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Results of Doxycycline Administration on Bee Colonies

Research Article

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ABSTRACT

The aim of the study was to estimate the impact of antibiotic doxycycline on the spring development of the bee colonies, worker bees' bodies chemical composition, development of hypopharyngeal glands and some physicochemical parameters of honey. The experiment was conducted during the spring stimulating feeding at apiary of Research Center of Stockbreeding and Agriculture, Smolyan, Bulgaria. Two groups of bee colonies were formed – control and experimental. The control group was fed with sugar solution (sugar/water 1:1) and experimental group with the addition of antibiotic doxycycline at a dose of 500 mg/L in sugar solution. Significantly lower values were obtained for the strength of the bee colonies and the amount of sealed worker bee brood fed with doxycycline than the control group. Strong correlations were found between the amount of sealed worker bee brood and the quantity of bee pollen and the quantity of honey and bee pollen in the both groups. From this study, it was concluded that development of hypopharyngeal glands was not positively affected after application of antibiotic doxycycline at a dose of 500 mg/L. Based on the results obtained there is no difference in physicochemical parameters of honey samples from the control and experimental group. In all cases testing for doxycycline residues in honey samples from the experimental group was relevant and high. Therefore, the authors do not recommend doxycycline in the beekeeping.

KEY WORDS doxycycline, honey, honeybees, hypopharyngeal glands, physicochemical parameters.

INTRODUCTION

To the honeybee colonies it is important to have enough amounts of carbohydrates, proteins, vitamins and minerals. They receive these components with the honey and bee pollen in order to perform their vital functions, to stimulate the egg-laying of the queen, to increase the quantity of the capped brood and mobilization of the bees with permanent employment in the beehive. The spring-summer period is characterized by increasing number of bees in the bee colony. For this reason, bee feeding is very important during spring period. A number of products for prophylaxis and stimulation of the development of the honey bee colonies are delivered through the bee food (i.e., sugar solution or mixture of honey and powdered sugar). In this regard, essential oils, herbs, plant extracts and organic acids (Hristov, 1995; Nenchev *et al.* 2006; Hristakov, 2012; Shumkova, 2016) are commonly used in the bee feeding.

Antibiotics are an important class of drugs. They represent a key component in the control of bacterial diseases. In this respect antibiotics should be given at recommended doses and under appropriate supervision (Menkem *et al.* 2019). It is also well known that antibiotics could be used also as growth promoters, when their action is exerted at gut microbiota level (Dibner and Richard, 2005). Sometimes low doses of antibiotics could be added to water and food of the animals to increase their body size (Menkem *et al.* 2019). In beekeeping the antibiotics are used at rela-

tively high doses to treat infections, or at low doses as "growth promoters" (Al-Waili *et al.* 2012). According to European regulations the use of antibiotics in beekeeping is not permitted in the European Union. In some cases, the policy "zero tolerance" for antibiotic residues in honey is not possible. Antibiotics are also used for control of bacterial diseases of plants such as fire blight of pear and apple trees (Stockwell and Duffy, 2012). If honeybees collect nectar from treated plants antibiotic residues could contaminate the honey. However, maximum residue limits (MRLs) have not been defined for antibiotics and their metabolites in honey according to European Community regulations. It means that honey containing antibiotics residues are not permitted to be sold (Forsgren, 2010; Al-Waili *et al.* 2012).

Some European countries (i.e., Switzerland, Greece, France, United Kingdom and Belgium), have established action limits for antibiotics in honey. Generally, these limits are between 0.01 to 0.05 mg/kg for each antibiotic group (Al-Waili *et al.* 2012). The antibiotics are not found only in Europe. For example, in China the antibiotics tetracycline, oxytetracycline, doxycycline, chlortetracycline and chloramphenicol were successfully determined by high performance capillary electrophoresis in honey samples.

The detection limits were 20 μg/L for tetracycline, oxytetracycline and doxycycline (Chen *et al.* 2001). The main reasons are high probability for antibiotic resistance to develop and high levels of antibiotic residues in honey. In all cases the antibiotics must be used with precaution in the honey production. Antibiotic rescues in honey may carry health problems such as development of antibiotic resistance.

