Body Protein Reserve and Possibilities for Improvement in Honey Bee Colonies - Review

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Abstract
The purpose of this review is to assess the body's protein reserve in honey bee colonies and the possibilities for improving it. Understanding the causes of changes in body proteinis is important for the beekeeping sector in order to improve bioproductive indices and ensure the health of bees.The body's protein reserves of bees are located in the fat body, hypopharyngeal glands and plasma proteins (vitellogenin). Its active role has been proven in the following aspects: the secretion of larval food (royal jelly), longevity, metamorphosis, the evolution and behavior of adults, immunity and detoxification of bees. Body protein values ranges from 21 to 67% of the dry matter. The values considered physiologically normal are over 40%. The factors that reduce the protein content are: the quantity and quality of protein feed, overuse (enzymatic, immune, toxic or various diseases). Body protein deficiency causes bees to fly at a younger age; they also quickly become bee-collectors and their longevity is shortened. Research has established the ideal protein for bees, which is close in value to the proportion of aminoacids in royal jelly. Pollen as a source of protein for bees was classified as follows: poor quality below 20% CP (crude protein), average quality between 20-25% CP and high quality with over 25% CP. The quality of pollen protein is given by the presence of essential aminoacids. No deficiencies of essential aminoacids are reported in pollen produced by species of the family *Rosaceae*, *Phacelia spp*., *Echium spp*., nor in most species of the families *Brasicaceae* and *Fabaceae*. It is noted that the plants produce pollen with a low content of protein and essential aminoacids in summer and autumn. In conclusion, it is necessary to supplement the bee families with protein feed in order to ensure the body protein reserve according to the requirements.

Keywords: aminoacids, pollen, additional feed, royal jelly, protein

1. Introduction

The protein reserve of the bee family is constituted on the one hand by the bee bread reserve in the honeycombs (less important) and on the other hand by the body reserve [1, 2]. Bees, beside the amount of constituent proteins of cells and muscle fibers, also have a quantity of reserve proteins, constituents of the fat body, proteins stored in the pharyngeal glands and plasma proteins. The fat body is an organ with multiple metabolic functions, largely similar to those of the vertebrate liver, but not only that. Its weight can represent up to 65% of the weight of the adult bee [3]. A main substance synthesized in the fat body is vitellogenin, present in hemolymph, pharyngeal glands, ovaries, which contains 91% proteins, 7% lipids and 2% carbohydrates [4]. The biological value of the fat body is: Reserve nutrient, from which, in the absence of oral supply of fresh proteins, the adult population secretes royal jelly for the growth of the next generation of bees [5]; Having a role in bee longevity, which varies from 200 days in

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overwintering bees that have not raised brood to 20-50 days in summer bees, depending on body protein reserve [5]. After exhausting the fat body, healthy bees have about 8-12 days left to live, a period they spend collecting nectar, pollen and propolis from the environment (become bee-collectors) [6]. Its consumption is achieved following the secretion of royal jelly, which is the source of food for the brood bees or of enzymes for the inversion of carbohydrates in the food, due to hunger or because of some diseases (Varroosis, digestive diseases) [2, 7, 8]. Due to a lack of protein resources, the fat body may not form during the first 7-10 days of the adult bee, after which the malnourished bees become bee-collectors without exercising their role as nurses [6]; Metabolic role of converting nutrients absorbed at intestinal level into accessible forms [3, 9]; Role in detoxification (metabolism of toxins that have reached the bee's body from food and environment) [8, 10]; Important role in immunity [11-13]. Eischen, quoted by Oliver R., showed an increase in resistance to Nosema for the bee's protein-fed in the spring, superior to that conferred by fumagillin treatment. [14-]. Both the intensification of the immune activity and the detoxification of some groups of toxins (both phenomena being increasingly common as consequences of overcrowded or polluted environments) are active consumers of the fat body, which in such situations is consumed and decreases by fighting the toxic or the pathogen, and the vitality and productive efficiency of the bees is diminished [17-19]. It has been demonstrated that there is a link between the environment polluted with pesticides and the weakening of resistance to Nosema and vice versa – the weakening of resistance to the toxin in the presence of the parasite [20, 21]. The purpose of this review is mainly related to the evaluation of the body protein reserve in honey bee colonies and the possibilities of its improvement.

