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Alterations in the lipid composition of pectoral muscles in honey bees (*Apis mellifera*) in relation to brood-rearing activity

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Colony preparation for hypobiosis is associated with the emergence of highly viable individuals. Autumn bees possess significantly greater longevity due to a physiological state defined by reduced metabolic activity. This preparatory process is evidenced by diminished brood rearing and decreased flight activity in response to natural factors. Consequently, young bees cease brood care and begin to accumulate increased quantities of proteins, lipids, and glycogen in the fat body. In this context, intensive brood rearing may be regarded as a stressor that significantly affects both energy metabolism and plastic processes within the insect organism. Research findings indicate that this factor determines both the total lipid content and the distribution of lipid classes in various tissues prior to the onset of hypobiosis, with particular reference to the thoracic flight muscles of bees. Given their principal role in thermogenesis, the structural organization of muscle tissue and its functional state substantially influence the metabolic activity and physiological condition of the insects, thereby affecting the overall success of colony overwintering. The pectoral musculature of honey bees constitutes a highly specialized, energy-demanding system whose activity is supported by the mobilization of reserves from the fat body. Accordingly, lipid accumulation within these muscles for winter survival does not take place. The lipids present are largely associated with mitochondria and serve as substrates for prompt oxidative metabolism, rather than as strategic reserves. Their proportion in muscle tissue is considerably lower than that of proteins and glycogen. Analysis revealed that the total lipid content in the pectoral muscles of worker bees ranged from 62.4 to 80.1 mg/g of tissue mass. The cessation of brood rearing in the experimental colonies led to significant quantitative and qualitative changes in muscle total lipid composition, affecting both total lipid levels and the relative distribution of lipid fractions. Specifically, total lipid content increased by 17.6% in the experimental group ($P < 0.001$). Additionally, the complete cessation of brood rearing resulted in a 2.75% increase in phospholipid content within the pectoral muscles ($P < 0.001$), a change that influences the functional stability of cellular membranes. The experimental group also exhibited a 4.6% reduction in free cholesterol levels, a key determinant of membrane fluidity. Furthermore, the 2.89% increase in triacylglycerol content observed in the experimental group suggests an adaptive response to low-temperature conditions. In general, the changes associated with the brood-rearing factor exert a detrimental effect on the physiological status of autumn bees. As a consequence, these individuals acquire features typical of aged summer bees and demonstrate a shortened lifespan, thereby reducing their capacity to successfully endure the winter period. This ultimately compromises colony overwintering success, spring development, and productivity in the subsequent season.

Keywords: honey bees; muscles; total lipids; lipid classes; brood quantity; hypobiosis; productivity level.

Introduction

In the phase leading up to hypobiosis, worker bees develop characteristics that differ in terms of their viability. Specifically, individuals preparing for hibernation exhibit increased longevity relative to those of the spring and summer generations. Researchers suggest that this phenomenon results from multiple factors, primarily the adaptation of the honey bee's body to prolonged inactivity associated with the flightless period (Hopkins et al., 2021). The length of this period varies with geographical conditions and may extend up to 160 days.

The preparation phase for winter is characterized by a marked decrease in brood-rearing activity. This phenomenon arises from the restricted supply of food to the nest under adverse environmental conditions. As worker bee flight activity diminishes, queens reduce their rate of oviposition. Consequently, younger bees that are not involved in larval care deposit larger quantities of reserve nutrients proteins, lipids, and glycogen within their own bodies. It should be emphasized that protein and lipid constituents are primarily introduced into the organism through the consumption of bee bread (Tawfik et al., 2020; Jang et al., 2022). Numerous studies have documented the influence of feed quality on the nutrition of honey bees and its consequent effects on their longevity (Basualdo et al., 2013; Begum et al., 2023). Several investigations have specifically addressed bee feeding strategies in preparation for the winter period (Guler et al., 2018). Furthermore, a clear relationship has been established between the quality of protein and the rearing of broods (Kovalskyi et al., 2021). The present data hold significant implications for the assessment of bee viability. It should be recognized that intensive brood rearing, in particular the feeding of larvae, places pronounced demands on the organism's physiological systems. Among these, the pharyngeal gland and the fat body structures of fundamental importance have been most thoroughly investigated. In functional terms, the fat body in insects, particularly in honey bees, is analogous to the liver in vertebrates. Bee longevity is closely correlated with the development and functional capacity of this organ (Aljedani, 2018).

