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## **Influence of the food protein on the development of hypopharyngeal glands, fat body, quality and lifespan of honeybees**

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**Abstract.** Research on the anatomical and physiological characteristics of bees (the state of fat body, hypopharyngeal glands) in connection with changes in natural and climatic conditions (soil composition, prolonged droughts, prolonged rains, cold weather, environmental disasters) impoverishment of fodder base for bees due to the decrease of sown areas of honey crops leading to the use of bees' feeding, is relevant. The research aims to study the influence of food protein on the development of hypopharyngeal glands, fat body and life expectancy of honeybees. The zootechnical

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(the setting of the scientific research, the feeding of the bee families, the power of the bee families, productivity), ethological (orientation in the bee-entrance area), micrometrical (physiological and morphological characteristics of the parts of the bee's body), microscopic (analysis of hypopharyngeal glands and fat body of bees) and statistical (biometric data processing) methods were used in the study. The significant difference in their degree of development depending on the carbohydrate or protein feeding was identified. After feeding sugar syrup to bees, signs of protein malnutrition and the state of development of hypopharyngeal glands corresponding to the I-II degrees were noted. Alveoli of hypopharyngeal glands were reduced, and underdeveloped, with marked spaces, whereas after feeding protein food in the form of sugar syrup with bee pollen the bees had well-developed hypopharyngeal glands. The alveoli of the hypopharyngeal glands of the researched bees filled out, grew milky, with no space between them, and were able to produce larval food. Such a state of the glands' development corresponds to the IV degree of the development of hypopharyngeal glands. The research results indicate that the development of the fat body of bees happens at a young age when bees consume protein food the most. It has been proven that protein feeding of the bee colonies of experimental groups contributed to the better development of hypopharyngeal glands in both old and young bees, which resulted in better provision of food for larvae and more active development of the bee colonies. Protein feeding of bees in early spring under conditions of limited supply of protein food contributes to the production of larger larvae, which in turn contributes to the production of more complete bees

**Keywords:** Ukrainian steppe bee; protein feeding; bee larvae; the power of the bee colony; hypopharyngeal glands; alveoli of hypopharyngeal glands; fat body

## INTRODUCTION

The creation of optimal conditions for the life of bee colonies requires various types of food for feeding. The search for the most effective food resources and their impact on bee productivity remains relevant. R. Brodschneider *et al.* (2019) and K. Khamid (2021) proved that increasing the number of bees in the colony in spring is of exceptional importance in preparing it for pollination of crops, using productive honey collections, as well as in autumn for increasing the number of young bees, which will have to overwinter and raise several generations of bees' next spring.

The life activity of the honeybee colony, particularly rearing of brood, successful overwintering, disease resistance, flight-collecting and pollinating activity depends to a large extent on the food stocks in the nest and the flow of the protein food – bee pollen. K. Ulutaş & A. Özkirim (2018), S. Ahmad *et al.* (2021), and M. Carroll *et al.* (2021) proved that for normal life activity, each bee colony needs a balanced diet, which should include a complex of proteins, fats, and carbohydrates, as well as mineral compounds and water. Honeybees use carbohydrates and lipids to replenish energy reserves and protein compounds – for growth and development; and minerals, vitamins and water are inseparable components of metabolic processes.

R. Pudasaini *et al.* (2020), M. Lee *et al.* (2019), and V. Corby-Harris *et al.* (2019) dealt with questions of dependence of the development and functional activity of hypopharyngeal glands on the age of bees and the availability of the protein food in the nest. There is a direct link between feeding, physiological state, and life expectancy of honeybees whereas the stocks of food are important (Lan *et al.*, 2021). The use of protein food has an impact on physiological development and contributes to the continuation of life of young bees. A statistically

valid correlation between the life expectancy of bees and their physiological state, particularly close between the degree of development of the fat body and life expectancy, is present. M. Thakur & V. Nanda (2020), Li *et al.* (2019), and U. Toprak *et al.* (2020) noted that the presence of a sufficient amount of protein compounds increases the lifespan of worker bees, has a positive effect on the development of young bees and brood.