According to the Bulgarian regulations the antibiotics are banned also in the beekeeping since 2003. Indeed, there are a few studies for determination of antibiotics in Bulgarian honey samples (Dinkov *et al.* 2003; Dinkov *et al.* 2005). In the past in Bulgaria the antibiotic doxycycline from tetracycline group is not widely used in the treatment of bee diseases. For the last 14 years new studies have not been published for antibiotics administration in beekeeping. We want to know the impact of doxycycline as a stressor on some parameters which characterize the development of the bee colonies in spring. Parameters such as development of hypopharyngeal glands that could be affected after treatment with antibiotics. If the bee colonies are fed with antibiotic they should not be used for honey production.

In this respect the aim of the study was to estimate the impact of antibiotic doxycycline on the spring development of the bee colonies, worker bees' bodies chemical composition, development of hypopharyngeal glands and some physicochemical parameters of honey.

MATERIALS AND METHODS

The spring stimulating feeding was conducted during the spring-summer period (23 April–06 June 2018). The experiments included the local bee colonies of *Apis mellifera macedonica* settled in Langstroth-Rut (multihull) hives. The experiment was conducted at apiary of Research Center of Stockbreeding and Agriculture, Smolyan, Bulgaria. The bee colonies were free from illnesses. The bee colonies were reared in experimental apiary. The honey was not sold or used for human consumption at all.

Two groups were used: control group (5 bee colonies) and experimental (treated) (5 bee colonies). Equal hive strength, brood and food supplies (honey and bee pollen) were applied in both groups. The control group was fed with sugar solution (sugar water 1:1) without doxycycline. The experimental group was fed with the addition of antibiotic doxycycline at a dose of 500 mg/L sugar solution (sugar water 1:1) 4 times at intervals of 7 days. This dose was chosen based on the previous experiments in Bulgaria (Dinkov *et al.* 2003; Dinkov *et al.* 2005). The volume of feeders of the hives was 500 mL. Each experimental bee colony received 2 L sugar solution with antibiotic. For each bee colony a total of 5 L sugar solution was provided. This was needed for spring stimulation feeding of the bee colonies. The feeding with the antibiotic started in April when the main pasture was not started. The all quantity of honey was used of the bees as a food.

For the whole experiment a total of 5 measurements were done in every 12 days with a measuring frame (size of the squares are 5×5 cm). The following parameters were determined:

1) amount of bees (strength of the bee colony) in kg-approximately in number of frames occupied by bees and calculating the mass in a statement of one frame the multihull hive contains approximately 200 g of bees after control measurements.

2) quantity of honey (in kg) and bee pollen in the beehives (cm^2).

3) amount of sealed worker bee brood (number of cells)-in 1 cm^2 there are 4 worker cells in the honeycomb. There are 100 worker cells in the area of 25 cm^2.

The degree of development of the hypopharyngeal glands (HPhGs) was determined in worker bees from each bee colony. The HPhGs were found with shallow cut in the head. They were removed and placed on glass slide. Both lobes of the HPhGs were evaluated with a binocular loupe LAB-20, OPTIKA-Italy. The degree of development of HPhGs was established using the 4-point scale of Hess. The method is described by Maurizio (1954).

Determination of the worker bees' bodies chemical composition

The bee body samples for chemical analysis were taken at the end of the experiment. The samples with worker bees were collected from each bee colony and from different honeycombs. The samples were stored in a freezer (–20 ˚C) until the beginning of the analyses.

The analyses for the chemical composition of the worker bees' bodies were carried out at the Laboratory of Institute of Animal Science (Kostinbrod, Bulgaria). The following parameters were determined: moisture (%) and ash (%)-weight analysis (gravimetry); protein (%)-Kjeldahl method, lipids (%)-Soxhlet extraction method.

Chemical composition of bee honey

The honey samples are taken directly from the wax combs immediately after the feeding. The physicochemical parameters (water content, diastase, electrical conductivity, pH, total acidity) were determined according to the European Honey Commission recommended methods (Bogdanov *et al.* 1997). Doxycycline in bee honey was determined by quality operating procedure (QOP) 504-1-IM-LC/MS in Laboratory Alimenti, Plovdiv, Bulgaria.

