10.1016/j.ejphar.2007.05.072

Tlak Gajger, I., Kozaric, Z., Berta, D., Nejedli, S., & Petrinec, Z. (2011). Effect of the herbal preparation Nozevit on the mid-gut structure of honeybees (*Apis mellifera*) infected with *Nosema* sp. spores. *Veternarni Medicina*, *56*, 344–351.

Tlak Gajger, I., Petrinec, Z., Pinter, L., & Kozarić, Z. (2009). Experimental treatment of *Nosema* disease with "Nozevit" phyto-pharmacological preparation. *American Bee Journal*, *149*, 485–490.

Tlak Gajger, I., Ribaric, J., Matak, M., Svecnjak, L., Kozaric, Z., Nejedli, S., & Smodis Skerl, I. M. (2015). Zeolite clinoptilolite as a dietary supplement and remedy for honeybee (*Apis mellifera* L.) colonies. *Veternarni Medicina*, *60*, 696–705.

Tlak Gajger, I., Vugrek, O., Grilec, D., & Petrinec, Z. (2010). Prevalence and distribution of *Nosema ceranae* in Croatian honeybee colonies. *Veterinarni Medicina*, *55*, 457–462.

Tlak Gajger, I., Vugrek, O., Pinter, L., & Petrinec, Z. (2009). "Nozevit patties" treatment of honey bees (*Apis mellifera*) for the control of *Nosema ceranae* disease. *American Bee Journal*, *149*, 1053–1056.

Tsagkarakis, A. E., Rokkas, C., & Katsimpoulas, I. (2015). Experimental treatment with the Natural Water Acidifier Provigoro® for *Nosema* spp. control: Preliminary results. *Advances in Entomology*, *3*, 83–85. https://doi.org/10.4236/ae.2015.33009

Vegetti, A., Rowlerson, A., Radaelli, G., Arrighi, S., & Domeneghini, C. (1999). Post-hatching development of the gut and lateral muscle in the sole, *Solea solea* L. *Journal of Fish Biology*, *55*(Suppl. A), 44–65. https://doi.org/10.1111/j.1095-8649.1999.tb01045.x

Vejsnæs, F., Neilsen, S. L., & Kryger, P. (2010). Factors involved in the recent increase in colony losses in Denmark. *Journal of Apicultural Research*, *49*, 109–110. https://doi.org/10.3896/IBRA.1.49.1.20

Veljaca, M., Lesch, C. A., Pllana, R., Sanchez, B., Chan, K., & Guglietta, A. (1995). BPC-157 reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. *Journal of Pharmacology and Experimental Therapeutics*, *272*, 417–422.

Vuksic, T., Zoricic, I., Brcic, L., Sever, M., Klicek, R., Radic, B., … Sikiric, P. (2007). Stable gastric pentadecapeptide BPC 157 in trials for inflammatory bowel disease (PL-10, PLD-116, PL14736, Pliva, Croatia) heals ileoileal anastomosis in the rat. *Surgery Today*, *37*, 768–777. https://doi.org/10.1007/s00595-006-3498-9

Zorat, T. (2015). *The efficiency of acaricides used in summer treatment of honeybee colonies against varoosis (Učinkovitost primjene akaricida u ljetnom tretiranju pčelinjih zajednica protiv varooze)*. In Croatian, Graduate Thesis, University of Zagreb Faculty of Veterinary Medicine.

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