The study of lipid metabolism has received much attention for many years (Feregrino-perez *et al.*, 2018; Chen, 2018). The fat body develops quickly among the bees that take part in feeding the larvae, it is highly developed among larvae and also old queen bees. It is the most developed in the body of bee larva where its proportion corresponds up to 60% of the mass of the larvae. The research data (De Souza *et al.*, 2019; Almasri *et al.*, 2020; Frizzera *et al.*, 2020) indicates that although in the adult bee, the fat body occupies a relatively small part, this organ plays an important role in the physiological state of bees. The fat cells of summer and winter generations of bees are distinct in their structure. G. Galvani *et al.* (2019), M. Kunc *et al.* (2019), and A. Strachecka *et al.* (2021) have proven that in summer the fat cells form a thin and almost clear layer and among winter bees they create a thick yellow-white cover where cells, apart from fat droplets, also contain protein bodies. The main biochemical processes take place in the fat body as well as stockpiling of nitrogenous substances in the autumn period that define the life expectancy and safekeeping of bees during overwintering. The degree of development of the fat body is a reliable physiological indicator of the state of bees and their resistance. The metabolism in the body of bees is closely linked to the state of the fat body as one of the important vital organs of bees.

The studies on the impact of the protein feed on the development of bee colonies and their physiological state play an important part both in the conservation of honeybees and the increase in their productivity and are relevant for the beekeeping of Ukraine. The research aims to study the influence of the protein feed on the development of the hypopharyngeal glands, fat body and life expectancy of bees during the period of the low level of flow of bee pollen into the nest of the bee colony.

## MATERIALS AND METHODS

This study is a part of the fundamental study and was conducted according to the plan of research work on the topic: "To study mechanisms and characteristics of the production of the bee wax and royal jelly by Ukrainian steppe breed and Carpathian breed of bees" (the number of the state registration 011U6001455) during 2016-2020.

The study was conducted on the experimental apiary of the National Scientific Centre "Institute of Beekeeping named after P.I. Prokopovych" on the standard bee colonies of the Ukrainian steppe breed (*Apis mellifera scossimai*). The bee colonies met the standard of the Ukrainian steppe breed, as confirmed by the results of the exterior evaluation.

The entire bee colonies as well as separate individually marked bees of the Ukrainian steppe bees were the objects of research. The caring for the experimental bee colonies was equal and conducted using a generally accepted method (Brovarskyi *et al.*, 2017). The research was conducted under the provisions of the "Basic Ethical Principles for the Animal Experiments", adopted at the First National Bioethics Congress (Reznikov, 2003) and "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (1986).

The following groups of the bee colonies were formed: I group (control) – the bees of this group received feeding in the form of sugar syrup in the ratio 1:1 both in larval stage and after birth; II group – bees in larval stage received protein feeding (sugar syrup in the ratio 1:1 with addition of 15% bee pollen that was soaked in water for 6 hours), and after birth went on sugar syrup nutrition;

III group – bees in larval stage received sugar syrup and after birth went on protein nutrition; IV group – bees received protein feeding both in larval stage and after birth.

Physiological characteristics were determined according to the degree of development of the fat body (on the Maurizio scale) and brood food (HP) glands (on the Hess scale) determined by 4 levels of their development. Fifty bees aged one day were placed in laboratory holding cages and were kept on the grid above the nest of the strong colony. Food and water were changed every two days. The holding cages were examined every day with an accounting of dead bees. To study the development of brood food (HP) glands, 10 bees from each holding cage were taken on the 10th, 20th, and 30th days. Hypopharyngeal glands were dissected and examined under a digital microscope and photographed afterwards. The studies were conducted with repeatability of two measurements. The mass of the newly born bees was determined on the torsion scale VT 500.

The amount of bee bread and sealed brood was determined with the help of a frame grid with the size of quadrants 5x5 cm. For the convenience of accounting for bee bread (given that it doesn't always occupy big solid areas) the frame grid was additionally divided into smaller quadrants 2.5x2.5 cm. Biometric data processing was conducted on a PC with the help of MS Excel software using built-in statistical functions.

