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Hypopharyngeal glands development of worker bees (*Apis mellifera* L.) during supplemental feeding with various types of sugar syrups

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Abstract

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For the normal vital activity of bees, a complete diet is necessary, providing sufficient proteins, fats and carbohydrates. The hypopharyngeal and mandibular (pharyngeal and maxillary) glands of bees secrete the royal jelly to feed the larvae and the queen bee. In the present experiment, the effect of feeding of bee colonies (*Apis mellifera* L.) during spring and autumn stimulating feeding with substitutes of carbohydrate food such as "Sugar syrup", "Honey syrup", "Inverted sugar syrup" and "Isosweet" on the development of hypopharyngeal glands of worker bees has been established. After wintering (before feeding), the percentage of worker bees with a second stage of development of the hypopharyngeal glands is the highest. After the end of feeding in the spring period, in the bee colonies that received a honey solution, 48% of the bees had the maximum fourth stage of development of the hypopharyngeal glands. During the autumn period (before the start of feeding), the percentage of worker bees with the first stage of hypopharyngeal gland development (54%) was the highest and 48% had the second stage. During the indicated period, but after feeding was completed, 60% of bees fed Apiinvert, 56% of bees fed sugar syrup 2:1, and 56% of those fed honey syrup had the fourth stage of hypopharyngeal gland development. For the four stages of development of the hypopharyngeal glands of the worker bees, statistically significant differences (at $p < 0.05$) have been established between the bee colonies fed with the different types of syrups and the control groups in the spring and autumn stimulating feeding.

Keywords: worker bees; stimulating feeding; sugar syrups
Abbreviations: Stage of Development of the Hypopharyngeal Glands (SHPG); Hypopharyngeal Glands (HPG)

Introduction

Nutrition is particularly important for the correct course of physiological processes in insects. Most insect species have similar nutritional needs (Etebari et al., 2004). Using supplements of natural feed in some insect species, such as *Apis mellifera* L. and *Bombyx mori* L., is of interest to science and practice from the point of view of increasing the amount of produce obtained from them (Dingle et al., 2005). In that regard, several authors have found that the diet composition, along with the breed characteristics of specimens affects the productivity and development of economically beneficial insects – honey bees (Balkanska et al., 2013; Zhelyazkova & Shumkova, 2013) and silkworms (Singh et al., 2012; Saviane et al., 2014; Guncheva et al., 2016; 2020; 2021).

Honey bees get their food (nectar and pollen) from nature. Nectar is the main source of carbohydrates that provide energy to worker bees, and pollen is the main source of

proteins, amino acids, lipids, sterols, vitamins and minerals – necessary for brood rearing, maturation and development of individuals (Brodschneider & Crailsheim, 2010; Wright et al., 2018). The development and survival of larvae and adult bees depend on the bee colony's appropriate nutrition and health status (Wright et al., 2018). During periods of carbohydrate scarcity in nature, the needs of bee colonies can be met by providing sugar syrup.

Royal jelly is a protein-based food that is fed to bee larvae and queen bees (Huang & Otis, 1989; Crailsheim, 1990; Knecht & Kaatz, 1990; Ohashi et al., 1999). Royal jelly is secreted by the hypopharyngeal glands (HPG), located in the frontal area of the worker bee's head (Hrassnigg & Crailsheim, 1998; Deseyn & Billen, 2005). The feeding of honey bee larvae with royal jelly is directly dependent on the development of the hypopharyngeal glands of the "nurse" bees. The development of the bee colony depends on these glands (Silva de Moraes & Bowen, 2000). The stage of hypopharyngeal gland development (SHPG) depends on the age of worker bees and determines different social behaviours (Liu et al., 2013; Ueno et al., 2015). Each gland consists of small oval bodies (acini) that are connected by secretory ducts. Hypopharyngeal glands are fully developed and with the greatest secretion of royal jelly in young worker bees aged 6–13 days (Lass & Crailsheim, 1996; Rahman et al., 2014). The HPG activity depends mainly on the acini size, which changes with age to express age polytheism in worker bees (Robinson, 1992; Deseyn & Billen, 2005; Johnson, 2010). These glands are vulnerable to various types of stress, such as hunger, heat, and Varroa infestation, which can lead to their reduction and degeneration (Khalil, 1992; Yousef et al., 2014).

