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THE USE OF WHEY FOR HONEY BEE FEEDING AND OBTAINING OF PROTEIN-CARBOHYDRATE BEE FEED

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Abstract

The content and quality of the dietary proteins determines its preferences and accessibility for honey bees, and represent the basis for the elaboration of protein additives for supplementary bee feeding, which is increasingly applied in beekeeping practices to compensate the amino acid deficiency when natural pollen is scarce. The use of sugar and whey syrup as a protein supplement for early spring feeding stimulates the growth of bee colonies by 13.1-14.5% and increases productivity in harvesting acacia honey by 24.7-44.8%. The result is explained by the fact that whey contains a higher amount of essential amino acids compared to pollen. The whey also contains all vitamins of group B, enzymes and minerals that increase the working capacity of bees, ensure an intensification of the queen's egg laying, improve the broad rearing. At the same time, sweetened whey may be used to obtain easily assimilable protein-carbohydrate food (honey) with a high content of amino acids for the nutrition of bee colonies in pollen-deficient periods in nature.

Key words*: honey bee, protein, amino acids, whey.*

INTRODUCTION

For normal growth and development, bees need proteins, carbohydrates, lipids, vitamins, minerals and water. The main sources of these nutrients are honey and pollen. If the honey is permanently present in the hive, then pollen (or bee bread) at some periods of the year may be in insufficient amounts, which may cause protein deficiency in the bee's nutrition, which in turn affects their health and resistance to infections and parasites. To compensate these deficiencies in bee's nutrition, the protein supplemental bee feeding are largely used depending on the seasonal specificity of the bee functional state and the bee colony's protein needs: in spring – for feeding and brood growth, and in autumn – for the accumulation of reserves in the fat body (fat bees), which ensures better survival of colonies during the winter (Brodschneider and Crailsheim, 2010; Eremia, 2009).

Some supplements for honeybees are also intended to enhance disease control, but their efficacy is not always scientifically confirmed (Botíasa et al., 2013).

Beekeeping specialists have been carried out investigations in pollen substitute proteins that would supplement the amino acid deficiency of bee colonies, especially in early spring. Various processes and technologies for obtaining protein supplements based on soybean, peas, corn, oats, barley meal, egg powder and egg whites, integrated milk or powdered milk, fish meal, dry yeast, etc. are known to be administered either in the form of "candy", which is a mixture of honey, powdered sugar and protein flour, or in the form of sugar syrup with the addition of proteins or amino acids (Malaiu, 1976; Standifer et al., 1977; Brodschneider and Crailsheim, 2010; Fleming et al., 2015).

However, these substituents did not have the same effect on various vital processes in the bee organism, and as a result contradictory data on the effects of protein supplements feeding on the growth and productivity of bee colonies were obtained. When different protein additives are analyzed, it is recommended to consider the biological value of the proteins and the amino acid content and composition compared to pollen or bee bread. Following these parameters, among the protein sources tested, the most positive results were obtained using dried yeast, soy bean meal and milk (Brodschneider and Crailsheim, 2010, Matilla and Otis, 2006).

Also the supplements can be obtained by mechanical mixing of the selected components, without taking into account the functional peculiarities of food consumption of adult bees. It is known that working bees only consume liquid food; solid protein particles (as flour) are hardly assimilated by bees (Wright et al., 2018). At the same time, the majorities of protein-enriched candies are costly and take a long time to get the final product. On the other hand, in the case of syrup administration in low temperature conditions of early spring and late autumn, the bees lose a lot of energy for its processing (evaporation of the syrup fluid), which in turn causes bee colonies weakening.

Recently, researchers and specialists in nutriation pay close attention to milk whey as a source of biologically and physiologically valuable protein. For these reasons whey proteins are currently used for various therapeutic purposes, as well as a basic component of balanced diet (Krissansen, 2007; Gupta and Prakash, 2017). However, data on the use of whey as a protein additive for bee feeding are fragmented or even lacking.

Thus, the aim of this study work was to reveal the influence of whey sugar syrup on the honey bee colony development and honey production and the use of whey syrup to obtain protein-carbohydrate food (honey) with a high content of amino acids for bee feeding.

MATERIALS AND METHODS

In order to reveal the influence of whey-based syrup (sweetened whey) on the honey bee colony development and honey production, the experiments were performed on a group of 8 bee colonies: 4 – the control group and 4 – the experimental group.

Syrup was administered in the spring (year 2017). Colonies in the control group were given sugar syrup in the ratio 1:1 water and sugar, and colonies in the experiment group– sweet whey sugar syrup in the same proportion (1:1 sweet whey and sugar). Each colony consumed 3 liters of syrup, given at most 300 ml at an interval of 2-3 days for one month. The recordings were performed until and after stimulation feeding. The size of bee colony was measured by the number frames with bees in hive and by the amount of capped brood cells.