At the end of the experiment and after all measurements, all honeycombs from the experimental bee colonies were removed from the hives and destroyed.

Statistical analysis

Statistical analysis of the results was performed using SPSS 20.0 for windows (SPSS, 2011). The Student's t-test was used to determine the significance of the differences. Level of statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The population of a bee colony is not static and varies with the season of the year. A colony of bees will have a low population in the winter (January and February) and can grow up to 60000 bees in the peak season (spring and mid-summer). Figure 1 shows the strength of the bee colonies. They grew which is a normal process in their spring development. The result showed that after the first measurement (4.05.) until the end of the experiment the control group bee colonies have higher value of the parameter strength of the bee colonies compared to the experimental group bee colonies fed with doxycycline as a supplement. At the last measurement (6.06) the strength of the bee colonies on the control group was 1.2 times more than the experimental group (Figure 1).

Significantly lower values were obtained ($P<0.05$) for the strength of the bee colonies fed with doxycycline versus the control group for the all measurements.

Although the strength of the experimental group fed with doxycycline was lower, it is well known that in very short period the bee colonies can increase their population. There is enough amount of sealed worker bee brood in the hive. At the end of the experiment (6.06.) all bee colonies from the experimental and control group were well developed and they had enough number of bees which can actively participate in the main pasture.

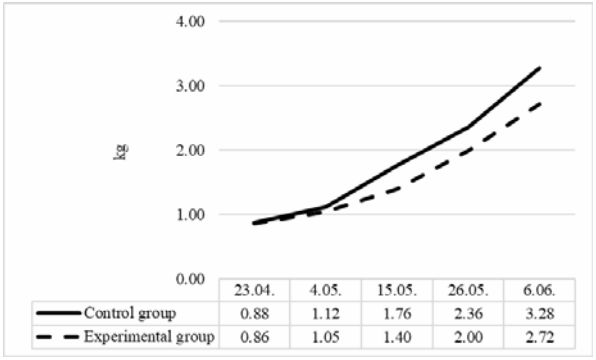

Figure 1 Strength of the bee colonies (kg)

This conclusion is based on our previous research for the same period and apiary (Shumkova and Zhelyazkova, 2013) when the strength of the bee colonies was lower compared to the present study.

The average value and standard deviation of honey in the bee hives at the beginning of the experiment is 1.65 ± 0.28 kg per bee colony. At this time there were flowering of fruit crops (*Prunus cerasifera*, *Prunus cerasus* and *Malus domestica*) and spring plants in the area. On 15.05 there was more quantity of honey in the group of family received doxycycline compared to the control group (Figure 2).

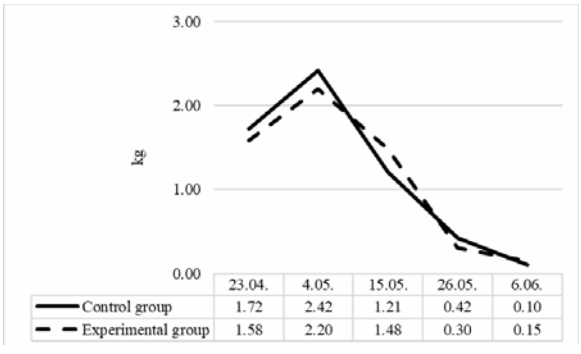

Figure 2 Quantity of honey (kg)

After this moment the amount of honey decreased because most of the fruit plants were overblown and the honey and sugar syrup were used to feed the young bees and the bee brood.

For the honeybees, pollen is the most important protein source. Pollen consumption dependents on the age and the function of the worker bee (Hrassnigg and Crailsheim, 1998). During spring and summer nurse-aged bees feed on large amounts of pollen because they produce royal jelly for the larvae and bee queen. A few studies have shown a correlation between the amount of brood in a colony and the amount of pollen returned to the hive (Jevtić *et al.* 2009; Taha El and Al-Kahtani, 2013; Mohapatra *et al.* 2008; Corby-Harris *et al.* 2015). Furthermore, the experiments of Eckert *et al.* (1994) with high and low brood indicate that the values of nectar and pollen resources to a colony depend on colony state and that individual foragers modify their behavior. In the present study the quantity of pollen in the control group bee colonies was higher than the experimental group for the whole period (from 4.05 to 6.06) (Figure 3).