2. Materials and Methods

The data presented in this review were collected from the scientific papers studied.

3. Results and discussions

The amount of body protein is 31-37% of the dry matter at the hatching of the bee and increases in the first 12 days of life up to 67% based on feeding the nurse bees with protein sources (pollen or pollen substitutes) [22]. Average body protein can range from 21 to 67% of body weight [3]. Dropping this percentage below 40%, automatically also reduces bee longevity from the normal 46-50 days in bees with more than 40% body protein to 20-26 days in bees with 21% body protein, so the longevity is halved [3, 5]. With the decrease of body protein in the adult population towards values of 20%, the viability of the grown larvae is proportionally reduced to extinction (larvae die at various stages before full development) [3, 23]. Apart from the normal seasonal oscillations of the body protein, it can also be influenced by the quality of the protein food (pollen or pollen substitutes), and by the wear (overloading of the enzymatic system of sucrose inversion) from periods with a high influx of nectar or carbohydrate food [5]. Physiological fluctuations are related to the age of the individuals, with a maximum around the 12th day after hatching and to the physiological state of the colony, with decreases during development and with maximum values in colonies ready for swarming or wintering [24-26]. The accumulation of body proteins is directly related to the ratio of the number of nurse bees to the number of larvae, increasing during periods when the colony has more nurses and fewer larvae (even with medium quality pollen as nurses are less demanding) [3]. The efficiency of protein accumulation is also dependent on the ratio between the quality of accessible food proteins and the amount of sucrose or maltose taken up by the family, being increased if the pollen is of good quality and the amount of carbohydrates taken up is moderate [27, 28]. Bees well fed with protein record higher bio-productive indices [29]. In diseases that decrease the protein reserve (Varroosis, virosis, Nosemosis), as well as in cases of starvation, they become bee-collectors, without going through the nursing stage completely or at all, starting at the age of 7 days, which added up to the 8-12 days that a bee has left to live after becoming a forager, reveals a possible shorter lifespan than a complete cycle of brood development (21 days) and thus the clear trend of family depopulation [5, 6, 30]. The problems of protein deficiency that can occur have been described in the last century by researchers as follows: Antonius de Groot, quoted by Oliver R. and Ricigliano, determined the essential amino acids for bees and the essential amino acid requirements of bees by feeding captive bees artificial amino acid diets, standardized to the amino acid profile of casein, using for each amino acid in nine different concentrations. He then analyzed the body concentration of nitrogen in the bees of each cage [29, 31]. The ratio of essential amino acids according to these requirements is close to the ratio of the same amino acids in the chemical structure of royal jelly (ranging up to 25.1%), with two exceptions: the amounts of lysine and phenylalanine are higher in relation to the rest of the amino acids in the structure of royal jelly compared to the ratio in the list of requirements, by 43% to lysine and by 32% to phenylalanine [5, 31]. The concentration of essential amino acids in royal jelly is 57% higher than ideal protein values [5]. Over time, researchers and beekeepers found differences in the longevity and bio productive performance of bee colonies depending on the type of pollen they had access to. Classification of pollen categories according to the longevity it gives to captive bees: Sunflower pollen extends the life of bees by 1.6 times, the sesame one by 1.7 times and rapeseed by 2.5 times compared to the control group [3]. Schmidt at all, quoted by Popovici discovered that compared to the control group fed with sugar (in the absence of protein - longevity was 19.5 days), the pollen of *Ambrosia artemisifolia* (ambrosia), the one of *Typha latifolia* (cattail), *Kallstroemia* and fungal spores shorten the life of bees by up to 3.9 days (they contain toxic substances in addition to low nutritional value), pollen varieties from the plants *Haplopappus*, *Baccharis* and *Taraxacum officinale* (dandelion) lead to an insignificant extension of the duration of life compared to the control group (by 6.4 days more), those of *Rubus spp.