Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 1, 2020
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

BIOCHEMICAL FEATURES OF PROTEIN NUTRITION OF HONEY BEES

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Abstract

The features of protein nutrition and protein requirements of honey bees must be taken into account, given that bees are social insects, and should be examined at three levels: bee colony nutrition; adult bee nutrition; larva nutrition, since disturbances in protein nutrition at the previous stages of development affect the subsequent stages and vice versa. The content of free amino acids in the main protein sources - pollen, bee bread royal jelly, as well as the content of amino acids in some bee protein additives and in whey was examined. The quality of the natural bee protein food, must be evaluated, depends on the essential amino acid content, especially leucine, isoleucine and valine. When replacing the natural protein food of bee colonies, with other protein additives and substitutes, it is necessary to consider the protein and essential amino content, and also the lysine/arginine ratio, as an index of the quality of the protein and the protein additive respectively.

Key words: *amino acids, honey bee, proteins.*

INTRODUCTION

It is known that for normal growth and development, honey bees need basic nutrients: proteins, carbohydrates and lipids, as well as vitamins and minerals. It has also been proven that adequate nutrition is the basis for the growth and development of bee colonies. Nutritional deficiencies and starvation are likely causes of bee colony loss worldwide (VanEngelsdorp et al., 2009*)*.

Proteins play the most important role in the health and vital functions of honey bees and honey bee colonies, respectively (Crailsheim, 1990):

• From a biological point of view, proteins are the main substances of a living cell, since the formation and growth of new organisms depends on them.

• From a physiological point of view, proteins take part in most of the vital processes of the body, as they form the basis of enzymes, hormones and many other biologically active substances.

The protein requirement of bees must be taking into account based on the fact that bees are social insects, often considered superorganisms (Seeley, 1989). Thus, protein nutrition should be regarded at three levels: features of protein nutrition of the bee colony as a whole; features of protein nutrition of an adult bee; features of protein nutrition of the larva, since, disturbance in protein nutrition at previous stages of development affect subsequent stages and vice versa (Brodschneider and Crailsheim, 2010).

Proteins are necessary for young bees to form wing muscles (Hersch et al., 1978). A suboptimal protein diet slows down the time needed to reach the maximum thoracic mass of a working bee (Hagedorn and Möller, 1968). Proteins are also necessary for the development of hypopharyngeal glands (Alqarni, 2006) and ovaries (Hoover et al., 2006). It has been shown that glucose oxidase activity (an indicator of social immunity) and fat body mass (an indirect indicator of the immunocompetence of the individual bees) depend on protein nutrition (Alaux et al., 2010). Protein-rich food is absolutely essential in early spring for the growth and development of brood, to ensure the duration and quality of life of working bees (Algarni, 2006; Brodschneider and Crailsheim, 2010). In addition, protein-rich feed during this period are beneficial for bee health and their ability to resist infections and parasites (Alaux et al., 2010).

First of all, proteins are necessary as a source of amino acids that are reused in the biosynthetic processes of the bee's body.