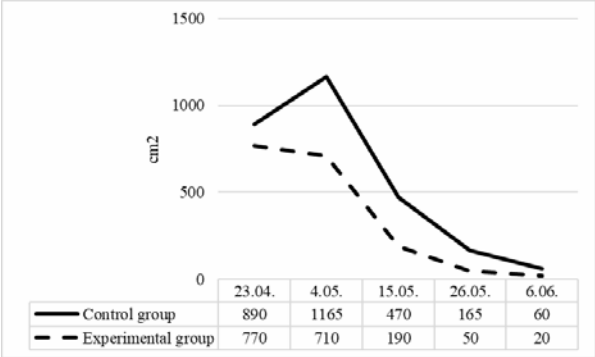

Figure 3 Quantity of bee pollen (cm^2)

It suggests that control group bee colonies have more young bees which collect bee pollen. The results for the quantity of honey and bee pollen are similar. Again, the quantity of the bee pollen reached its peak on 4.05. These results were expected, as during this period there is abundance of flowering plants and the honey bees collect nectar and bee pollen. After this period the quantity of bee pollen stored in the hives gradually decreases and at the end of the experiment it was close to zero. All quantity of bee pollen was used as a food for the bee brood. In general, sufficient supplies of honey and bee pollen in the bee colonies always have positive influence on the intensity of the bee's development and productivity (Zhelyazkova, 2005; Ivanova, 2005; Parvulov, 2006).

The results of the amount of sealed worker bee brood are presented in Figure 4. After the first measurement on the 12th day (4.05) to the end of the experiment the control group bee colonies have higher values for this parameter compared to the experimental group. The highest value (13220 number of cells) is almost at the end of the spring period.

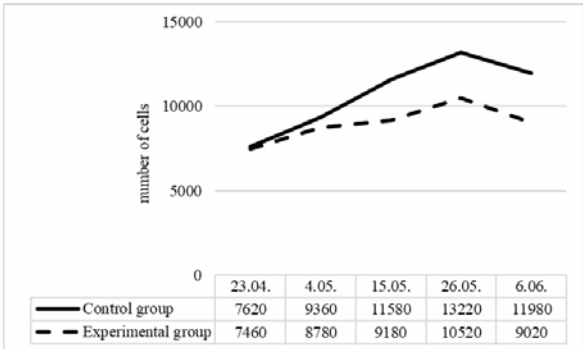

Figure 4 Amount of sealed worker bee brood (number of cells)

The spring development of the bee colonies provides possibility of preservation of the species. The number of bees in the colonies increases and this largely depends on amount of worker bee brood. The amount of worker bee brood from the experimental group bee colonies showed significantly lower values ($P<0.05$) than the control group for every control date.

Correlations between the amount of sealed worker bee brood and the quantity of bee pollen ($r=-0.61$, $P<0.01$) and the quantity of honey and bee pollen ($r=0.91$, $P<0.01$) were found in the control group bee colonies. Similar correlations were found in experimental bee colonies $r= -0.71$, $P < 0.01$ and $r =0.80$, $P < 0.01$, respectively (Figures 5 and 6).

The negative correlations showed that the supplies of bee pollen were rapidly decreased due to extensive consumption for brood feeding.

When the bee pollen is not sufficient in the bee colony the hypopharyngeal glands are underdeveloped. The lower pollen consumption has an impact on the development of the hypopharyngeal glands (Lebedev and Billas, 1994). It may result in the development of smaller HPhGs (Di Pasquale *et al.* 2013), less brood reared (DeGrandi-Hoffman *et al.* 2008) and reduced lifespan of the bees (Khoury *et al.* 2011). Pollen consumption is necessary for the glands to develop fully and to produce high quality protein-rich royal jelly for the brood. The hypopharyngeal glands in worker bees are a biological indicator for assessing the efficacy of the substances which the bees are fed (Omar *et al.* 2017). Renzi *et al.* (2016) studied the effect of thiamethoxam on hypopharyngeal gland development and protein content in *Apis mellifera*. The results showed that the bees had smaller and irregularly shaped acini.

