THE IMPORTANCE OF AMINO ACIDS FOR THE DEVELOPMENT OF BEE COLONIES

VRABIE Valeria, DERJANSCHI Valeriu, CIOCHINĂ Valentina

Abstract. For a normal growth and development, bee colonies need a balanced nutrition in essential and functional amino acids. The bees' necessity of amino acids varies depending on the age, being higher for young bees. Preferences in certain amino acids are caused by the maintenance of the various functions of the bees' body and their social activity. Amino acids have an essential role in the formation of nutritional motivation and in the selective collection of pollen from various floral species, which are the main source of amino acids. The comparative analysis of free amino acids in three types of pollen revealed a greater amount in acacia pollen in comparison to poly flower and sunflower pollen. The amino acids leucine and lysine have the highest percentage in total essential amino acid content, and aspartic acid, glutamic acid and proline from the total non-essential amino acids pattern. Also, the content and the correlation of essential and functional amino acids in protein feed determines its preferences and accessibility for bees and underlies the prospecting of protein feed, which is increasingly applied in agricultural practice to compensate the deficiency of amino acids in case of lack or insufficiency of pollen in nature.

Keywords: amino acids, pollen, honey bee, nutrition, development.

Rezumat. Importanța aminoacizilor pentru dezvoltarea familiilor de albine. Pentru o creştere şi dezvoltare normală, familiile de albine au nevoie de o nutriţie echilibrată în aminoacizi esenţiali şi funcţionali. Necesarul în aminoacizi variază în dependenţă de vârsta albinelor, fiind mai mare pentru albinele tinere. Preferinţele în anumiţi aminoacizi sunt cauzate de menţinerea diverselor funcţii ale organismului albinelor şi de activitatea lor socială. Aminoacizii au un rol esenţial în formarea motivaţiei nutriţionale şi în colectarea selectivă a polenului de la diverse surse florale, care reprezintă principala sursă de aminoacizi. Analiza comparativă a aminoacizilor liberi în trei tipuri de polen a relevat un conţinut mai mare al acestora în polenul de salcâm spre deosebire de polenul poliflor şi polenul de floarea-soarelui. Aminoacizilor, leucina și lizina, le revine o pondere mai mare din conţinutul total al aminoacizilor esenţiali, iar acidului aspartic, acidului glutamic şi prolinei – din cel al aminoacizilor neesenţiali. De asemenea, conţinutul şi co-raportul aminoacizilor esenţiali şi funcţionali în hrana proteică determină preferinţele şi accesibilitatea acesteia pentru albine şi stau la baza prospecţiunii furajelor proteice, care tot mai des se aplică în practica agricolă pentru a suplini deficitul de aminoacizi în cazul lipsei sau insuficienţei polenului în natură.

Cuvinte cheie: aminoacizi, polen, albine, nutriție, dezvoltare.

INTRODUCTION

The requirement of bee colonies regarding certain nutrients varies according to the levels of social organization of the bees, and it depends on the seasons of the year. It is known that the autumn feeds with sweet sugar syrup are primordial for the bee colonies to survive the winter conditions, while early spring feeding is necessary to restore the strength of the bee colony after winter and increase its productivity during the active harvesting period. Early spring feeding with high protein content is absolutely necessary for the brood growth and development, and also determines the bee lifespan (HAYDAC, 1970; ALGARNI, 2006; MATTILA & OTIS, 2006; BRODSCHNEIDER & CRAILSHEIM, 2010). Also, protein-rich food during this period is beneficial to the bees' health and the ability to resist infections and parasites (ALAUX et al., 2010).

Proteins are necessary as a source of amino acids that are reused in the biosynthetic processes of the bee organism. In many studies, bee protein requirement depends on age: for larvae – 40 mg per day (HRASSNIGG & CRAILSHEIM, 2005) and for adult bees – 0,68 mg per day (SCHMIDT & BUCHMANN, 1985; BRODSCHNEIDER & CRAILSHEIM, 2010). Also, brood and young bees consume more essential amino acids compared to mature bees, which require amino acids predominantly to maintain somatic functions (enzyme production, tissue regeneration, immunity formation) or reproduction (PAOLI et al., 2014). It has been established that royal jelly factors, namely protein and amino acid composition, can play a crucial role in honey bee development (MALESZKA, 2018).