To obtain the protein-carbohydrate food (honey) the bee colonies were feeding with whey sugar syrup in the period: from the end of the picking of acacia honey to the beginning of harvesting of lime honey, daily 3-5 liters per colony in the evening.

Determination of the free amino acid content in obtained honey was performed at the amino acid analyzer AAA-339M (Czech Republic) by the ion exchange chromatography method (Moore et al., 1958).

The analysis is performed in the standard procedure for the determination of free amino acids using lithium buffer solutions, pH 2,90; 2,95; 3,20; 3,80 and 5,00; with a flow rate of 12,0 ml/hr. On the basis of the qualitative calculation of amino acid content in the liquid studied it is stated that the amount of an amino acid in the sample is proportional to the surface of the pick of the chromatogram. The calculation consists in the fact, that sample and standard mixture of amino acids with the same content is analyzed. The amount of amino acids dosed on the ionic column in the test sample is given by the formula below:

$$C_{i(doz.)}=k \cdot n \cdot S_{i(prob.)}/S_{i(st.)} \cdot M_i \cdot 10^{-6} \text{ (mg)}$$

where $C_{i(doz.)}$ – the ionic concentration of amino acids in the volume of the dosed node; n – the amount of the amino acids in the analyzed mixture; $S_{i(prob.)}$ – the tip(pick) surface of the amino acids in analyzed mixture; $S_{i(st.)}$ – the tip(pick) surface of the amino acids in standard mixture; k – correction coefficient considered to be changing the detector sensitivity; M_i – the ionic molecular weight of the amino acid.

The automatical analyzer AAA-339M detects ninhydrin positive components within 1-100 nanomoles concentration. The duration of the analysis of the physiological fluids is 3.5 hours.

RESULTS AND DISCUSSIONS

For normal growth and development, bees need ten (10) essential amino acids, namely valine, leucine, isoleucine, threonine, methionine, lysine, arginine, phenylalanine, tryptophan, and histidine (De Groot, 1953).

The main source of amino acids and proteins for bees is pollen. However, the pollen from different floral sources has different nutritional values for bees (Corby-Harris et al., 2018).

In the conditions of lack of pollen in nature, there is more than necessary early spring protein feeding to supplement the protein and amino acids deficiency in food, which are also necessary for the honey bee colony growth and increasing of acacia honey production. Such feeding is a frequent apicultural practice for spring build-up of bee colonies, that stimulate the egg laying by queen in early spring, restore the number of bees in colony and start developing more rapidly (Eremia, 2009; Fetea et al., 2011).

It should be noted that when replacing pollen with other protein-rich feed, it is advisable to consider their nutritional value derived from the amino acids pattern, as well as their amount, especially of essential ones.

On the other hand, Herbert et al. (1977) demonstrated that the optimum protein content in bee feed should be 20-30%. At the same time the 50% level should be avoided.

Choosing of milk whey as the basis for the syrup for honey bee feeding has emerged from the exclusively significance of whey as a dietary super food of new generation (Barth & Behnke, 1997).

Whey proteins make up 20% of milk proteins (the remaining 80% is casein) and have biological and physiological importance (Krissansen, 2007, Gupta & Prakash, 2017). Thus, the whey is valuable not only in terms of the amount of protein but also of its quality. Whey proteins have various therapeutic applications for humans due to its immuno-regulatory, detoxifying, anti-inflammatory, antimicrobial and antioxidant properties (Krissansen, 2007).

Comparative analysis of whey and pollen amino acid patterns revealed that sweet cow's milk whey contains all the spectrum of amino acids present in the pollen. However, their amount in whey is higher than in pollen from different plant species (Table 1).

In whey, as in pollen, a higher content of glutamic and aspartic acids was established. It is considered that glutamic acid is important for the formation of bee's olfactory memory (Locatelli et al., 2005), and glutamine serves as a "fuel" for rapidly proliferating cells, and is considered "conditional essential" in metabolic stress conditions (Krissansen, 2007).

Whey proteins contain a significant amount of branched chain amino acids (BCAAs) – leucine, isoleucine and valine. It was determined that leucine and isoleucine enhance protein synthesis (Kimball & Jefferson, 2006), and for bees, they are key amino acids in the formation of haemolymph proteins. Also these amino acids ensure the functional balance of the internal secretion glands and play an important role in the transition from larva to pupa. Valine is also essential in function of nervous system (Malaiu, 1976; Woltedji et al., 2013).