The main source of amino acids and proteins for bees are pollen and bee bread. Royal jelly is a source of proteins for larvae and queen.
The nutritional value of pollen primarily depends on the protein content. It has been proven that the content of proteins and amino acids in pollen depends on the type of plant and can vary from 3 to 61%, but an average make up about 25% (Roulston et al., 2000). Relatively protein-rich pollen is observed in plants most often pollinated by bees or other insects (dandelions, fruit trees, clover, alfalfa and other plants). Low protein pollen is the majority of herbs, sedges, conifers, ragweed, and other plants that are pollinated by the wind. Thus, pollen collected from different flower sources has different nutritional values for honey bees. It has also been proven that pollen quality directly affects the bee health (immunity, metabolism, brood, lifespan and fertility). The life span of working bees increases significantly when the pollen with a high percentage of protein (25-30%) is consumed - compared with lower quality pollen (less than 20% protein) (Schimdt L.S et al., 1995). Brood growth also depends on the quality of pollen (amount of protein): increases - when only pollen with an optimal content of protein and essential amino acids is available; decreases - when only low protein pollen is available. Pollen with a high nutritional value contributes to the resistance of bee colonies to: parasites, pathogens, stress factors.
The amount of pollen consumed by the bee colony largely depends on the percentage of protein. In his studies, Anderson et al. (2014) found that in order to receive 10 g of bioavailable protein, the bee colony must process 48 g of pollen with 30% protein or 72 g of pollen with 20% protein. That is 2 kg of pollen with 30% protein is equal to 3 kg of pollen with 20% protein. During the year, the bee colony consumes from 25 to 55 kg of pollen.
In addition, many other factors influence the amount of consumed pollen (Anderson et al., 2014). Eating only one type of pollen, which has a small amount of protein, does not cover the bee's body needs for essential amino acids. Schimdt et al. (1995) showed that only a mixture of various types of pollen has a beneficial effect on the development and productivity of bee colonies.
Thus, the nutritional value of pollen also depends on the content of essential amino acids. According to the results obtained by De Groot (1953), honey bees need 10 (ten) essential amino acids to support vital processes - arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The absence or low content of one of 10 essential amino acids in pollen significantly reduces the development of the bee colony. Most of all, honey bees need leucine, isoleucine and valine, or the so-called BCAA (Branced Chain Amino Acids).
For example, bees need 4% isoleucine from their protein intake. In one type of pollen, only about 2% of isoleucine is contained, which leads to the consumption of twice amount of pollen to meet the requirements of the bee colony or to the mixing of different types of pollen containing a large amount of essential amino acids. In addition, these poly flower pollen mixtures enhance certain immune functions and provide antiseptic protection for honey bees (Alaux et al., 2010).
It was determined that the lysine/arginine ratio, as well as, the high lysine content, is the factor that determines the quality of pollen proteins and bee preferences in some types of pollen (Szezęsna, 2006). In pollen collected from various flowers, this ratio ranges from 1.24 to 3. For acacia pollen, the Liz/Arg ratio is 1.38, and for rape pollen - 1.84. It is considered that those plants that contain more essential amino acids in pollen are more beneficial for honey bees and are more often visited by them.
Amino acids also play an important role in the formation of food motivation and the selective collection of pollen from various plants. It has been established that pollen amino acid composition affects the sensitivity of special bee receptors, thereby contributing to the formation of conditioned reflexes between the quality of food and its aroma and determining the selective preference of honey bees to certain plant species. Some researchers believe that glutamic acid is the main amino acid for the formation of the olfactory memory of bees (Hendriksma and Shafir, 2014).
Thus, the one aim of this work was the comparative study of free amino acid content in

the main sources of protein for the bee colony: pollen, bee bread and royal jelly.
The other objective of this study was the comparative analysis of amino acid content in dried yeasts and whey in order to argue the utilization of whey (whey proteins) as alternative supplemental protein feeding.

MATERIALS AND METHODS

The amino acid content was determined in three types of pollen - acacia, poly flower and sunflower, in bee bread, royal jelly and in whey and dried yeast - as alternative protein feed for bee colonies. Pollen samples were collected from the central zone of the Republic of Moldova.
Amino acid analysis was performed on an AAA 339 M amino acid analyzer by ion exchange chromatography (Moore et al., 1958) at the Institute of Physiology and Sanocreatology, Republic of Moldova.
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:
$C_{i(doz.)}=k \cdot n \cdot S_{i(prob.)}/S_{i(st.)} \cdot M_i \cdot 10^{-6}$ (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

The amino acid analysis in the pollen samples taken in the study according to the ion exchange liquid chromatography method revealed 17 amino acids. Tryptophan in pollen samples was identified in extremely small amounts, which did not allow its comparative analysis with other amino acids in the samples. Aspartic acid includes both aspartic acid and asparagine and glutamic acid includes both glutamic acid and glutamine (in the process of detection asparagine is combined with aspartate and glutamine with glutamate and so they have the identical picks that reflect the quantity of extraction).
In the investigation of the content of free amino acids in acacia pollen, poly flower pollen and sunflower pollen, a higher amount was determined in acacia pollen, namely 13.2 mg/100 mg. In poly flower pollen this value is 11.95 mg/100 mg, and in sunflower pollen - 8.35 mg/ 100 mg (Figure 1).

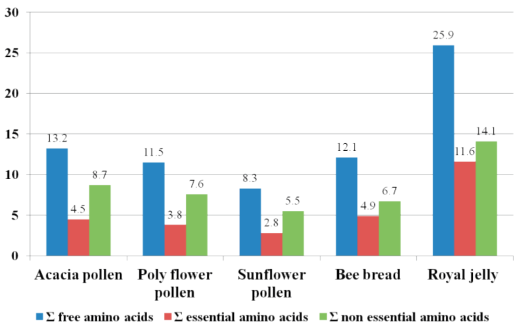

Figure 1. The total content of free amino acids, essential and non-essential amino acids in pollen, bee bread and royal jelly (mg/100 mg d.w.)