For normal growth and development, bees need ten essential amino acids: valine, leucine, isoleucine, threonine, methionine, lysine, arginine, phenylalanine, tryptophan and histidine (DE GROOT, 1953). These amino acids are the same as those needed by other animal taxa. Thus, it can be assumed that bees do not vary in their nutritional requirements concerning amino acids (VANDERPLANCK et al., 2014). The main sources of amino acids and proteins for bees are pollen and bee bread. Pollen collected from different floral species has different nutritional values for bees.

Often the need of amino acids for bees is analyzed in terms of their content in different sources of pollen. It has been demonstrated that the content of proteins and amino acids in pollen depends on the plant and can vary between 3.8 and 40.8%, the average being 25% (SZEZĘSNA, 2006). It was noted that the maximum concentration of one or another amino acid is not identical to one and the same plant. Nutrition with only one type of pollen, which has an insufficient amount of protein, does not cover the essential amino acid requirements of a bee's body. SCHIMDT et al. (1987, 1995) have shown that only a mixture of different types of pollen has a beneficial effect on the development and performance of the bee colony. For example, according to the results obtained by DE GROOT (1953), bees require 4% of isoleucine

from the total available amino acids. Only 2% of isoleucine is contained in one type of pollen, which results in the consumption of a double quantity of pollen to supplement it, or to mix different types of pollen containing a larger amount of essential amino acids (STACE, 1996). Thus, it seems that amino acid composition has a greater influence on the amount of pollen required by bees than crude proteins content (NICOLSON, 2011). The plant species that contain in pollen proteins a higher amount of essential amino acids are considered to be more valuable from the nutritional point of view for bees and are more frequently visited by them. Also, these poly flower pollen mixtures increase some immune functions and confer antiseptic protection to bees (ALAUX et al., 2010). It has been established that amino acids from pollen sources influence the sensitivity of the glucose receptors of the amino acids themselves, thus contributing to the formation of conditional reflexes between food quality and its flavor (ZACEPILO et al., 2012). The content of proteins and amino acids in food is absolutely essential for the development of the hypopharyngeal gland and enzyme secretion respectively, which in turn depends on the amount of nectar harvested. A direct correlation between the activity of the secretory glands (enzyme activity), the ability to process nectar within the hive (hive) and the production of honey has been demonstrated (JEREBKIN, 1965).

Thus, amino acids play an important role in the formation of nutritional motivation and in the selective collection of pollen from various floral sources.

Based on the information mentioned above, the purpose of the work is to compare the free amino acid content in different pollen samples in order to identify those sources that contain the optimal content and spectrum of amino acids needed for the growth and development of bee colonies.

MATERIAL AND METHODS

Three types of pollen –acacia, poly flower and sunflower – were taken in the study. Honey samples were collected from the Center Zone of the Republic of Moldova.

The amino acid analysis was performed at the AAA-339M (Czech Republic) amino acid analyzer by the ion-exchange chromatography method (MOORE et al., 1958).

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 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 (Fig. 1).

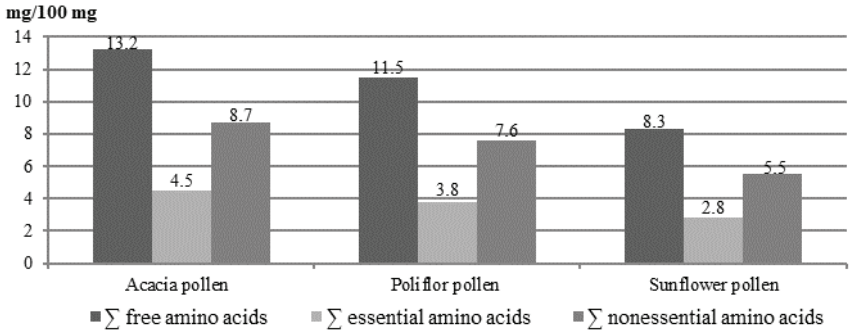


Figure 1. The content of free, essential and non essential amino acids (mg/100 mg) in acacia pollen, poly flower pollen and sunflower pollen.

The total content of essential and non-essential amino acids is also higher in acacia pollen (4.5 and 8.7 mg/100 mg, respectively) compared to poly flower pollen (3.8 and 7.6 mg/100 mg, respectively) and sunflower pollen (2.8 and 5.5 mg/100 mg, respectively) (Fig. 1).