Table1. The content of some amino acids (mg/g dry weight) in pollen from various plant species (Szezęsna, 2006) and milk whey (Markus et al., 2002; Nilsson et al., 2007)

Amino acids	**Pollen from flower sources**					**Milk whey**
	Onagraceae	*Caryophyllaceae*	*Brassica*	*Sinapis alba*	*Chelidonium maius*	
Essential						
Valine	11.93	9.81	10.70	11.79	11.60	59.3
Leucine	16.09	18.80	21.49	22.26	23.10	79.8
Isoleucine	10.23	8.82	8.92	9.88	9.72	57.3
Threonine	9.62	9.53	12.50	11.65	11.44	61.1
Methionine	2.65	3.33	4.30	4.16	4.52	19.4
Lisine	14.22	14.19	20.23	21.14	16.19	76.1
Arginine	9.45	8.80	11.00	11.26	10.72	22.0
Phenilalanine	10.24	9.71	10.75	11.46	11.08	21.3
Histidine	4.63	6.16	5.35	5.57	5.72	18.7
Tryptophan	2.0					12.3
Non eseential						
Alanine	11.47	10.41	12.61	12.94	12.54	42.1
Cysteine	3.4					22.8
Tyrosine	5.20	4.77	5.87	5.62	5.85	20.8
Glycine	9.76	9.58	11.47	12.76	10.79	13.8
Proline	24.97	28.79	28.02	26.98	19.18	46.7
Serine	10.43	11.43	13.27	14.20	11.94	38.8
Aspartic acid	24.85	21.52	25.45	30.22	27.01	94.1
Glutamic acid	26.43	22.47	26.89	29.25	27.90	141.4

In whey (compared to pollen), there is a higher amount of methionine and histidine. It is known that methionine is actively involved in the regulation of protein and lipid metabolism, and in the neutralization of toxic substances, and histidine is particularly necessary for growing brood (Malaiu, 1976; Di Pasquale et al., 2016).

As well, as a result of decarboxylation, histidine is converted to histamine, which is a component of bee venom (De Groot, 1953). In whey, a higher level of tryptophan was found. In pollen only trace amount of this amino acid was detected. Tryptophan is important in maintaining of reproductive functions, producing of nicotinic acid, synthesizing of proteins for larvae feeding and contributing to pigmentation of the bee's body (Zhao et al., 2015). Some of non-essential amino acids – glycine and proline (that have a higher content in whey in comparison with pollen), exert a stimulating effect on growth in unfavorable conditions and on the flying capacities of honey bees (Haydac, 1970; Malaiu, 1976; Micheu et al., 2000; Di Pasquale et al., 2016). The ratio of lysine and arginine determines the nutritional value of bees' protein (Szezęsna, 2006). In pollen from various floral sources this ratio is 1,87, while in whey – 3,45, which is another argument in using whey as a protein pollen substitutes.

Based on the nutritional potential of whey, the experiments of bee colonies feeding with whey-based syrup in early spring were conducted (Derjanschi et al., 2014). Recordings were made until and after stimulation feeding.

The analysis of the obtained results reveals that the use of sugar and whey syrup stimulates the growth of bee colonies and the brood growth with 13,1-14,5% compared to the control group which receiving water sugar syrup (Table 2).

Table 2. Capped brood and honey production after stimulation feeding of bee colonies with whey sugar syrup

Variants	Capped brood		Honey production	
	Cells x 10^2	%	kg	%
Control	124.1	100	10.5	100
Experimental group				
1	142.1	114.5	15.2	144.8
2	140.5	113.2	13.6	129.5
3	141.6	114.1	14.8	140.9
4	140.4	113.1	13.1	124.7

Similar data were obtained by other authors, who noted the increase in the number of rearing broods in bee colonies feeding with pollen supplements or pollen protein substitutes in early spring (Doull, 1980; Mattila and Otis, 2006). Previous studies have established that various protein substitutes as well as bee brad contribute to the protein content in haemolymph, development of hypopharyngeal gland, and bee life span (Algarni, 2006; De Jong et al., 2009).

It should be noted that balanced feeding with supplements or with protein substitutes influences not only the brood rearing and some bee morphological and physiological parameters, but also the production of honey.

Thus, it was established that in the experiment with whey-based syrup administration, the productivity of acacia honey increased by 24.7-44.8% compared to the control variant (Table 2). So, we can assume that whey as a source of valuable protein can influences the colony's gathering capacity (Derjanschi et al., 2014).

Some authors consider that increasing honey production in colonies fed with protein supplement may be the result of increasing the bee longevity (Doull, 1980; Mattila and Otis, 2006). Other authors state that honey production is closely correlated with the some physiological abilities of bees, which depends on the feed protein content (Al-Sherif et al., 2012; Malaiu, 1976; Ohashi, 1997). As mentioned above, proteins are absolutely essential for the hypopharyngeal gland development and for the enzyme secretion respectively, which in turn determine the amount of harvested nectar. A direct correlation between the activity of the secretory glands (enzyme activity), the ability to nectar process and the production of honey has been demonstrated (Jerebkin, 1965).