The production of diverse enzyme complexes, as well as the structural components of royal jelly and wax, occurs via the secretion of plastically active substances by the fat body. Simultaneously, the physiological condition of worker bees involved in larval feeding is significantly compromised under conditions of extensive brood rearing. In preparation for the hypobiosis period, this may result in a notable reduction in colony strength and negatively influence the deve-

lopment of worker immunity. Additionally, an increased production of royal jelly by young bees is associated with a shortened lifespan. Under these conditions, the prospect of preserving optimal physiological health in workers, and thereby prolonging their lifespan, through the regulation of brood-rearing intensity, is of considerable interest. Insight into the mechanisms of energy and plastic metabolism (Westerterp, 2017), the accumulation of longevity-influencing substances in bees, and the colony's inherent capacity to sustain these traits provides a scientific basis for technological approaches to producing long-lived worker generations during colony preparation for wintering (Koval′s′kyy et al., 2024).

Published data indicate that significant physiological and biochemical modifications have been observed in the bodies of autumn-generation bees. Specifically, individuals preparing to endure low temperatures exhibit a higher accumulation of total lipids (Deeter et al., 2023). At the same time, alterations in the relative proportions of lipid classes within their tissues are observed (Wegener et al., 2018, 2022), which is of considerable importance for the preparation of bee colonies for the winter period. This is particularly relevant because phospholipids, for example, are integral to lipid metabolism, serving a variety of essential metabolic functions (Arien et al., 2020). Modern scientific studies prioritize the identification of patterns capable of forecasting the course of wintering (Wright et al., 2018). Given the role of the pectoral muscles in heat production, research into the metabolic characteristics of muscle tissue and methods for their regulation continues to be of significant interest.

The objective of this work was to evaluate the influence of brood-rearing on the lipid composition of the pectoral muscles in honey bees in the period preceding hypobiosis.

Materials and methods

The research was conducted between 2023 and 2025 at the Department of Production and Processing of Small Animal Products of Stepan Gzhytsky Lviv National University of Veterinary Medicine and Biotechnology. Carpathian bee colonies were used as the experimental material. All colonies were established according to the analogue method to ensure comparability between groups. At the onset of the experiment, each colony contained 2.1–2.3 kg of carbohydrate feed and 450–500 g of bee pollen. The total live weight of bees per experimental colony ranged from 1200 to 1250 g. All queen bees originated from a single maternal lineage and were reared under uniform conditions within the same foster colony. The bee colonies were maintained in six-frame hives (435 × 300 mm frame size). After the termination of the hypobiosis period, all colonies were relocated to eight-frame case hives. Honey yield was quantified at the conclusion of flowering of each melliferous crop through gravimetric assessment of the extracted marketable honey. The harvested honey originated solely from supers isolated from brood chambers by means of queen excluder grids. Throughout the beekeeping season, the experimental colonies were involved in nectar collection from several honey plants, notably winter rapeseed (*Brassica napus*), which, under the agro-climatic conditions of Ukraine, flowers from the first through the third decade of May. The next significant nectar source is the small-leaved linden (*Tilia cordata*). Flowering duration is temperature-dependent, averaging 10–14 days, commonly occurring from June 25 to July 10. The final honey plant in the season is the sunflower (*Helianthus annuus).* Its phenology varies annually, typically with flowering from late July through early August.

The age of worker bees was determined by applying unique colored markers to the thorax immediately upon emergence from the brood cells. Approximately 500 individuals per colony were observed. Two experimental sets, each comprising five colonies, were established. The control colonies contained open broods with an area of 1250–1270 cm² as of August 27. In the experimental group, a broodless condition was induced by confining the queens from August 14, resulting in the absence of open brood. The degree of physiological development in bees is primarily determined by the characteristics of their functional activity, with larval nutrition during brood rearing representing a critical factor, as it heavily depletes the energy reserves of nurse bees. The absence of an open brood stage maximizes the preservation of these young bees, which subsequently constitute the core of winter clusters and serve as a fundamental determinant of colony wintering success. Queen isolation beginning on August 14 effectively eliminates the open brood phase in colonies from August 23 onward. To terminate egg-laying, queens in the experimental group were confined within isolators. The experimental insulators were fabricated with dimensions of 20 × 20 cm and a thickness of 1 cm. The active surface was constructed from plastic film featuring elongated oval apertures 4.4 mm wide, facilitating unrestricted passage of worker bees to provision and care for the queen within the central compartment. In all hives, insulators were installed at the nest center, and queens were retained until the initial cleansing flight, after which they were liberated. To prepare colonies for overwintering, each received 2 L of 50% (w/w) sucrose solution (1:1 sugar-to-water ratio) following depletion of previous stores, resulting in a cumulative sugar supply of 15 kg per colony.

For biochemical analyses, thoracic muscles of worker bees were collected. Prior to dissection, bees were rendered immobile by exposure to –18 °C for 20 minutes. The thoracic segments were then dissected, and the direct and indirect muscles were separated from the chitinous covering with a scalpel. The resulting muscle tissue was processed to quantify water content, total lipids, and the relative distribution of lipid classes.