In order to reveal the biological value of pollen collected by bees, the ratio of essential and non-essential amino acids to the total free amino acid content was analyzed. Thus, the share of essential amino acids compared to the total free amino acid content in acacia pollen is 34% and of nonessential amino acids – 65%. This ratio respectively in poly flower pollen is 33% and 65.2%, and in sunflower pollen – 33.7% and 65%.

Hence, the nutritional value of the pollen for bees is primarily defined by its absolute and relative content of essential amino acids. All analysed pollen types have about the same share of essential and non-essential amino acids, indicating that they have nearly the same nutritional value for bee colonies. However, acacia pollen in according to essential amino acid share denotes better nutritional qualities than poly flower pollen and sunflower pollen.

At the same time, the significance of pollen proteins and their preference for bees depends on the content of certain amino acids with key functions for the optimal growth and development of bee colonies.

For these reasons it was proposed to analyse the content of each amino acid in part in the pollen samples taken in the study (Figs. 2 and 3).

The analysis of essential amino acids revealed a higher content of leucine and lysine amino acids in acacia pollen, valine, isoleucine, threonine, methionine, phenylalanine in poly flower pollen and histidine in sunflower pollen (Fig. 2). Thus, obtained data determined that the leucine and lysine have the highest percentage from the total essential amino acid content, data which coincides with other studies (SZEZĘSNA, 2006).

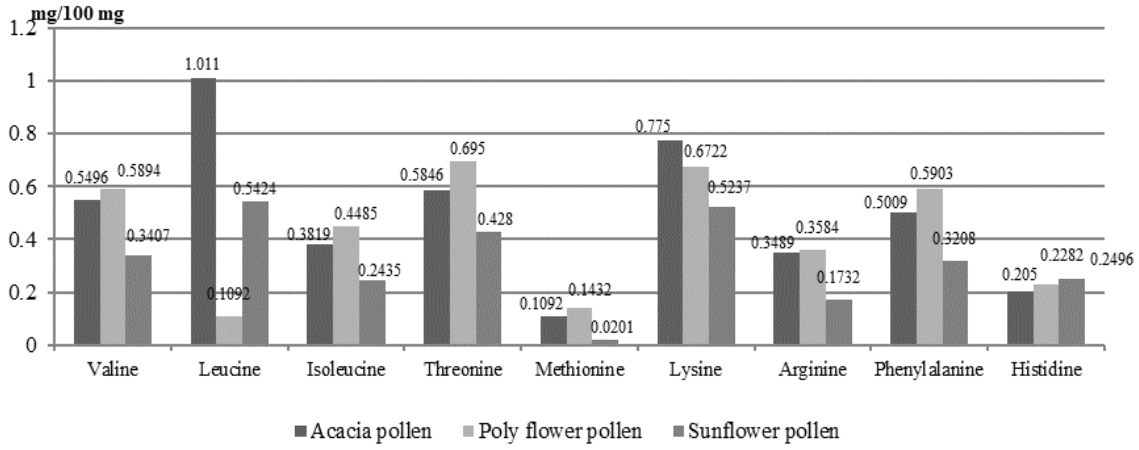


Figure 2. The comparative content of essential amino acids (mg/100 mg) in three types of pollen.

It is known that a significant index for the bee's vital activity is the content of branched chain amino acids (valine, leucine and isoleucine), which in acacia pollen is about 14.6%, in poly flower pollen – 10.03%, and in sunflower pollen – 13.5%. It has been established that leucine, isoleucine and valine are the most required amino acids for bees, and their deficiency limits the development of the bee colony. Also, isoleucine is the necessary nutritional factor in bee feed, which should account for no less than 4% of the protein content (HAYDAC, 1970). In acacia and sunflower pollen this ratio is 2.9%, and in poly flower pollen – 3.9% compared to the total free amino acid content.

It was determined that the lysine-arginine ratio as well as the high lysine content is an important index, determining the protein quality required for bee feeding and bee preferences for certain types of pollen (FETEA et al., 2011). In acacia pollen, this index is 2.22, in poly flower pollen – 1.87, and in sunflower pollen is 3.02.

Some pollen sources contain more methionine (1.2% in poly flower pollen) and histidine (2.9% in sunflower pollen).

Recently there is growing interest in biochemistry, physiology and nutrition of amino acids in growth, health and disease of humans and other animals. The new findings in this field of research led to the new concept of functional amino acids (WU, 2013). The functional amino acids which are also some non-essential amino acids participate in key metabolic pathways of living organisms.