The objective of the present paper is to study the effect of supplemental feeding to bee colonies with different types of sugar syrups on the development of hypopharyngeal glands in worker bees (*Apis mellifera* L.).

Material and Methods

The study used 12 bee colonies from the local honey bee (*Apis mellifera* L.) housed in 10 Dadan-Blatt frame hives at the Educational and Experimental base of the Beekeeping section at the Faculty of Agriculture of Trakia University, Bulgaria. Spring and autumn feeding of the bee colonies was carried out. Colonies have been pre-equalized by strength, amount of sealed worker brood, food supply (honey and pollen) and queen bee age.

Groups

A total of 5 experimental groups and 1 control group of bee colonies have been formed along the provided scheme:

C – Control group – without supplemental feeding – 2 bee colonies;

1st Experimental group – supplemental feeding with sugar syrup at 1:1 ratio (sugar: water) – 2 bee colonies;

2nd Experimental group – supplemental feeding with sugar syrup at 2:1 ratio (sugar: water) – 2 bee colonies;

3rd Experimental group – supplemental feeding with inverted syrup – 2 bee colonies;

4th Experimental group – supplemental feeding with glucose-fructose syrup "Isosweet" – 2 bee colonies;

5th Experimental group – supplemental feeding with honey syrup at 1:1 ratio (honey: water) – 2 bee colonies;

Each bee colony from the experimental groups has been provided with 2 litres of syrup per week for supplemental feeding 3 times.

Determining the stage of hypopharyngeal gland development of worker bees

To determine the hypopharyngeal gland (HPG) development, from each bee colony, flightless worker bees were sampled at 5–10 days of age before and after feeding. For the two periods of the study (spring and autumn), the hypopharyngeal gland development of a total of 1200 worker bees has been determined. The method of Maurizio (1954) was applied using the 4-point Hess scale (in points).

The stage of hypopharyngeal gland development (SHPG) of each bee has been determined using an MBS-1 binocular magnifier.

Statistical analysis of the data

The "x^2 – analysis" (chi-square) method has been used to study the effect of feeding bee colonies with different types of syrups on the hypopharyngeal gland development of worker bees during spring and autumn stimulating feeding. The significant differences between the groups have been calculated using Univariate ANOVA with Post Hoc procedure with Tukey test at $p < 0.05$. Variations in the data have been examined using Levene's test.

The data obtained have been processed with the statistical software IBM SPSS Statistics 26.0 (NY, USA).

Results and Discussion

Spring period

Table 1 contains the results of the x^2-analysis regarding the stages of hypopharyngeal gland development of worker bees during the spring period – before feeding (immediately after wintering) and after feeding. After wintering in the experimental groups relatively high percentages of bees (48%; 46%; 38%; 36%; 36%) with HPG at a second stage of devel-

Table 1. Relationship between the type of feeding and the stages of hypopharyngeal glands development of worker bees for the periods Before Spring feeding (after wintering) and After Spring feeding

Season	Groups according to the type of feeding	Stage of hypopharyngeal gland development, %			
		SHPG 1	SHPG 2	SHPG 3	SHPG 4
Before Spring feeding (After wintering) (n = 300)	1:1 (honey/water)	28.0	48.0	20.0	4.0
	1:1 (sugar/water)	40.0	46.0	10.0	4.0
	2:1 (sugar/water)	28.0	38.0	26.0	8.0
	Apiinvert	28.0	36.0	30.0	6.0
	Isosweet	22.0	36.0	34.0	8.0
	Control	10.0	24.0	46.0	20.0
	Cramer's V = 0.207; Sig. (p) = 0.001				
After Spring feeding (n = 300)	1:1 (honey/water)	6.0	16.0	30.0	48.0
	1:1 (sugar/water)	12.0	28.0	30.0	30.0
	2:1 (sugar/water)	10.0	26.0	24.0	40.0
	Apiinvert	20.0	42.0	24.0	14.0
	Isosweet	8.0	8.0	42.0	42.0
	control	36.0	34.0	16.0	14.0
	Cramer's V = 0.256; Sig. (p) = 0.001				