* (mulberry), *Populus spp*. (poplar), *Ephedra*, *Prosopsis* extend the life of bees from 28 up to 38 days compared with control group, and some pollen mixtures extend it up to 40.6 days (a mixture of pollen from 5 plant species in equal proportion: *Populus*, *Prunus*, *Prosopsis*, *Larreus*, *Cerea*) [1]. Also, another study shows that pollen from *Taraxacum officinale* (dandelion) increases the lifespan of adult bees with 1-4 days, while pollen from *Vaccinus spp.* (blueberry, cranberry) increases lifespan by 16-21 days, in conditions where *Vaccinus spp.* produce poor quality pollen with 13-19% crude protein (CP) [3]. Dandelion pollen is a pollen that has been proven unable to support brood growth either [1, 3]. A study of *Bombus terrestris* (bumblebee) finds that the pollen of *Castanea sativa* (chestnut), *Papaver spp.* (poppy) and *Rubus spp.* (blackberry) is of good quality, and that of *Helianthus anuus* (sunflower) and *Cistus* is poor quality [32]. The pollen of species from the *Compositae* family is generally inferior in quality and with lower digestibility compared to the other varieties [33-35]. Apart from the disappointing information on sunflower pollen, Somerville states that prolonged picking in *Carduus nutans* (thistles) after 2 generations (over 40 days) can have adverse effects of reducing body protein and lifespan. The CP in *Carduus nutans* pollen is only 15.1% [36]. Kleinschmidt makes the following observations in relation to *Helianthus anuus* (sunflower) pollen, which is of poor quality– 12.9-18.5% CP [3, 36]: The families that harvest sunflowers, although they do not suffer depopulation, they suffer a decrease in body protein such that a subsequent harvest associated with a lack of protein quality leads to serious depopulations or they are unable to raise brood to maintain their production capacity for an extended harvest in the following months at locations other than if they are additionally fed with protein food, this aspect of weakening the capacity of bees being also valid for the harvest of eucalyptus species [36]. There are several plant species that secrete a large amount of nectar, but the quality of the pollen is poor [3]. This category includes the pollen varieties of Eucalyptus spp. (eucalyptus), Fagopyrum esculentum (buckwheat) (11.5% protein), Lavandula spp. (lavender) and Medicago sativa (alfalfa) has pollen with less than 20% CP and deficient in isoleucine, *Compositae* and even *Robinia pseudoacacia* (acacia) has pollen with 22% CP and unbalanced especially in that pollen is produced in small quantity and is associated with abundant nectar secretion. It is well known to Romanian beekeepers that an abundant harvest of *Robinia pseudoacacia* (acacia) can cancel the swarming instinct, and this phenomenon is based precisely on the "wear and tear" of the population by decreasing the body's protein reserve [3, 29, 36]. In relation to the wear caused by the abundance of nectar (overstraining the body through the secretion of invertase), associated with the poor quality of the pollen, we have the following description by Kleinschmidt of the situation in Australia: A strong family with 60,000 bees, after producing 138 kg of eucalyptus honey during 12 weeks of harvesting, at the end of the period, attrition led to a population reduction of up to 45,000 bees. In certain situations, at the end of the eucalyptus nectar collection, the bee's vitality decreases so much that they stop collecting the nectar that is still on the flowers. Egg laying decreases to its complete cessation in some of the queens. The longevity of the bees decreases from 45-50 days to 20-26 days. The body protein of the remaining bees can decrease to its lowest possible of 21% of the dry matter. Protein losses amount to 1.5 kg/family by decreasing the number of bees and the body protein of the existing ones [5]. Decreases the