## RESULTS AND DISCUSSION

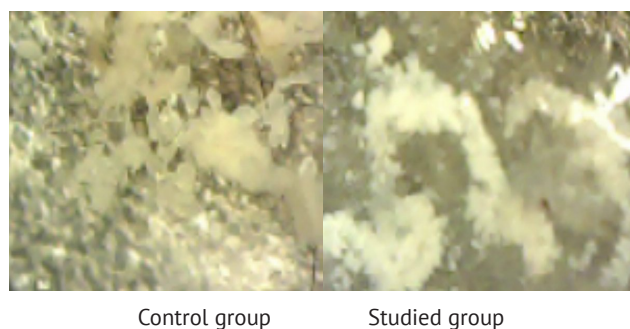
According to the results of the biometrical analysis of the level of development of hypopharyngeal glands of the bees from the research group colonies prevailed per degree of development of these glands over the bees of the control group on average by 0.86 points ( $P > 0.01$ ). Among young bees that were born in spring, the variance by the degree of development of hypopharyngeal glands was by 0.92 points more in the research group with probability  $P > 0.01$  (Table 1).

After examining the microscope of dissected hypopharyngeal glands of bees, authors identified the significant difference in the level of their development depending on feeding with carbohydrate or protein food (Fig. 1)

**Table 1.** Physiological development of bees in research and control colonies

Rates	Old bees		Young bees			
	The level of development of hypopharyngeal glands, points		The level of development of hypopharyngeal glands, points		Live body weight of bees, mg	
	Research	Control	Research	Control	Research	Control
n	100	100	100	100	80	80
Lim	2-5	1-4	2-4	1-4	78-116	70-100
X±m	3.4±0.10	2.6±0.15	3.1±0.14	2.1±0.13	93.2±1.12	90.8±1.10
Cv, %	21.4	39.95	26.88	36.35	9.55	9.42
P	≤0/01		≤0.01		≥0.05	

**Source:** compiled by the authors



**Figure 1.** Hypopharyngeal glands of bees of control and research group

The figure above shows that among the bees of a control group that received sugar syrup, there are signs of protein deficiency and the level of the development of hypopharyngeal glands meets the criteria of degree I-II. The Alveoli of hypopharyngeal glands

are underdeveloped, with significant gaps and they remained clear throughout the whole research.

Quite a different situation was observed among bees of a research group that were fed with the protein mix in the form of sugar syrup with bee pollen both in the larval stage and after birth. They have sufficiently well-developed hypopharyngeal glands. The Alveoli of the hypopharyngeal glands of the researched bees became rounded and cloudy, no gaps were observed between the alveoli, and they had a milky white colour and were able to excrete brood food. Such a state of glands meets the criteria of IVth degree of development of hypopharyngeal glands of bees with sufficient stocks of protein feed in the nest. The fat body covers the internal organs and body walls of bees and contains fat, glycogen, and protein. In spring those substances flow into the brood food (HP) glands and are spent on producing the food for larvae. The data of research on the development of the fat body is provided in Table 2.

**Table 2.** The dynamics of development of the fat body of bees

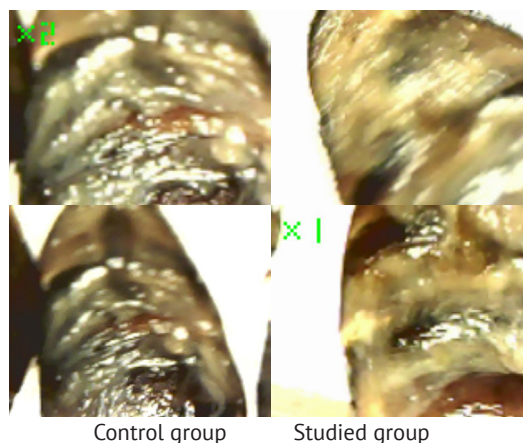
Bees' age, days	Research Group		Control group	Reliability of the differences, td
	M±m	% before the control	M±m	
1	1.2±0.03	86	1.5±0.04	4.0
3	1.7±0.05	85	2.1±0.05	4.1
6	2.1±0.05	78	2.5±0.05	7.5
12	2.1±0.06	95	2.2±0.02	1.0
18	2.0±0.05	94	2.1±0.05	1.4
24	2.1±0.06	100	2.1±0.82	0.0

**Source:** compiled by the authors

As seen from the data in the table, the highest rate of the development of the fat body among bees, 2.5 points, was observed at the age of 6 in the control group where the frames filled with protein food were not removed. At the same time, the degree of development of a fat body of the 6-day-old bees in the research group was 2.0 points. Statistical processing allowed to identify sufficiently stable accuracy of difference (td=7.5).