Muscle tissue mass was measured with an analytical balance, and dry mass was determined following oven-drying at 102 °C to constant weight. Water content was calculated from the difference between fresh and dry masses. Total lipid content was assessed using the method of Folch (1957), while individual lipid class distribution was analyzed according to Tkachuk et al. (2011). All biochemical measurements are reported in accordance with the International System of Units (SI) for clinical laboratory practice. Data were analyzed using Statistica 7.0. One-way ANOVA was performed to identify significant differences between groups, with significance defined as $P < 0.05$. In the tables, statistical data are given as mean ± standard error of the mean (SE).

Results

At the first stage of the investigation, the body mass of worker bees in the experimental and control colonies was assessed. Newly emerged individuals from brood combs in all colonies of both groups were collected and subjected to gravimetric analysis. Each bee was placed in a paper container, weighed individually, marked for identification, and subsequently returned to its original colony. The initial measurements were performed on September 4, corresponding to 21 days following queen isolation, coinciding with the emergence of workers from the final brood cycle in the experimental colonies. The analysis demonstrated that the mean body mass of workers emerging in the control group was 102.4 ± 1.33 mg. In contrast, workers from the experimental group exhibited a mean body mass of 104.1 ± 1.9 mg, with values ranging from 99.6 to 105.9 mg. The subsequent weighing of bees from the control group colonies was conducted on October 26, when the last brood emerged. The mean body mass of the bees was recorded as 103.6 ± 1.6 mg. Given that the mass of newly emerged bees was nearly identical between both experimental and control groups, irrespective of the timing of emergence, it can be inferred that this parameter is not significantly influenced by the specific functional dynamics of the colonies during brood rearing. It is apparent that the body mass of newly emerged bees is primarily determined by the availability and adequacy of the colony's food resources, as well as the workload placed on nurse bees, which is directly related to the volume of brood they are tasked with rearing. Concurrently, body mass is influenced by the microclimatic conditions within the nest, particularly temperature, which has been shown to play a critical role (Kovalskyi et al., 2021). Nest temperature is directly associated with colony strength and its capacity for thermoregulation. The final series of weighings was designed to assess the effect of functional characteristics of autumn-generation bees, specifically their involvement in brood rearing on adult body mass immediately prior

to the onset of hypobiosis. These measurements were conducted on November 26. During the study period, ambient temperatures ranged from –1 °C at night to +6 °C during daytime hours. Under these environmental conditions, the bees had already formed overwintering clusters ("winter clubs"). According to the obtained results, the mean body mass of worker individuals in the experimental colonies was 109.8 ± 5.4 mg, whereas in the control colonies it was 108.2 ± 4.4 mg. The findings indicate that brood-rearing activity performed by autumn-generation bees did not exert a measurable effect on the body mass of worker individuals prior to overwintering.

In this context, colony strength defined as the total biomass of worker individuals appears to be the primary determinant of successful overwintering performance. The subsequent phase of the study involved quantifying the total lipid content in the thoracic muscles of insects during the period of colony preparation for hypobiosis. This preparatory stage is biologically significant, as it is associated with the accumulation of reserve nutrients within the cells of the fat body. In honey bees, the fat body serves a central role in metabolic regulation and functions as the primary repository of energy and structural macromolecules, particularly lipids. At the time of physiological activity, these nutrient reserves are mobilized from the fat body and transported via the hemolymph to the thoracic musculature.

Lipids entering muscle cells are metabolically transformed and utilized chiefly for structural purposes, with a subsidiary role in energy production. As membrane constituents, they modulate fluidity and maintain mitochondrial enzymatic activity, thereby supporting muscle integrity and recovery. Seasonal variation in fatty acid composition promotes cold adaptation. This metabolic interdependence between the fat body and pectoral muscles ensures effective physiological and seasonal adaptation in the bee. The autumn generation of bees is characterized by a greater degree of fat body development, attributable to the particularities of their functional specialization (Azeez et al., 2014). Such enhancement contributes to extended longevity and supports post-winter reproduction. The developmental status of the fat body is largely defined by its protein, lipid, and glycogen content. Increased fat body reserves likely promote more efficient lipid mobilization and transport toward the pectoral muscles.

The results obtained on August 27 indicated that the gradual reduction in the open brood in the experimental group resulted in a slight, yet highly significant ($P < 0.001$), increase in the lipid content within the pectoral muscles, which rose by 8.9% compared to the control group. As the winter period commenced, notable changes were observed, including a further increase in total lipid content in the tissues under investigation. Specifically, by November 26, the total lipid content in the bees of the experimental group had increased by 10.2% relative to the values measured on August 27. Over the same time period, this parameter in the control group exhibited a modest increase of only 2.1%. In contrast, restrictions on brood rearing imposed on the experimental group resulted in a significant elevation of total lipid content in the pectoral muscles, such that by November 26, levels were 17.6% higher than those observed in the control group ($P < 0.001$). The observed trends in lipid accumulation, as well as the intergroup differences, are likely attributable to the role of lipids as the primary energy substrate for the synthesis of milk by the active individuals, which is essential for larval nourishment. Furthermore, brood rearing entails energy expenditure to regulate and maintain stable microclimatic conditions. In the experimental group, the constraints on brood rearing resulted in reduced utilization of fat reserves to fulfill these energetic demands, as evidenced by the total lipid content measured in the pectoral muscles (Fig. 1).