size of the bees upon hatching [37]. It has been determined that rebuilding a similar family to be able to cope with another production harvest takes 12 weeks for those with a body protein of 20% of dry matter or 4 weeks for those with a body protein of 40% of dry matter [5]. Or after other research between 14 and 30 days, depending on protein quality and other factors [3, 29]. The lifespan of worker bees is 30-50 days during the warm season, which means that the mentioned 12-week recovery takes much longer than one generation of bees. The fact that protein deficiency is inherited from one generation to another, while the recovery of the body's protein reserve usually takes more than one generation, is also confirmed by a team of researchers from the USA who found that bees that were raised during the larval stage in families with limited access to pollen, they have much reduced performance in picking, orientation, searching for food sources and their longevity is reduced, compared to others that benefited in the larval stage from an abundance of pollen, which are more long-lived and more efficient in their activities, regardless of whether or not they had an active pollen collector on the hive during this abundance [38, 39]. Thus, the efficiency of the next generation's food supply is affected by the inherited handicap of the adult generation and a vicious circle appears. Research has established that pollen with less than 20% CP leads to malnutrition over time and classifies pollen varieties as qualitatively inferior - with less than 20% CP (they cannot support the development of colonies and lead to a decrease in the body protein of adults over time), of medium quality (they support the development and health of bees only in conditions of low intensity nectar collection) - 20-25% CP and superior quality - with more than 25% CP. They also find that medium quality pollen with less than 25% CP cannot protect bees from attrition in case of nectar gathering or heavy sugar feedings, which in the absence of protein will lead to a decrease in the lifespan of bees that process carbohydrates. The maximum amount of brood is increased only with pollen whose CP exceeds 25% [3, 5, 37]. During abundant nectar gathering (or high sucrose feedings), even pollen with more than 30% CP may be insufficient to maintain health, body protein, development [3, 36]. During the period of abundant supplemental feedings with non-inverted carbohydrates, the simultaneous supply of quality pollen is beneficial, or it is recommended to combine the carbohydrate feed with an additional feeding with protein feed that has a role to prevent wear. Low quality pollen assortments (below 20% CP): all *Gramineae* family, all *Gymnosperms* (conifers), *Robinia pseudoacacia* (acacia), *Lavandula spp.* (lavender), *Fagopyrum spp.* (buckwheat), *Medicago sativa* (alfalfa), *Vaccinum spp.* (blueberry, cranberry), *Ambrosia artemisifolia* (ambrosia), *Corylus spp.* (hazel), *Cornus spp*. (corn), *Alnus spp.* (alder), *Ulmus spp.* (elm), *Salix alba* (white willow), *Actinidia spp.* (kiwi), *Tilia spp.* (linden), *Typha spp.*, *Citrus spp*. (citrus), *Cistus spp.*, the *Asteraceae* family in general: *Helianthus anuus* (sunflower), *Helianthus tuberosus* (turnip) *Carduus spp.* (thistles), *Arctium spp.* (scallions), *Cirsium* (cornflower), *Taraxacum officinale* (dandelion), *Hypochaeris radicata* (hog weed, false dandelion), *Cynara* (artichoke), *Rudbekia*, *Carthamus tinctorius* (safflower), *Cichorium spp.* (chicory), *Centaurea spp.* (bluegrass), *Artemisia spp.* (wormwood), *Tanacetum vulgare* (vetrice) [5, 29, 36]. Assortments of medium quality pollen (20-25% CP): family *Rosaceae* (apple, blackberry, raspberry, rosehip, hawthorn), family *Brasicaceae* (rapeseed, mustard, cabbage), *Trifolium spp.* (clover), *Papaver spp.* (poppy), *Acacia carolae* (currawong wattle), *Vicia spp.* (vetch, bean), *Salix spp.* (willow), *Westringia fruticosa*, *Asphodelus fistulosus* (onionweed), *Angophora floribunda*, *Acacia longifolia*, *Eucaliptus delegatensis*, *E. bridgesiana*, *E. viminalis*, *E. polyanthemos*, *E. syderoxilon*, *E. macrorhyncha*, *E. camaldulensis*, *E. robusta*, *E. albens*, *E. dumosa*, *E. longifolia* [3, 35, 36].