opment have been reported, while in the control group, the percentage of bees whose hypopharyngeal glands reached a third stage of development has been 46%. The results for the spring period after feeding the bee colonies show that with the addition of honey syrup, the highest percentage of bees (48%) with the fourth stage of development of the hypopharyngeal glands has been recorded. In the group fed sugar syrup 1:1, an equal amount of bees (30% each) whose glands were developed to the third and fourth stages have been recorded. Similar are the results about the bee colonies fed Isosweet syrup – an equal number of bees (42% each) have been reported whose glands had developed to SHPG 3 and SHPG 4. For the bee colonies receiving Apiinvert, it has been found that the highest percentage of bees (42%) with a second stage of HPG development. For the control group, the percentage of bees whose glands reached the SHPG 1 development stage has been the highest (36%).

The comparative analysis of the results for the hypopharyngeal gland development of worker bees (Table 1) shows that before spring stimulating feeding in all groups (except for the control one), the percentage of bees with a second stage of gland development has been maximum (36-48%), and after the end of feeding in four of the groups the percentage of bees with the third and fourth stage of HPG development has been the highest. In the control group, the best development has been reported for the first and second stages (36% and 34%, respectively). Taking into account the fact that the bees from all bee colonies included in the study have been placed under the same climatic conditions and bee pasture, it can be commented that the differences in the hypopharyngeal gland development of the bees from the analysed samples are due to a certain extent to supplemental feeding. A confirmation of the stated opinion are Cramer's V coefficients (0.207 and 0.256), which show that the effect of the type of feeding on hypopharyngeal gland development of worker bees is weak but statistically significant.

Figure 1A outlines the significant differences between the bee colonies included in the study fed the different types of syrups before the spring stimulating feeding for the different stages of their hypopharyngeal gland development. It can be seen from the figure that for all stages of HPG development of bees, there are reliable differences between the individual groups. Significant differences have been reported between the control and all experimental groups.

For bee colonies whose hypopharyngeal glands reached SHPG 1 development stage after spring feeding (Figure 1B), significant differences have been found between bee colonies fed Apiinvert and all other groups. The control group is statistically different from all the experimental groups that received the different types of syrups. In bee colonies with SHPG 2, significant differences have been found between the group fed honey syrup and the groups fed sugar syrup 1:1, Apiinvert and Isosweet. The 2:1 sugar syrup fed group has been statistically different from the Apiinvert and Isosweet fed groups. The control group was statistically different from all experimental groups fed the different syrups. In bee colonies with SHPG 3, differences have been reported between those fed Isosweet and all other groups, as well as between the control and all other groups. In bee colonies with SHPG 4, significant differences have been found between the group fed sugar syrup 1:1 and all other groups. The 2:1 sugar syrup and Isosweet fed groups differed from the control and Apiinvert fed groups.

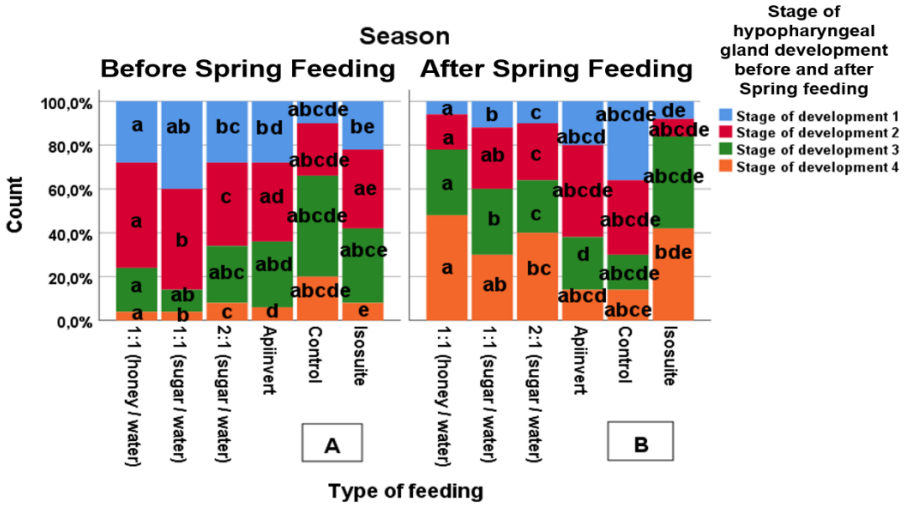

Fig. 1. Significant differences between bee colonies fed with various syrups during the spring stimulating feeding on the four stages of development based on their hypopharyngeal glands