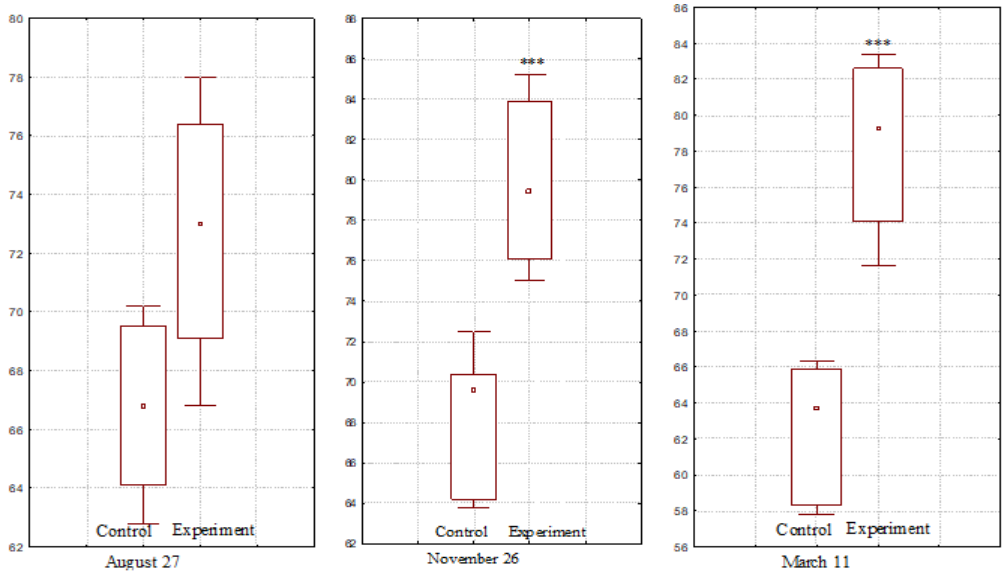

Fig. 1. Total lipid content in the pectoral muscles of worker bees (mg/g, n = 5):
here and below, the differences in the indicators of bees of experimental colonies compared to the control ones are probable (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$); 40 workers were selected for each experimental sample

From the perspective of the impact of queen oviposition intensity during colony preparation for hypobiosis on both the quality of bee overwintering and their physiological condition, the analysis of total lipid content in the pectoral muscles following the hypobiosis period was of particular interest. To this end, in the spring, on March 11, total lipid levels in the pectoral muscles of bees from the experimental colonies were re-evaluated. At this point, the control colonies already contained a brood of various developmental stages. Following the flightless period, a reduction in total lipid content was observed in the muscle tissue of bees from both experimental colony groups. Throughout wintering, bees in the control group exhibited a more substantial reduction in lipid reserves. Relative to measurements at the beginning of the winter period, total lipid content in the muscle tissue of control bees declined by 8.4% ($P < 0.001$). In the experimental group, this decrease was only 2.4% ($P < 0.001$), indicating a slower rate of lipolysis. At the end of hypobiosis, pectoral muscle lipid content in bees from experimental colonies was 78.2 mg/g dry matter, which is 25.3% higher than the 62.4 mg/g recorded in the control group.

The subsequent phase of the study focused on examining and quantifying the distribution of individual lipid classes in the pectoral muscles of bees from the experimental groups (Table 1). Using thin-layer chromatography, total lipids from both the direct and indirect muscles of the pectoral region were fractionated into six distinct classes. Table 1 demonstrates that phospholipids and triacylglycerols were the dominant lipid components in the pectoral muscles of bees during the pre-wintering period. Their cumulative contribution to the total lipid pool increased from 50.2% in the control to 55.8% in the experimental group. This represents a statistically significant enhance-

ment of 5.64% in the experimental condition compared to the control ($P < 0.001$). Triacylglycerols are the dominant storage lipids in the fat body of bees. In contrast, mono- and diacylglycerols occur chiefly as intermediate compounds produced during triacylglycerol hydrolysis under conditions of energy demand. Consequently, the measured difference in mono- and diacylglycerol concentrations was relatively small (0.91%).