Among the bees older than 6 days the development of the fat body with the removal of the frames with protein food or without there was almost no difference. Such phenomena point to the fact that the development of this organ takes place at a young age when bees mostly consume protein food.

The dissected bee terga abdomen is presented in Figure 2.



**Figure 2.** Fat body of bees of research and control group

**Source:** compiled by the authors

Among the bees of the control group, the fat tissue has white rounded cells without visible inclusions, with visible chitin and meets the criteria of the I-st degree of development. Among the bees of the research group, the fat tissue is multi-layered, plicate, with yellow-filled inclusions and meets the criteria of III-IV degree of development. As a result of the study, the life expectancy of

bees with protein feeding in the larval stage and after birth turned out to be significantly higher than among the bees of the control group that received the sugar syrup as extra-nutrition. The life expectancy of bees accounted for  $47.09 \pm 1.72$  days and  $22.8 \pm 1.75$  days in the research and control group respectively. The difference is statistically accurate ( $P < 0.001$ ), (Table 3).

**Table 3.** The life expectancy of bees

Groups	n	lim	M±m	Cv, %	P
I	100	1.0 – 33.0	22.80±1.75	44.1	-
II	100	1.0 – 41.0	27.67±1.61	37.2	0.001
III	100	1.0 – 70.0	37.00±2.65	38.4	0.001
IV	100	1.0 – 73.0	47.09±1.72	30.5	0.001

**Source:** compiled by the authors

The life expectancy of bees in the second group was significantly higher even though these bees received sugar syrup after birth but protein food in the larval stage. The bees of the third group had a relatively long lifespan –  $37.00 \pm 2.65$  days. As for the behaviour of bees, the bees from all groups behaved differently, the restless behaviour was observed among the bees with a short lifespan.

Life expectancy is a breed characteristic with seasonal variation and is directly proportional to the state of development of hypopharyngeal glands and fat body where the reserve nutrients are stored. Therefore, the study of the influence of alimentary factors on the life expectancy of bees is an important rate in their breeding. Studying the effect of feeding with pollen from 38 plant species on bees, M. Kunc *et al.* (2019) showed that pollen prolongs the life expectancy of bees by up to 100% compared to control, and improves some indicators of their physiological state. Ukrainian and foreign science and practice have a great deal of experience in the field of visual qualitative evaluation of the degree of development of the fat body of honeybees.

According to L. Bortoloṭti *et al.* (2020), the fat body is an important metabolic organ and is decisive in the quality and activity of the course of physiological and biochemical processes in the body of honeybees. That is why the degree of fat body development is the main indicator of the biological state of the body of honeybees and affects their lifespan.

R. Balkanska *et al.* (2018) and B. Moumeh *et al.* (2020) proved that a sufficient amount of protein compounds increases the lifespan of worker bees, and has a positive effect on the development of young bees and brood. Scientific research dedicated to studies of the life of separate specimens and bee colonies as a whole demonstrates that during the overwintering with favourable conditions, the biological lifespan of bees is directly related to the presence of the reserve nutrients in their body. Meanwhile, what matters the most is the

degree of development of the fat body that also determines the ability of bees to rear the brood intensely in early spring. That is why in spring they consume a large amount of protein food to provide the physiological development of the biological mode of the overwintering process and prepare the young spring generation to replace them.

F. Azzouz-Olden *et al.* (2018), I. Kumari & R. Kumar (2019) it has been proven that meeting the nutritional needs for protein compounds is critically important for the general condition of the bee colony, especially during the period of active development. The main area of accumulation of the reserve nutrients is the fat body of the bee. In its granules, the fatty-protein compounds appear where the reserve nutrients (proteins, fats, and carbohydrates) are stored and later used in the process of overwintering the bee colony. The fat body feeding only carbohydrate food remains relatively thin, with a limited amount of nutrients. With protein feeding it develops as multi-layered and contains large amounts of protein, fat, and glycogen. The fat body of bees serves as an area where extra amino acids are preserved which are accumulated in the active period and used under protein deficiency. In that regard, it can be concluded that the fat body is one of the most important elements in the process of metabolism and has an influence on the activity of all physiological processes. That is why the degree of the development of the fat body is one of the most important characteristics that defines the biological state of the body of a bee and its life expectancy. It is experimentally proven that the protein food in the form of a mixture of sugar syrup with bee pollen has a positive influence on the life expectancy of bees.