Assortments of high quality pollen (over 25% CP): *Echium Plantagineum* (Patherson's curse)–over 30% and potentially toxic, *Echium vulgare* (viper's bugloss) – over 30% and potentially toxic, *Prunus spp.* (plum, cherry, sour cherry, loft, almond, peach, apricot, cherry plum), *Pyrus comunis* (pear), *Brasicaceae* Family (rape, mustard, cabbage), *Phoenix dactylifera* (date palm)–over 30%, *Cucurbita spp*. (pumpkin), *Trifolium michelianum* (Balansa clover), *Trifolium pratense* (red clover), *Trifolium repens* (white clover), *Lupinus spp.* (lupine)–over 30% CP and potentially toxic, *Vicia spp.* (vetch, bean), *Onobrichis vicifolia* (parsnip)–over 30% CP, *Phacelia spp.* (phacelia), *Salix caprea* (buck willow)-over 30%, *Banksia ericifolia* (health lived banksia), *Banksia serrata* (saw banksia)-over 30% CP, *Eucaliptus globoidea*, *E. socialis*, *E. mannifera*, *E. punctata*, *E. saligna*, *E. haemastoma*, *Corymbia maculate* [3, 36]. There can be variations in the percentage of protein in pollen and in general the chemical composition depending on the soil, rainfall, temperatures, plant variety, etc [3]. Analyzing the lists of existing polliniferous plants species in most regions of the globe, we most frequently find that the number of species with low-quality pollen is much higher than that of species with medium-quality pollen, and the number of species with high-quality pollen, with more than 30% CP is very small [3, 29, 36]. The biological effect of pollen is given on the one hand by the nutritional value, by the presence or absence of toxic substances in the pollen (including those of a plant nature) and by the amount of pollen consumed (which is in direct correlation with its attractiveness) [40]. The attractiveness of pollen is not always related to its nutritional value, but also to the proportion of certain substances attractive to bees, both nurses and foragers [41, 42]. It is also known that the nurses do not communicate with the pollen collectors [40]. CP in feed is a general quantitative guideline parameter for quality assessment. Of great importance is the quality of the protein, given by the percentage of essential amino acids and the ratio between them [1, 3, 31]. This aspect explains part of the differences in population development efficiency in batches fed with different pollen substitutes [43]. Researchers have described families with a high amount of brood, a low amount of honey accumulated in combs and body protein accumulation at the level of 40-60%, after 6 weeks of feeding with pollen at the level of only 12-15% CP, originating from the arid areas of the state of Arizona. The good biological effect despite the low protein content is ensured by the high quality of the protein, rich in well-balanced essential amino acids [3]. French beekeepers know that after picking *Castanea sativa* (chestnut), the condition of bee colonies is much better than after picking *Lavandula angustifolia* (lavender) or *Medicago sativa* (alfalfa) [29, 36]. *Castanea sativa* (chestnut) pollen is proven in a study to be of very good quality, unlike that of mustard, sunflower or asparagus [44]. Another study highlights the protein quality of *Castanea sativa* (chestnut) pollen, characterized by an extraordinarily good ratio between essential amino acids, according to the concept of ideal protein for bees, and also a good proportion of essential amino acids in the protein structure. Chestnut pollen contains 16.9% CP, but the small percentage is well compensated by the excellent quality of this protein [45]. Also in this field, Black J., states that analyzing the data provided by Somerville, 74% of the pollen samples are deficient in isoleucine and analyzing the same bibliographic sources, valine, methionine, lysine or histidine deficiencies can also be found in some pollen species [5, 29, 36]. Lipinski Z. summarizes a series of data such as the deficiency of tryptophan in pollen of *Helianthus anuus* (sunflower), of isoleucine in *Medicago sativa* (alfalfa) and *Lavandula spp.* (lavender), of histidine in *Zea mays* (corn), of arginine, valine, isoleucine and leucine in *Taraxacum officinale* (dandelion) [3, 46]. In general, studying the data on the protein composition of the pollen of plant species, no deficiencies of amino acids due to imbalance, according to the concept of the ideal protein, are reported in the pollen produced by the species of the family *Rosaceae*, *Phacelia spp.*, *Echium spp.*, nor in most species of the families *Brassicaceae* and *Fabaceae* [3, 29]. Contrary to the traditional information present in the specialized literature and to the old explanations based more on logic than on scientific observations, it is the fact that nurse bees prefer to consume fresh pollen within 1 to a maximum of 4 days after its collection and avoid consumption of pasture. It will be consumed late, only in periods of protein shortage or its consumption is completely avoided [47]. Protein feeding in the winter-spring period, apart from increasing the number of larvae by 36-46%, also ensured a protection of adult bees, so that protein-deficient colonies suffered visible depopulation [48]. And other authors found the reduction of the adult population during the winter by 78% in the colonies wintered without pollen compared to those wintered with pollen where the population was reduced by only 6% [3, 49]. Several studies reveal that in temperate zones, pollen during spring was of medium and high quality (above 20% CP), but during summer and autumn, pollen quality has a natural tendency to decrease in many years to levels of 13.88% or 15.09% CP in certain periods [3, 50, 51]. This fact is also doubled by the reduction in the abundance and diversity of spontaneous flora, being replaced by large areas of agricultural crops whose pollen is of poor quality during the summer like *Helianthus anuus* (sunflower), *Zea mays* (maize), *Sorghum bicolor* (sorghum), *Fagopyrum esculentum* (buckwheat), *Lavandula angustifolia* (lavender) [46, 52, 53]. Autumn supply deficiencies, if not remedied by access to quality protein, can affect the overwintering population, resulting in overwintering depopulation, weakened brood growth, weakened resistance to Nosema and viruses [11, 12, 14]. The colonies that, for various reasons (genetic or technical-physiological, like too young queen, population too small) that raise brood until late autumn, have a high degree of risk of protein deficiency by decreasing the pollen supply in the environment. To obtain acceptable benefits from a colony, it is necessary that it has more than 50,000 adult bees. There are situations in which the number of bees drops below 25,000/family: in the spring, after abundant nectar collections associated with poor quality pollen or after periods of lack of collection (hunger). These families only recover by collecting good quality pollen or by supplemental feeding with protein food starting more than 4 weeks before the intended collection [2, 5, 37]. These data are also valid in connection with the preparation of the population for production picking, but also for wintering. Deficiency in pollen protein quality can sometimes be compensated for by increased consumption if the colony population, both nurse and forager, is sufficiently abundant. Thus, in well-populated families, especially during the period of growth of generations for wintering, the poorer quality of the pollen can be compensated at least partially by the greater amount of pollen used and by the greater number of nursemaids compared to the number of larvae [3, 5]. Quantity of 244-400 g pollen with 25% protein can be replaced by 250-500 g of pollen with 20% protein or 340-680 g if the pollen has 15% protein [3, 34]. But for this phenomenon, first of all, there is an abundance of pollen in the environment and, secondly, sufficient foraging bees [3]. These compensatory possibilities against the poor quality of protein sources are nullified in underpopulated colonies.