Table 1
The effect of brood rearing on the ratio of individual lipid classes in the pectoral muscles of worker bees at the beginning of wintering (%, n = 5)

Indicator	Experimental groups	
	control	experimental
Phospholipids	30.66 ± 2.76	33.41 ± 1.34***
Mono and diglycerides	5.04 ± 1.26	5.95 ± 0.02*
Free cholesterol	13.67 ± 0.78	9.07 ± 0.82***
Non-esterified fatty acids	13.85 ± 0.46	14.08 ± 1.13
Triacylglycerols	19.53 ± 1.99	22.42 ± 1.14***
Esterified cholesterol	17.24 ± 1.09	15.06 ± 1.92**

Note: here and below, there are significant differences in the indicators of bees of experimental colonies compared to the control ones (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$); 40 workers were selected for each experimental sample.

Assessment of lipid composition revealed that free cholesterol, a key determinant of cellular physiological state, was significantly lower in the experimental group by 4.6% ($P < 0.001$) compared with the control group. In contrast, non-esterified fatty acid content did not differ significantly between groups.

According to the ratio of lipid classes, a highly probable higher content of triacylglycerols by 2.89% ($P < 0.001$) was found in the experimental group. At the same time, a lower content of esterified cholesterol by 2.18% ($P < 0.01$) was found in this group.

Alterations in the total lipid content and in the proportional distribution of individual lipid classes within the direct and indirect flight muscles of worker bees exposed to a stress factor suggest stress-induced modifications in metabolic processes. These changes are likely associated with the regulation of lipid phase microviscosity in sarcolemmal membranes. To further evaluate the effects of hypobiosis and brood-rearing intensity on the content and distribution of lipid classes in the thoracic muscles of worker bees, a second analogous experimental series was conducted on March 11 in both control and experimental colonies (Table 2).

Table 2
The ratio of individual lipid classes in the pectoral muscles of worker bees at the end of wintering (%, n = 5)

Indicator	Experimental groups	
	control	experimental
Phospholipids	27.61 ± 1.22	32.17 ± 1.55***
Mono and diglycerides	5.01 ± 0.67	5.41 ± 0.32
Free cholesterol	14.87 ± 1.29	9.91 ± 1.02***
Non-esterified fatty acids	11.18 ± 0.53	12.48 ± 1.86
Triacylglycerols	18.99 ± 1.61	20.97 ± 2.04***
Esterified cholesterol	22.33 ± 2.08	19.06 ± 1.63**

A comparative analysis of individual lipid fractions in the pectoral muscles of bees following emergence from hypobiosis and at the onset of the wintering period revealed distinct alterations in lipid composition. The results demonstrated a reduction in the levels of total lipids, phospholipids, mono- and diacylglycerols, non-esterified fatty acids, and triacylglycerols. Conversely, an increase in the concentrations of free cholesterol and esterified cholesterol was observed. This pattern was observed in both experimental groups; however, the temporal dynamics of changes in individual lipid classes differed significantly between the experimental and control colonies. These findings suggest that the functional activity of bees specifically brood rearing under the conditions of this study exerts a notable influence on lipid metabolism during the pre-wintering period.

The practical implication of the present research is that implementing a technological method involving restricted brood rearing during the preparation of bee colonies for hypobiosis positively influences their subsequent honey yield (Fig. 2).