The analysis of non-essential amino acids in pollen samples revealed a higher content of alanine, glycine, proline, aspartic acid and asparagine, glutamic acid and glutamine in acacia pollen; of cysteine, serine and tyrosine in poly flower pollen. The content of individual amino acids in sunflower pollen is lower compared to pollen of acacia and poly flower (Fig. 3). From the data obtained in presented study as well as from the data obtained by other authors, it was established that in pollen amino acids – proline, glutamic acid and aspartic acid have the highest percentage from the total non-essential amino acid content.

In the conditions of the lack of pollen in nature, early spring protein feeding is more than needed to supplement the protein and amino acid deficiency in food, which are necessary for the growth and accumulation of workers in bee colonies at the acacia harvesting. Such stimulating feeding are an ever more frequent bee-keeping practice, that make the earlier egg laying by queen, the restoring of bee colony size and the rapidly colony development (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. HERBERT et al. (1977) demonstrated that the optimal protein level in bees feed should be 20-30%. At the same time the 50% content should be avoided.

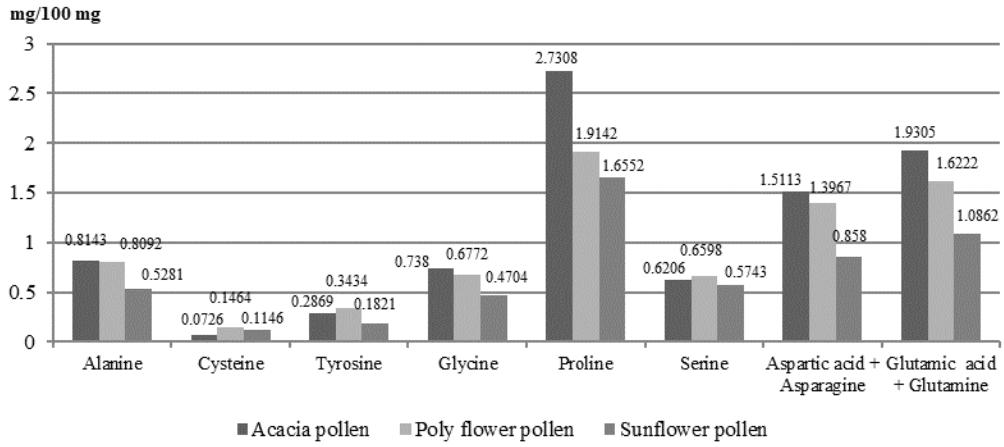
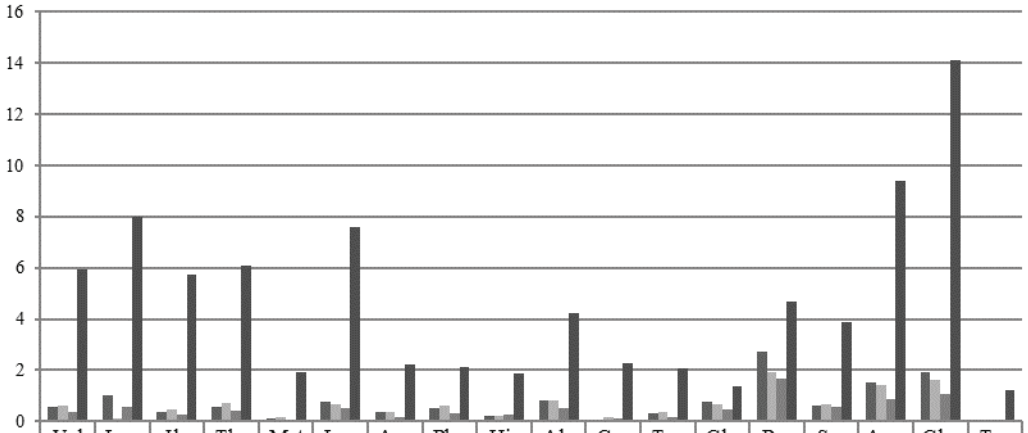


Figure 3. The comparative content of non-essential amino acids (mg/100 mg) in three types of pollen.

Pollen of poor quality has a negative effect on brood rearing, offspring size and can lead to additional nectar foraging. This aspect determines activities for the selection of good quality protein feed for bee colonies.

In exploratory work, the biochemical peculiarities of whey were studied as a protein supplement and a source rich in essential and functional amino acids for the early spring bee colony feeding, and proved the advantage of this product compared to pollen or other protein feeds (VRABIE et al., 2013; DERJANSCHI et al., 2014).