The obtained results are explained by the fact that whey contains the same nutrients that pollen, but in higher quantities (Table 1). The whey also contains B complex vitamins, calcium, potassium and other mineral, which can influence the bee's working capacity, activate the egg laying by queen and brood growth.

Taking into account the nutritional qualities of whey and the high content of essential amino acids for bee growth and development it have been carried out the experiments to obtain whey protein-based food (honey) that corresponds to the natural nutrition of the bees and can be proposed as a protein supplement in the absence of pollen in nature.

The protein-carbohydrate bee feed (whey honey) is obtained as a result of whey sugar syrup administration to bee colonies between the end of the acacia honey harvesting and the beginning of lime honey harvesting and the extraction of "whey honey" after 5-7 days of the last syrup administration. The expected result was to obtain easily assimilable bee food (honey) with an increased amino acid content or protein-carbohydrate bee feed (Vrabie et al., 2019).

In the obtained "whey honey", the content of free amino acids was determined and analyzed in comparison with sunflower honey, most frequently administered to bee colonies during the cold period of the year (January-February) and pollen, which is the main source of protein for bee nutrition (Table 3).

Table 3.The content of free amino acids in protein-carbohydrate bee feed, obtained from whey syrup, in sunflower honey and in pollen (mg/g dry weight)

Aminoacids	**„Whey honey”**	**Sunflowe rhoney**	**Pollen**
Aspartic acid + Asparagine	0.372	0.197	13.967
Threonine	0.156	0.059	6.950
Serine	0.176	0.091	6.598
Glutamic acid + Glutamine	0.429	0.256	16.222
Proline	0.736	0.479	19.142
Glycine	0.088	0.055	6.772
Alanine	0.141	0.084	8.092
Valine	0.135	0.075	5.894
Cysteine	0.030	0.017	1.464
Methionine	0.003	0.001	1.432
Isoleucine	0.148	0.056	4.485
Leucine	0.220	0.092	1.092
Tyrosine	0.028	0.009	3.434
Phenylalanine	0.116	0.149	5.903
Lysine	0.180	0.087	6.722
Histidine	0.053	0.027	2.282
Arginine	0.058	0.042	3.584
Tryptophan	0.007	0	0
Σ of free amino acid	3.076	1.776	114.03
Σ of essential amino acids	1.076	0.588	38.344
Σ of non essential amino acids	1.999	1.189	75.691
Σ of immunoactive amino acids	1.446	0.779	59.187
Σ of BCAAs	0.503	0.223	11.471

The protein-carbohydrate bee feed (whey honey) has a higher free amino acid content compared to sunflower honey.

The ratio of free essential amino acids to the total content of free amino acids in honey obtained from whey is 35.0%, in sunflower honey – 33.1% and in pollen –33.6%; of the immunoactive amino acids respectively is in "whey honey"– 47.0%, in sunflower honey – 44.0% and in pollen – 51.9%.

A significant index for bee's vital activity is the content of branched chain amino acids (valine, leucine and isoleucine), which in "whey honey" is 16.35%, in sunflower honey – 12.55% and in pollen – 10.06%.

Also, isoleucine is the major limiting factor in bee nutrition, which should be not less than 4% of the protein content (Stace et al., 1994). In the protein-carbohydrate bee food obtained from whey the isoleucine content is 4.8%, in the sunflower honey – 3.1%, and in the pollen – 3.9% of the total amino acid content.

It was determined that the lysine-arginine ratio as well as the high lysine content is an important index that determines the protein quality required for bee feeding and the bees' preferences for certain types of pollen (Szezęsna, 2006). In "whey honey" this index is 3.1, in sunflower honey – 2.0 and in pollen – 3.0.

Thus, the protein-carbohydrate bee feed (honey) obtained from the sweetened whey, by exploiting the physiological-metabolic potential of the bee colonies, is an organically valuable product in terms of amino acid content to fill its needs for bees in pollen-deficient periods in nature, which is also accessible and inexpensive.

CONCLUSIONS

Whey contains all the spectrum of amino acids present in the pollen.

The amount of free amino acids in whey is higher than in pollen from different plant species.

Due to the balanced content of proteins and amino acids in comparison with pollen whey can be used for bee stimulating feeding in early spring.

The administration of whey as a protein substitute encourage the brood rearing and, respectively, the bee colonies size in the spring, which contributes to the increase of acacia honey production.

Protein-carbohydrate bee food (whey honey) obtained from sweetened whey has an increased content of amino acids, is easily assimilable, inexpensive for bee colony stimulating feeding.

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