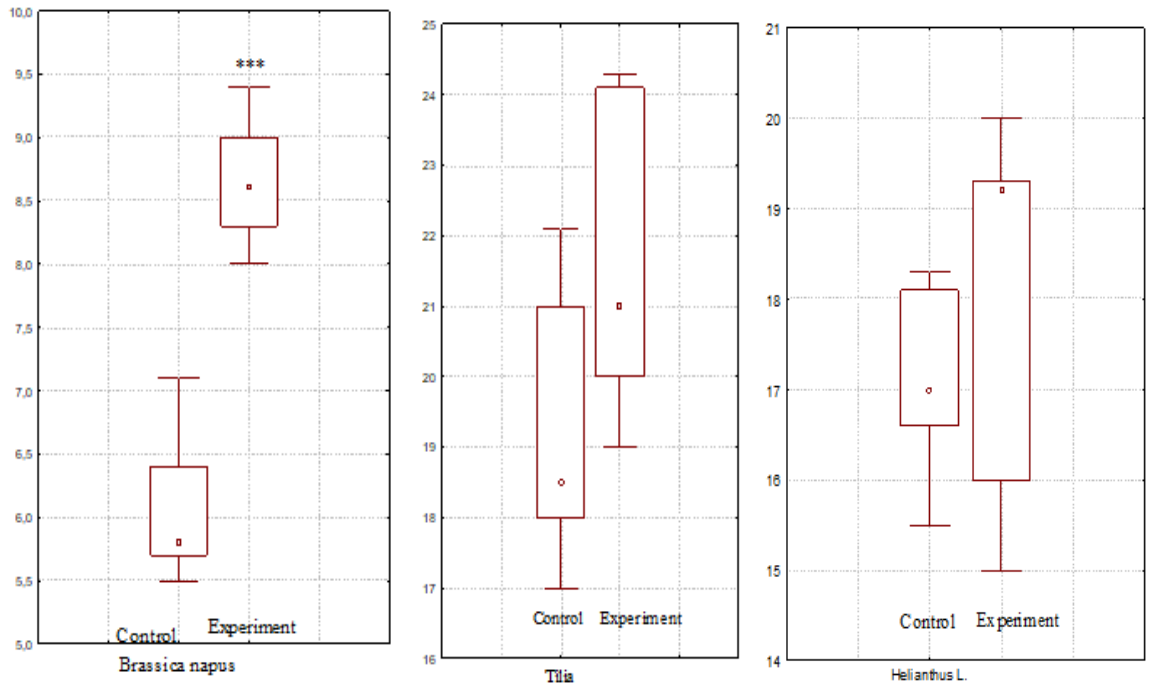

Fig. 2. Level of honey productivity at the main commercial honey harvests (kg from 1 bee colony, n = 5)

In the control colonies that maintained brood rearing during the autumn, rapeseed honey yield averaged 6.10 ± 0.65 kg per colony, whereas experimental colonies achieved a mean productivity of 8.66 ± 0.55 kg per colony. Consequently, the applied intervention resulted in an average increase of 2.56 kg in rapeseed honey production, representing a 42% enhancement relative to the control group ($P < 0.001$). These findings indicate that autumnal brood regulation promotes the development of physiologically more robust winter bees, which subsequently exhibit greater efficiency in early-season honey collection following emergence from hypobiosis. The subsequent commercial honey harvest was derived from lime. Analysis of colony productivity during this harvest demonstrated that experimen-

tal colonies maintained a measurable advantage over controls, yielding on average 16.1% more honey ($P < 0.05$); however, this effect was less pronounced than that observed during the rapeseed harvest. In the final sunflower honey harvest, differences between experimental and control groups were negligible, with productivity nearly equivalent. These findings suggest that the impact of limiting autumn brood is most significant for early spring honey harvests, when the quality of overwintered bees is a critical factor, whereas its influence diminishes in later-season harvests. Therefore, the implementation of this technological approach is suitable for enhancing apiary productivity, particularly during the initial honey harvests following the hypobiosis period.

Discussion

The development of honey bee broods is facilitated through intricate cooperative behaviors that sustain favorable conditions within the nest. A stable microclimate, with temperatures between 33 and 35 °C, is maintained primarily by heater bees a subgroup of nurse bees. Heat production occurs through sustained pectoral muscle contractions, and the mobility of these bees between brood cells allows precise thermal regulation, directly influencing the growth and survival of larvae and pupae.

Maintaining a stable microclimate within the nest of honey bees represents one of the most intricate and energy-demanding social behaviors. This thermoregulatory process primarily relies on the heat generation of worker bees through microvibrations of the pectoral muscles, which operate in a detached, wingless mode. These microvibrations are produced by isometric contractions of fibrillar muscles situated in the thoracic region. Unlike the contractions associated with flight, these muscle activities are specialized for the efficient conversion of chemical energy into thermal energy (Heinrich, 1980).

Approximately 50% of a colony's yearly honey reserves are devoted to brood care (Debnam et al., 2024). In brood-containing nests, honey bees regulate both temperature and humidity with remarkable precision, regardless of hive structure or seasonal conditions (Kutby et al., 2024). This homeostatic control is predominantly facilitated by thoracic musculature, particularly the pectoral muscles, which possess exceptionally high rates of energy metabolism relative to other animal tissues (Williams et al., 2008). These results indicate that the bee organism is characterized by a maximal rate of energy production and utilization, supporting essential physiological functions and locomotor activity. Energy metabolism comprises the entirety of biochemical processes responsible for energy generation and expenditure, including nutrient catabolism, adenosine triphosphate (ATP) synthesis, and its subsequent utilization in cellular functions. The energy released through these metabolic pathways is allocated to anabolic processes, thermogenesis, molecular transport, and other vital functional activities.

The preparation of honey bee bodies for hypobiosis involves a series of critical physiological processes that enable their survival under low-temperature conditions and limited food availability. During the wintering preparation phase, honey bees store reserve lipids, which serve as both an energy source and structural components throughout the winter, and are mobilized for brood rearing during the spring (Güneşdoğdu et al., 2024). The degree of pharyngeal gland development and their capacity to secrete royal jelly are closely linked to lipogenesis in nurse bees (Corby-Harris et al., 2019). The storage of energy in the form of lipids is metabolically advantageous, as the β-oxidation pathway of fatty acids yields a substantial amount of ATP, making it one of the most efficient mechanisms of energy production (Azeez et al., 2014). In insects, the fat body acts as the central organ for lipid storage and mobilization (Sheng et al., 2019).This organ is essential for the physiological adaptation of honey bees to adverse environmental stressors. Moreover, the fat body and hemolymph are functionally integrated systems, exhibiting strong biochemical interdependence. Accordingly, modifications in the fat body composition are reflected in corresponding alterations in the chemical composition of the hemolymph (Bryś et al., 2014, 2025).