Legend: The same letters on the lines show significant differences between groups at $p < 0.05$, as follows: a-a 1:1 (honey/water) and the remaining groups; b-b 1:1 (sugar/water) and the remaining groups; c-c 2:1 (sugar/water) and the remaining groups; d-d Apiinvert and the remaining groups; e-e Control group and the remaining groups

Autumn period

Table 2 shows the results of the x^2-analysis regarding the stages of hypopharyngeal gland development of worker bees during the autumn period – before the autumn stimulating feeding and after its completion. Before the start of feeding in the experimental groups, a relatively high percentage of bees with HPG in the first stage of development (34%, 38% and 54%) and the second stage of development (42%, 44% and 48%) has been reported. After the end of the autumn feeding, the highest stage of hypopharyngeal gland development (SHPG 4) has been recorded in bee colonies fed honey syrup (56%), sugar syrup 2:1 (56%) and Apiinvert (60%). For the group fed sugar syrup 1:1, the percentage of bees whose glands developed to SHPG 3 has been the highest (34%). For the control group and the one fed Isosweet, the highest number of bees whose glands developed to SHPG 2 has been reported (42% and 50%).

The data in Table 2 shows that before the autumn supplemental feeding, the percentage of bees with the first (34%, 38% and 54%) and second (42%, 44% and 48%) stages of HPG development has been the maximum. After the completion of feeding in three of the groups (fed honey syrup, sugar syrup 2:1 and Apiinvert) the percentage of bees with a fourth stage of HPG development has been the highest – 56%, 56% and 60%, respectively. In bees from the control group and those receiving Isosweet, the best gland development has been reported for the second stage, regardless of the study period spring or autumn. Based on the results

Table 2. Relationship between the type of feeding and the stages of hypopharyngeal glands development of worker bees for the periods Before Autumn feeding and After Autumn feeding

Season	Groups according to the type of feeding	Stage of hypopharyngeal gland development, %			
		SHPG 1	SHPG 2	SHPG 3	SHPG 4
Before Autumn feeding (n=300)	1:1 (honey/water)	34.0	32.0	26.0	8.0
	1:1 (sugar/water)	34.0	42.0	18.0	6.0
	2:1 (sugar/water)	38.0	36.0	24.0	2.0
	Apiinvert	54.0	32.0	12.0	2.0
	Isosuite	26.0	48.0	18.0	8.0
	control	24.0	44.0	18.0	14.0
	Cramer's V = 0.156; Sig. (p) = 0.011				
After Autumn feeding (n=300)	1:1 (honey/water)	0.0	16.0	28.0	56.0
	1:1 (sugar/water)	18.0	32.0	34.0	16.0
	2:1 (sugar/water)	0.0	12.0	32.0	56.0
	Apiinvert	6.0	10.0	24.0	60.0
	Isosuite	6.0	42.0	34.0	18.0
	control	26.0	50.0	20.0	4.0
	Cramer's V = 0.338; Sig. (p) = 0.000				

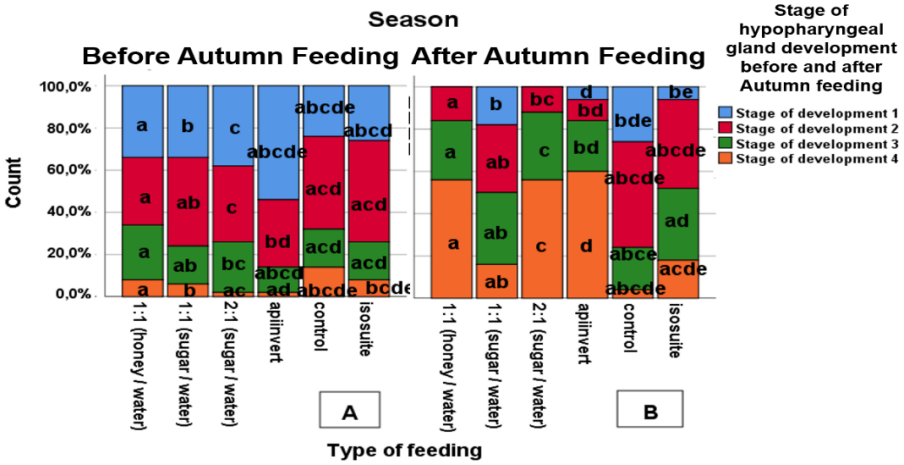

Fig. 2. Significant differences between bee colonies fed with various syrups during the autumn stimulating feeding on the four stages based on their hypopharyngeal gland development