In bee bread, the total content of free amino acids, as well as of the essential and non-essential amino acids, corresponds to that in pollen. In royal jelly the level of free amino acids, especially is much higher compared to pollen and bee bread (Figure 1).
In order to identify the value of a protein or protein product, a comparison of the proportion of essential amino acids to the total content of free amino acids is used (Table 1).

Table 1. The proportion of essential and non-essential amino acids to the total content of free amino acids in pollen, bee bread and royal jelly

Samples	Proportion, %	
	Essential amino acids	Non-essential amino acids
Acacia pollen	34.0	65.0
Poly flower pollen	33.0	65.2
Sunflower pollen	33.7	65.0
Bee bread	40.0	55.0
Royal jelly	44.0	54.0

Thus, in all types of pollen studied, the proportion of essential and non-essential amino acids is the same: 34.0% and 65.0% in acacia pollen; 33.0% and 65.2% in poly flower pollen; 33.7% and 65.0% in sunflower pollen, respectively. In bee bread, the ratio of essential amino acids is higher (40.0%) than in pollen. In royal jelly, the highest percentage (proportion) of essential amino acids (44.0%) and the lowest percentage of non essential amino acids (54.0%) were detected.

These data indicate that royal jelly is more valuable in terms of the content of essential amino acids of all of the studied protein sources for the bee colony (Postolati et al., 2012).

The other aim of this work was a study of amino acid content in alternative protein sources for bee colonies.

In some periods of the year, pollen (or bee bread) may be in insufficient quantities, which can cause a protein deficiency in the bee's diet, and, in turn, affect their health and resistance to infections and parasites. Most often, lack of pollen and/or low-quality pollen is caused by intensive cultivation of monocultures in agriculture. Since pollinating services for honey bees are often provided within a human-defined ecosystem, the basic nutrient needs of bees cannot be adequately provided (Naug, 2009).

To compensate for the lack of protein in bee nutrition, additional protein feeding of bees is widely used, depending on the seasonal specifics of the functional state of the honey bee and the requirements of the bee colony in protein: in spring - for feeding and brood growth; and in the fall - for the accumulation of a fat body, which ensures better survival of colonies during the winter (Brodschneider and Crailsheim, 2010; Eremia, 2009).

There are various methods and technologies for producing protein supplements based on soy, peas, corn, oats, barley flour, egg powder and egg whites, whole or dried milk, fish flour, dry yeast etc. These protein additives are introduced 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).

When replacing pollen with other protein-rich feeds, it is recommended to take into account the nutritional value of these substitutes, based on the spectrum of amino acids, as well as of their amount, especially of essential ones. Also, Herbert et al. (1977) showed that the optimal protein content in feed for bees should be 20-30%. At the same time, a 50% level should be avoided.

In 2011, studies of whey as an alternative source of protein and amino acids for bee colonies were started at the Institute of Physiology and Sanocreatology.

The choice of whey as the basis for syrup for honey bee feeding is due to the extremely important value of whey as a source of bioavailable protein with a high biological index (Barth and Behnke, 1997).

Whey proteins make up 20% of milk proteins (the remaining 80% is casein) and are characterized by high nutritional and biological value, having a nutritional index of 1 (casein has an index of 0.8), exceeding egg albumin, which long time has been considered protein No. 1 in terms of biological values (Krissansen, 2007; Gupta and Prakash, 2017). Thus, whey is valuable not only in terms of the amount of protein, but also its quality.

A comparative analysis of the amino acids from whey and pollen showed that whey contains the entire spectrum of amino acids present in pollen, bee bread and royal jelly. However, their amount in whey is higher than in samples taking into study (Figures 1, 2).

It was detected a higher level of essential amino acids especially of BCAA - valine, leucine, isoleucine, and also lysine, methionine, threonine, histidine and tryptophan - amino acids that determine the quality of protein feed for bees (Figure 2).

These data denote the possibility of using whey for supplemental protein feeding of bee colonies.