The onset of the overwintering period in bees is characterized by a decline in functional activity, which serves to conserve energy. This physiological adaptation supports the enhanced accumulation of lipid and protein stores (Amdam et al., 2009). Notably, variations in muscle tissue lipid composition are closely linked to functional activity patterns and play a significant role in determining bee lifespan (Brejcha et al., 2020). At the time of hypobiosis, bees exhibit reduced activity and remain within the hive, forming dense clusters that provide thermal insulation and facilitate heat conservation. These behavioral adaptations enable effective survival under harsh winter conditions and support colony viability until the subsequent season. However, the environmental and physiological factors regulating metabolic intensity during this period remain poorly understood. In this context, it is pertinent to investigate how the intensity of brood rearing during the pre-winter preparation of the colony influences the total lipid content and the distribution of lipid classes in the bodies of nurse bees. From a practical point of view, these data allow us to expand our understanding of the mechanisms of bee viability during hypobiosis, as well as the organism's resistance and resistance to toxicosis. It is also known that lipids and immune parameters are different aspects of the organism's biology, and they cannot be identified. Lipids serve as energy reservoirs and structural components of cell membranes, while immune parameters determine the state and ability of the immune system to protect the organism from infections and diseases. Although both categories of biomarkers are essential for maintaining overall physiological health, they represent distinct dimensions of bodily function. Notably, lipid homeostasis plays a significant role in modulating immune activity. Dysregulation of lipid classes, particularly increased concentrations of saturated fatty acids or cholesterol, has been associated with pro-inflammatory states and may alter immune responsiveness.

The results obtained in this research indicate that specific technological interventions in colony management, particularly the restriction of brood rearing during the preparatory phase preceding hypobiosis, exert a pronounced positive effect on overwintering survival in honey bees. The process of autumnal lipid deposition and physiological adaptation to winter conditions exhibits distinct characteristics, including modifications in the thoracic musculature, which serves as the principal organ of thermogenesis. Given the central role of the flight muscles in heat production during winter clustering, changes in their biochemical composition are of particular importance. In the experimental group, the peak total lipid concentration in the thoracic muscles during autumn reached 80.1 mg/g. This is consistent with the pre-winter increase in lipid metabolism: in worker bees of the autumn generation, triacylglycerol reserves increase, primarily in the fat body, as well as a small proportion of structural and reserve lipids in the pectoral muscles. This phenomenon provides membrane hypothermic adaptation – maintaining the fluidity of the sarcolemma and mitochondrial membranes at low temperatures due to the redistribution of phospholipids and, probably, a shift towards unsaturated fatty acids. Experimental restriction of brood rearing in colonies resulted in a 17.6% increase in lipid content of the pectoral muscles prior to the onset of overwintering compared to control colonies ($P < 0.001$). Thermogenesis during the wintering period is achieved through microvibratory contractions of the pectoral muscles (shivering thermogenesis). Although carbohydrates, primarily trehalose and glycogen, constitute the principal substrates for muscular activity in bees, lipids play a critical role in maintaining membrane integrity and serve as an essential reserve energy source under conditions of prolonged stress and food deprivation. Accordingly, a higher degree of lipid conservation during the hypobiosis period was observed in the experimental group. Bees of this group maintain a high lipid content in the pectoral muscles (78.2 mg/g at the end of wintering), which may indicate lower oxidative stress in the muscles (better antioxidant support and improved lipid transport). Another reason is related to the metabolic "saving" of lipids due to carbohydrate supply of the club in winter. In the control, a greater depletion of lipids is observed in spring (62.4 mg/g), which indicates a more intensive use or peroxide damage of cells during hypobiosis.

Compared with other tissues, muscle tissue is distinguished by a predominance of phospholipids in its membrane lipid composition. These lipids are fundamental building blocks of the plasma mem-

brane, forming a bilayer that not only shields the cell from external stimuli but also orchestrates numerous subcellular processes. Research increasingly underscores that the structural heterogeneity of membrane lipids contributes to a wide array of cellular functions, such as signal transduction, organelle maintenance, physiological regulation, and disease progression (Dai et al., 2021). It should be noted that these vitamins are mainly obtained through the consumption of pollen. Therefore, when preparing bees for hypobiosis, special attention should be paid to providing families with fresh portions of flower pollen. Phospholipids help transport these substances through cell membranes, ensuring their entry into cells. Phospholipids are involved in the transmission of signals between cells, which allows the coordination of various physiological processes (Wegener et al., 2018). They provide protection for cells from harmful effects, maintaining the stability of membranes and protecting intracellular structures. Previous researches have demonstrated that phospholipid content in muscle tissue during the pre-hypobiosis period ranges from 30.66 ± 2.76% in control groups to 33.41 ± 1.34% in experimental groups. Limitation of brood rearing results in a significant increase in phospholipid levels by 2.75% ($P < 0.001$). These findings suggest that metabolic alterations associated with enhanced functional activity in bees reflected by shifts in the relative proportions of lipid classes, including a reduction in cellular phospholipids and an increase in other lipid fractions such as cholesterol may serve as indicators of physiological aging (Yang et al., 2018; Dai et al., 2021). Exposure to oxidative stress induces lipid peroxidation, resulting in the degradation of lipids and the generation of oxidative byproducts. This process decreases the overall pool of functional lipids within tissues. Variations in phospholipid content in honey bee muscle tissue reflect alterations in the functional integrity and activity of cellular membranes.