Whey proteins contain a significant amount of such essential amino acids for bees as leucine, isoleucine and valine or branched chain amino acids and also of methionine and histidine. In comparison with pollen, whey contains a higher level of tryptophan. In pollen only trace amount of this amino acid was detected. In whey, as in pollen, the higher content of glutamic and aspartic acids was established (Fig. 4).



	Val	Leu	Ile	Thr	Met	Lys	Arg	Phe	His	Ala	Cys	Tyr	Gly	Pro	Ser	Asp	Glu	Trp
Acacia pollen	0.55	1.011	0.382	0.585	0.109	0.775	0.349	0.501	0.205	0.814	0.073	0.287	0.738	2.731	0.621	1.511	1.931	0
Poly flower pollen	0.589	0.109	0.449	0.695	0.143	0.672	0.358	0.59	0.228	0.809	0.146	0.343	0.677	1.914	0.66	1.397	1.622	0
Sunflower pollen	0.341	0.542	0.244	0.428	0.02	0.524	0.173	0.321	0.25	0.528	0.115	0.182	0.47	1.655	0.574	0.858	1.086	0
Whey	5.93	7.98	5.73	6.1	1.94	7.61	2.2	2.13	1.87	4.21	2.28	2.08	1.38	4.67	3.88	9.41	14.14	1.23

Figure 4. The comparative content of amino acids (mg/100 mg) in three types of pollen and whey.

As it was mentioned above, the ratio of lysine and arginine determines the nutritional value of bees' protein. In pollen from various floral sources this ratio is vary from 1.87 to 3.0, while in whey – 3.45, which is another argument in using whey as a protein pollen substitutes.

Taking into account the nutritional qualities of whey and the high content of essential amino acids for bee growth and development, whey and whey products can be proposed as a protein supplement in the absence of pollen in nature.

Thus, amino acids play an important role in the vital activity of honey bees. Leucine, isoleucine and valine have been shown to enhance the protein synthesis process (KIMBALL & JEFFERSON, 2006). On the other hand, leucine and isoleucine are key amino acids in the formation of haemolymph proteins and regulate the functional balance of the internal secretion glands. Along with valine, leucine and isoleucine play an important role in the transition from larva to pupa. Valine is also essential in functional nervous system (MALAIU, 1976). Methionine is actively involved in the regulation of protein and lipid metabolism, and in the neutralization of toxic substances, while histidine is particularly necessary for growing brood (MALAIU, 1976). Also, as a result of decarboxylation, histidine converts to histamine, which is a component part of bee venom (DE GROOT, 1953). Tryptophan, however, is present in trace

amounts in pollen and it is important in maintaining reproductive functions, producing nicotinic acid, synthesizing proteins for feeding larvae and contributing to pigmentation of the bee's body (DI PASQUALE et al., 2016). It was demonstrated that proline as well as glycine, that are in significant amount in all types of investigated pollens (Fig. 3), exert a stimulating effect on growth in unfavourable conditions (HAYDAC, 1970; MALAIU, 1976) and the amount of these amino acids in pollen represent an attraction factor for bees. Proline significantly influences the flying capacities of honey bees (MICHEU et al., 2000). Some non-essential amino acids are indispensable for certain physiological and biochemical processes. Thus, it has been established that glutamic acid is important for the formation of olfactory memory in bees (LOCATELLI et al., 2005) and glutamine serves as a "fuel" for cells that divide and is considered "essential condition" in metabolic stress cases (KRISSANSEN, 2007).

CONCLUSIONS

Essential and functional amino acids are necessary for the normal growth and development of bee colonies, especially for the growth of brood and young bees. The balanced content and composition in these amino acids contributes to maintaining of basic somatic functions and reproductive functions in adult bees.

Amino acids have an essential role in the formation of nutritional motivation and in the selective collection of pollen from various floral sources

The comparative analysis of free amino acids in three types of pollen revealed a greater content of them in acacia pollen compared to poly flower pollen and sunflower pollen. Amino acids leucine and lysine have the highest level from the total essential amino acid content, and aspartic acid, glutamic acid and proline – from the total non essential amino acid content.

The content and ratio of essential and functional amino acid in protein diet determine its preferences and accessibility for bees and underpin the prospecting of protein feed, which is increasingly applied in agricultural practice to compensate the deficiency of amino acids in case of lack or insufficiency of pollen in nature.

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