The protein substances at the bee colony level also directly influence: the amount of brood, [29]; the number of drones raised, as well as the quality and quantity of semen [29, 54, 55]. If protein deficiency reduces lifespan, population and colony resistance to pathogens, toxins, weathering, protein supplementation in such conditions naturally ensures positive effects and increased bioproductive performance [3, 56, 57].

4. Conclusions

Proteins and especially essential amino acids are very important for the good development of bee colonies. Recent trends have shown that bees prefer attractive nutritional supplements with high-quality ingredients that provide adequate protein quantity and quality. Intensive research must also be done on the digestibility of proteins and the bioavailability of essential amino acids from the pollen of different species of bee intrest and from potential sources of pollen substitute proteins. Standards can be created for the control of the bee population and the feed intended for them. The need for toxicological and teratological studies of the pollen of different plant species and of the potential sources of pollen substitute proteins is foreseen, in order to prevent possible unwanted effects. Preparing families for production, apart from ensuring the size of the population, also requires adequate protein feeding to ensure the protein reserve in the bees' body. Knowing that the process of colony development automatically also leads to a decrease in body protein reserve (except in rare situations where the bees have access to very good quality protein feed), it would be desirable that the development of the swarms of bee is completely completed and colony size to be large or medium when the overwintering generations of bees begin to grow. It would be recommended during the period of growth of bee generations for the winter, to monitor the quality of the pollen and to correct the deficiencies by additional feeding. In the absence of monitoring, preventive supplementary feeding with protein is an equally viable solution knowing that the appetite of the nurses for protein is directly proportional to the deficiency. The production of biological material is done in conditions of nutritional abundance. Additional feeding of families with protein is good to comply with the nutritional requirements of the species.

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