In the muscles of honey bees, the proportion of non-esterified fatty acids in the total lipid content is actually small and amounts to about 13–14%. Free fatty acids are important for the rapid supply of energy during intense physical exertion, but they do not actually accumulate in large quantities. In the control group, the content of triacylglycerols is lower due to the fact that stress factors apparently activate the body to use accumulated fat reserves for energy production. In this process, triacylglycerols are broken down into fatty acids and glycerol, which the cells then use to support life. Thus, the increased activity of triacylglycerol mobilization in the control group of colonies can be interpreted as a vital adaptation of bees to various stress factors, which may include intensive physical activity associated with brood rearing, maintaining an appropriate temperature regime, etc. In experimental colonies under conditions of limited brood rearing, such use of triacylglycerols was not observed. After wintering, the higher amount of triacylglycerols in the muscles of worker bees in the experimental group is likely due to increased adipocyte proliferation in intermuscular fibers.

In insects subjected to winter stress, cellular membranes may exhibit reduced fluidity, manifesting as increased rigidity, primarily due to deficiencies in nutrients, particularly unsaturated fatty acids. This decrease in membrane fluidity impairs the capacity of insects to acclimate to low-temperature environments, as membrane rigidity disrupts the normal dynamics of cellular processes.

Lipid metabolism exerts a significant influence on hydration dynamics within muscle tissue. Water content, particularly in muscular tissue, is closely linked to physiological demands that emerge during hypobiosis. In honey bees, a key biological characteristic is the cessation of foraging activity during winter. During this period, nutrient intake is restricted to endogenous reserves within the hive, primarily honey and pollen. Notably, metabolic processes contribute to endogenous water production, which constitutes a critical component of water balance. Under these conditions, the regulation of water balance plays a vital role in preserving muscle tissue hydration. This adaptation allows bees to optimize the use of their energy reserves during periods of minimal motor activity. In hypobiosis, insects demonstrate a significant reduction in movement compared to their active state. Water plays a key role in thermoregulation, enabling bees to sustain optimal physiological conditions in cold environments. Research has shown that the water content in muscle tissue remains largely stable at the onset of hypobiosis, ranging from 89.65% to 89.75%. During the process of lipolysis, water is generated as a metabolic byproduct, ensuring the maintenance of water balance in the absence of external sources.

In the analysis of research data, consideration of their practical applications is imperative. The preparation of honeybee (*Apis mellifera*) colonies for the overwintering period represents a critical determinant of wintering success, which subsequently exerts a direct influence on the productivity achieved in the following season. High-intensity brood rearing by autumn-generation bees has been identified as a physiological stressor, negatively affecting colony condition (Castaños et al., 2023). The present study demonstrates that technological interventions can modulate colony physiology. In particular, the controlled regulation of brood-rearing intensity provides an effective strategy for maintaining and optimizing the overall biological status of colonies throughout the annual cycle. Implementation of brood-rearing restrictions during the preparatory phase of bee colonies entering hypobiosis is recommended, as it promotes enhanced apiary productivity, particularly during early honey yields following emergence from hypobiosis. The findings of the present study extend the currently limited body of data in this research domain (Brodschneider et al., 2010).

Conclusion

The results of the present research indicate that brood rearing by honey bees in preparation for the hypobiosis period constitutes a stressor that impacts the physiological condition of the insect. This conclusion is supported by the observed effects of brood-rearing intensity on both the total lipid content and the distribution of lipid classes within the fat body and muscle tissues of honey bees.

Alterations in total lipid content and the relative proportions of individual lipid classes in both direct and indirect muscles of worker bees in response to a stressor reflect the activation of metabolic pathways, manifested as lipid mobilization required to preserve optimal microviscosity of the membrane lipid phase in the sarcolemma. This, in turn, supports the stability of ion transport systems and facilitates lateral protein diffusion. However, the underlying biological mechanisms linking the functional activity of bees to changes in lipid class composition remain incompletely understood, representing an area of scientific interest that warrants further investigation.

The authors report they have no conflicts of interest.

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