Thus, this research proved that protein feeding of bee colonies of the research groups contributed to the better development of hypopharyngeal glands both among old and young bees. This in turn contributed to the better release of brood food for larvae and more active development of the research group of colonies.

## CONCLUSIONS

According to the conducted research, it was established that protein feeding of bees in early spring under the conditions of a limited supply of protein food contributes to the obtaining of larger larvae, which in turn contributes to the production of more complete bees.

During the study of the influence of protein food on the development of hypopharyngeal glands and fat body, when comparing the physiological state of bees, it was established that the hypopharyngeal glands and fat body of bees that consumed pure candy, that is, had only protein supplements, and where a deficiency of protein food was observed, had the lowest degree of development. In addition, it has been confirmed that the development of the fat body occurs at a young age when bees consume protein food.

The results of the study show that the average lifespan of bees with protein feeding in the larval stage and after birth was significantly longer. The average

lifespan of bees with protein feeding in laboratory conditions was more than two times longer than the lifespan of bees with protein starvation. The rational feeding of bees is a decisive factor in their productivity. Meeting nutritional needs for proteins is an important condition for the general state of the bee colony, especially during the period of active development.

The studies of the influence of various factors including food, state of the colony, pesticides, and honeybee races that affect the physiology of bees are essential for the further investigation of the development and functionality of the hypopharyngeal glands and fat body of bees.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

None.

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## Вплив протеїну корму на розвиток підглоткових залоз, жирового тіла, якість та тривалість життя медоносних бджіл

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**Анотація.** Дослідження анатомо-фізіологічних особливостей бджіл (стан жирового тіла, підглоткових залоз) у зв'язку зі змінами природно-кліматичних умов (склад ґрунту, тривалі посухи, затяжні дощі, похолодання, екологічні катаклізми), збідненям кормової бази бджіл через зменшення посівних площ медоносних культур, що призводить до використання бджолами підгодівлі, є актуальним. Метою дослідження є вивчення впливу кормового протеїну на розвиток підглоткових залоз, жирового тіла та тривалість життя медоносних бджіл. У роботі використовували зоотехнічні (постановка наукових досліджень, годівля бджолиних сімей, сила бджолиних сімей, продуктивність), етологічні (орієнтування в зоні бджолосімей), мікрометричні (фізіолого-морфологічна характеристика частин тіла бджоли), мікроскопічні (аналіз підглоткових залоз і жирового тіла бджіл) та статистичні (біометрична обробка даних) методи дослідження. Виявлено достовірну різницю в ступені їх розвитку залежно від вуглеводної чи білкової годівлі. Після згодовування бджолам цукрового сиропу відмічали ознаки білкової недостатності та стан розвитку гіпофарингеальних залоз, що відповідає I-II ступеню. Альвеоли підглоткових залоз були зменшені, недорозвинені, з помітними проміжками, тоді як після згодовування білкового корму у вигляді цукрового сиропу з бджолиним пилком бджоли мали добре розвинені підглоткові залози. Альвеоли підглоткових залоз досліджуваних бджіл наповнилися, стали молочними, без проміжків між ними, і були здатні виробляти личинковий корм. Такий стан розвитку залоз відповідає IV ступеню розвитку підглоткових залоз. Результати досліджень свідчать, що розвиток жирового тіла бджіл відбувається в молодому віці, коли бджоли найбільше споживають білкового корму. Доведено, що білкова підгодівля бджолосімей дослідних груп сприяла кращому розвитку підглоткових залоз як у старих, так і у молодих бджіл, що призвело до кращого забезпечення личинок кормом і більш активного розвитку бджолосімей. Білкова підгодівля бджіл ранньою весною в умовах обмеженої пропозиції білкового корму сприяє отриманню більших личинок, що, в свою чергу, сприяє отриманню більш повноцінних бджіл

**Ключові слова:** українська степова бджола; білкова підгодівля; личинки бджіл; сила бджолиної сім'ї; підглоткові залози; альвеоли підглоткових залоз; жирове тіло