In this respect we examined the effect of the antibiotic doxycycline on development of the HPhGs on the worker bees in the both groups bee colonies. Figure 7 shows that the control group has a higher rate of development of the HPhGs compared to the experimental group.

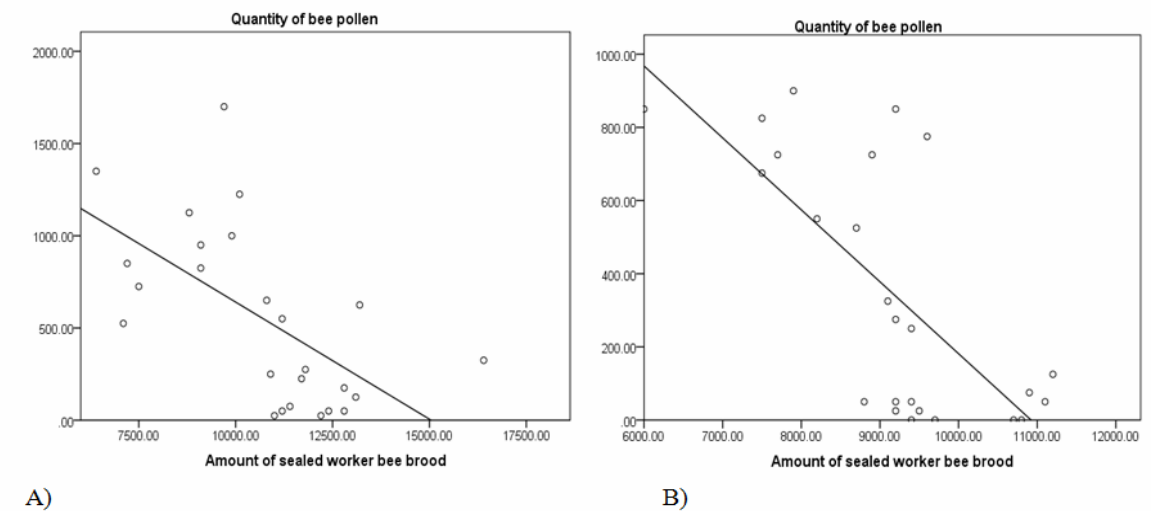

Figure 5 Correlation between the amount of sealed worker bee brood and the quantity of bee pollen A) control group (r=-0.61, P<0.01); B) experimental group (r=-0.71, P<0.01)

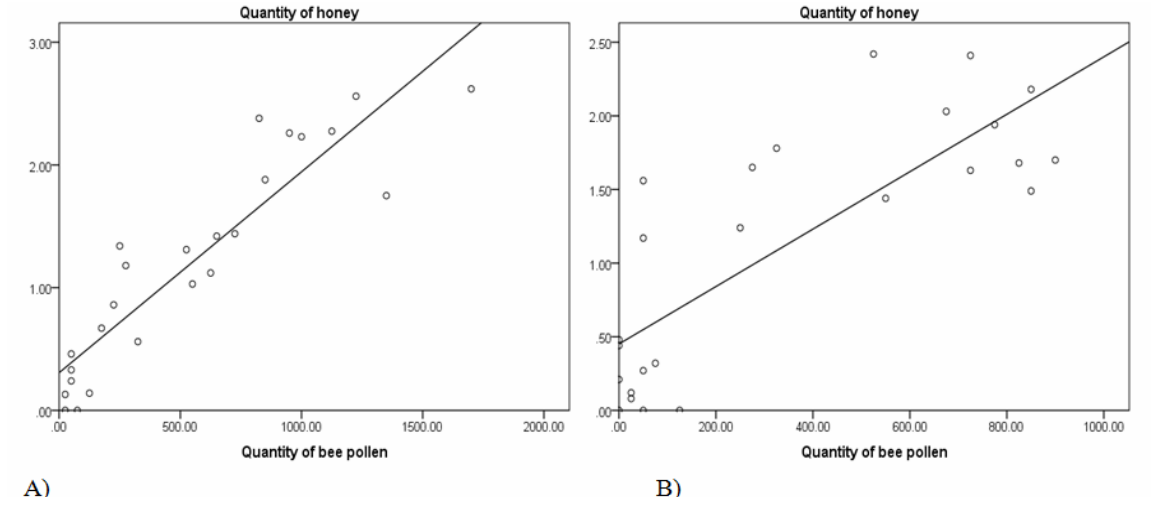

Figure 6 Correlation between the quantity of honey and bee pollen A) control group (r=0.91, P<0.01); B) experimental group (r=0.80, P<0.01)

Furthermore, the development of HPhGs could be a good criterion for evaluating the nutritive value of supplements for bees (Zhelyazkova and Shumkova, 2013; Shumkova and Zhelyazkova, 2015).