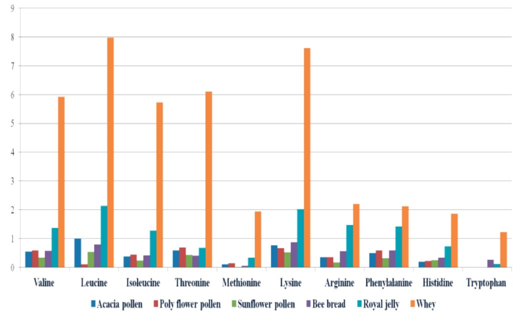

Figure 2. The comparative content of essential amino acids in pollen, bee bread, royal jelly and in whey (mg/100 mg d.w.).

Also, in whey there is a high content of non-essential amino acids, some of which have a significant role in physiological and biochemical processes and are called functional amino acids. Those are: glutamic acid and glutamine, aspartic acid and aspargine, proline, cysteine (Figure 3).

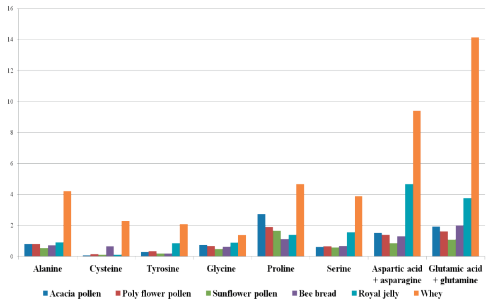

Figure 3. The comparative content of non-essential amino acids in pollen, bee bread, royal jelly and in whey (mg/100 mg d.w.).

As mentioned above, in order to compensate the lack of protein in the diet of bee colony, protein additive feeding is widely used, most often based on soy flour or dried yeast. In order to identify the possibility of using of whey as a source of amino acids and biologically valuable proteins for the bee colony, we compared the content of essential amino acids in whey and dried yeast.

Thus, in dried yeast, the total content of essential amino acids is 4.91 g/100 g d.w., and in whey - 4.85 g/100 g d.w. In comparison with dried yeast, whey contains a higher content of BCAA amino acids – valine, leucine and isoleucine, that is, those amino acids that are most needed for vital processes (Figure 4).

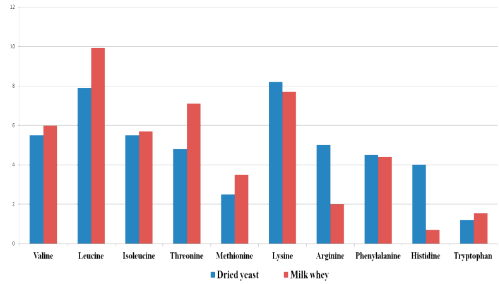

Figure 4. Comparative content of essential amino acids in whey and dry yeast (g/100 g d.w.)

The lysine/arginine ratio as an indicator of the quality of the protein for the bee was also higher in milk whey compared to pollen, bee bread, royal jelly and also dried yeast (Table 2).

Table 2. The ratio of lysine/arginine in pollen, bee bread, royal jelly and whey

Samples	The lysine/arginine ratio
Acacia pollen	2.22
Poly flower pollen	1.87
Sunflower pollen	3.0
Bee bread	1.57
Royal jelly	1.37
Dried yeast	1.64
Whey	**3.45**

Thus, this index (lysine/arginine ratio) can be used to argue the quality of protein feed for bees, and whey, in this regard, has the best characteristics.

Based on whey properties, it was obtained a patent MD 4284 "Method for feeding bee colonies" (Derjanschi et al., 2014) and a patent MD 1312 "Process for producing protein-carbohydrate food for bee colonies" (Vrabie et al., 2019).

CONCLUSIONS

Protein nutrition of the bee colony needs to be considered at three levels: features of protein nutrition of the bee colony as a whole; features of protein nutrition of an adult bee; features of protein nutrition of the larva, since, disturbances in protein nutrition at the previous stages of development affect the subsequent stages and vice versa.

Protein nutrition of bee colony is seasonal: in spring, protein-rich foods are needed primarily for brood rearing; the end of summer and early autumn - for the accumulation of a fat body, which ensures better survival of colonies during the winter.

The quality of the main source of protein for bee colonies - pollen, depends on the protein content and essential amino acids, especially leucine, isoleucine and valine and the lysine/arginine ratio.

According to the invention, “A method for bee colonies feeding” Patent MD 4284 (2014) and “Process for producing protein-carbohydrate food for bee colonies”, Patent Md 1312 (2019), whey can be used as protein additive for bee colony feeding in early spring to intensify brood rearing and bee colony development.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and also was financed from Project 15.817.04.01F.

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