Legend: The same letters on the lines show significant differences between groups at $p < 0.05$, as follows: a-a 1:1 (honey/water) and the remaining groups; b-b 1:1 (sugar/water) and the remaining groups; c-c 2:1 (sugar/water) and the remaining groups; d-d Apiinvert and the remaining groups; e-e Control group and the remaining groups

from the autumn stimulating feeding, it can be assumed that the differences in the hypopharyngeal gland development of the bees from the analysed samples are due to some extent to the supplemental feeding as well. Cramer's V coefficients (0.156 and 0.338) show that the strength of the relationship between the studied variables varies from weak to moderate while maintaining its statistical significance.

Figure 2 shows the significant differences between the bee colonies fed with the different types of syrups in the autumn stimulating feeding for the various stages of their hypopharyngeal gland development. It can be seen from Figure 2A that before autumn feeding for all stages of HPG development of bees there were significant differences between individual groups. The control group was statistically different from all the experimental groups.

For bee colonies with hypopharyngeal glands at SHPG 1 stage of development after autumn supplemental feeding (Figure 2A), significant differences have been found mainly between the control group and bee colonies fed with sugar syrup 1:1, Apiinvert and Isosweet. There is also a significant difference between bees fed sugar syrup 1:1 and those receiving Isosweet. Bee colonies with SHPG 2 fed 1:1 sugar syrup are statistically distinguished from all other groups. Statistically significant differences have been found between the groups fed sugar syrup 2:1 and Apiinvert with the control group, as well as with the group fed Isosweet. In bee colonies with SHPG 3, differences have been found between the group fed honey syrup and the groups fed sugar syrup 1:1, Isosweet and the control group, respectively. In addition, the 1:1 sugar syrup-fed group is statistically different from the Apiinvert-fed group and the control group. The 2:1 sugar syrup-fed group differed only from the control group, and the Isosweet-fed group differed from both the Apiinvert-fed group and the control group. In bee colonies with SHPG 4, significant differences have been mainly found between the control group and all other groups fed the different syrups.

Conclusions

When studying the effect of the different types of carbohydrate food substitutes on the hypopharyngeal gland development of worker bees during spring and autumn stimulating feeding, the following results have been established.

After wintering (before spring feeding), the highest percentages of worker bees with a second stage of hypopharyngeal gland development have been reported. An exception is the control group, where 46% of the studied bees have hypopharyngeal glands in the third stage of development.

After completion of the spring feeding of bee colonies, the highest percentage (48%) of bees with the maximum fourth stage of hypopharyngeal gland development has been found in colonies that received honey syrup.

In the autumn period before feeding, the highest percentage of worker bees (54%) with hypopharyngeal gland development in the first stage and 48% with a second stage of gland development has been recorded in the bee colonies.

After supplemental feeding in the autumn period, the highest stage of hypopharyngeal gland development (fourth) has been reported in bee colonies fed honey syrup (56%), sugar syrup 2:1 (56%) and Apiinvert (60%).

Statistically significant differences (at $p < 0.05$) have been found between bee colonies fed different types of syrups during the spring and autumn stimulating feeding and the control groups for the four stages of their hypopharyngeal gland development.

Based on the results of the spring and autumn stimulating

feeding of the bee colonies with different types of carbohydrate foods (syrups), it can be assumed that the differences in the hypopharyngeal gland development of the bees from the analysed samples are to some extent also due to the feeding. Confirmation of the assumption are Cramer's V coefficients (0.207 and 0.256 for the spring period and 0.156 and 0.338 for the autumn period), which show that the type of supplemental feeding affects the hypopharyngeal gland development of worker bees, and the strength of the relationship between the studied variables varies from weak to moderate while maintaining its statistical significance.

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