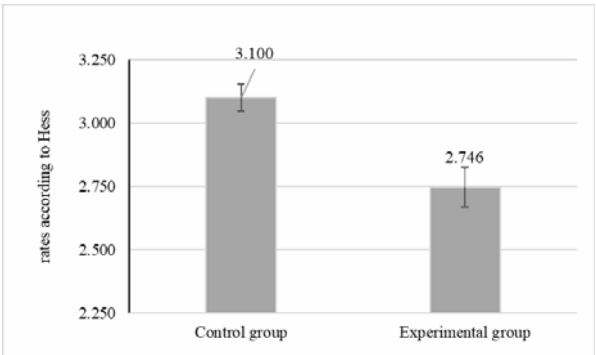

Figure 7 Development of the HPhGs of worker bees (rates according to Hess)

The results for the chemical composition of the worker bees' bodies after the spring feeding are given in Table 1.

The experimental group has a higher protein content and slightly greater mineral content than the control group. No significant differences were found for the studied parameters.

The results for some components from chemical composition of worker bees' bodies are comparable to previous studies (Shumkova *et al.* 2017). To date, more comprehensive data on chemical composition on the bees by adding different antibiotics as supplements are, however, limited. The results for the physicochemical parameters of bee honey obtained from the experimental and control group bee colonies are similar (Table 2). These values are in accordance with acceptable ranges for honey (EU Council, 2002) and similar to those obtained with others Bulgarian honeys (Shumkova and Balkanska, 2017).

Table 1 Chemical composition of the worker bees' bodies (Mean±SD)

Parameter	Control group	Experimental group	Significance
Water content, %	70.47±1.25	72.25±1.60	NS
Protein, %	52.10±3.70	55.40±4.81	NS
Lipids, %	11.95±0.55	11.51±0.77	NS
Mineral content, %	4.95±0.35	5.40±0.29	NS

NS: non significant.

Table 2 Physicochemical parameters of bee honey (Mean±SD)

Parameter	Control group	Experimental group	Significance
Water content, %	18.12±0.30	18.06±0.36	NS
Diastase, Gothe units	21.86±0.03	19.00±5.32	NS
Electrical conductivity, mS/cm	0.893±0.071	0.923±0.074	NS
pH	3.70±0.12	3.67±0.12	NS
Content of doxycycline	< 5.00 mg/kg	91.23 mg/kg	-

NS: non significant.

The quantity of doxycycline is relatively high in the honey samples received from the experimental group bee colonies. The reason for this is that the honey samples are taken immediately after the treatment with antibiotic. Sometimes the bees only transfer the sugar solution from the feeders of the hives in the honeycombs. As can be said before this honey is not for human consumption. Indeed, we expected lower values for doxycycline in honey. Therefore, according to present study doxycycline in a dose 500 mg/L is not suitable for the bees.

CONCLUSION

Significantly lower values were obtained for the strength of the bee colonies and the amount of sealed worker bee brood fed with doxycycline than the control group. Strong correlations were found between the amount of sealed worker bee brood and the quantity of bee pollen and the quantity of honey and bee pollen in the both groups bee colonies. From this study, it was concluded that development of HPhGs was not positively affected after application of antibiotic doxycycline at a dose of 500 mg/L. Based on the results obtained there is no difference in physicochemical parameters of honey samples and chemical composition of the worker bees' bodies from the control and experimental group. In the present study doxycycline residue in the honey samples were high. The experimental bee colonies were not used for honey production. In all causes testing for antibiotic residues in honey is always relevant particularly when the honey will be used as a food. The authors do not recommend doxycycline in the beekeeping.

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