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EFFECT OF GROSS ENERGY AND PROTEIN NUTRITIONAL VALUE OF POLLEN SUBSTITUTE ON SELECTED PHYSIOLOGICAL INDICES AND SURVIVABILITY OF WORKER BEES KEPT IN LABORATORY CONDITIONS

Summary. The aim of the performed experiments was to determine the impact of gross energy and protein nutritional value of the pollen substitute fed in laboratory conditions on the condition of worker bees. The experimental pollen substitutes were prepared on a uniform isonitrogenous (from 16.41 to 16.78 MJ/kg), the crude protein content in the pollen substitute level from 25.1 to 25.95. The physiological condition of worker bees kept in laboratory conditions was influenced by protein and other chemical compounds contained in the consumed feed. The survivability of worker bees in laboratory conditions was strongly influenced by gross energy contained in the feed consumed by them. The more gross energy they ingested, the higher was their survivability.

Key words: honeybee, feeding, pollen substitute, gross energy, survivability

Introduction

Similarly to other animals, one of the main purposes of bee feeding is to supply them with appropriate quantities of energy which is essential to maintain life processes, in particular, to perform muscle motions, to heat the body and to synthesise body tissue. A certain part of the supplied energy can be stored in the organism in the form of reserve fat and glycogen. Basic energy sources for bees can be found in essential nutrients such as: carbohydrates, fats and protein (GMEINBAUER and CRAILSHEIM 1993).

Thermal energy which is created in the course of complete combustion of the feed to such constituents as: carbon dioxide, water and other gases is referred to as gross energy. The main source of energy for the bee family is honey and then pollen, although in the case of the latter, its annual consumption is three times smaller than that of honey (SEELEY 1985). The consumption of pollen by an individual bee worker depends on the age and the fulfilled function (CRAILSHEIM et AL. 1997). In order to heat its thorax to the temperature of 40°C and maintain it at the surrounding temperature typical for the summer period, a bee uses 65 mJ of energy per second. During the entire period of brood rearing bees use nearly 2 mln kJ of the produced heat which constitutes over two thirds of the total energy consumption in the family during summer. Therefore, it can be said that bees' demand for energy is considerable. Bees need energy not only for proper course of physiological processes of their organisms but also as energy for flying, brood heating or to maintain constant temperature of the nest (TAUTZ and BASILE 2007).

The aim of the performed experiments was to determine the impact of gross energy and protein nutritional value of the pollen substitute fed in laboratory conditions on the condition of worker bees.

Material and methods

The elaboration of the results of nutritional experiments conducted earlier was carried out at the Institute of Zoology, Poznań University of Life Sciences in 2009.

Material

Preparation of the pollen substitute

Pollen substitute was prepared at the Experimental Station of the Technology of Feed and Aquaculture Production in Muchocin. The raw material composition of the substitute is presented in Table 1.

Table 1. Raw material composition of pollen substitutes (%)
Tabela 1. Skład surowcowy namiastek pyłku kwiatowego (%)

Specification	Pollen substitute		
	I	II	III
Fish meal	6.0	8.0	9.0
Blood meal	–	8.0	8.0
Hydrolysed casein	9.0	–	–
Powdered milk	10.0	10.0	10.0
Powdered eggs	5.0	–	–
Torulla yeasts	8.0	10.0	10.0
Soybean meal	7.0	9.0	8.0
Extruded maize	18.0	19.0	–
Hydrolysed wheat	–	–	19.0
Sugar	35.0	35.0	35.0
Soybean oil	1.0	–	–
Vitamin mixture	1.0	1.0	1.0
Total	100.0	100.0	–100.0

Experimental substitutes were made from individual feed raw materials comminuted to particles measuring 100 μm. Casein and wheat were hydrolysed enzymatically, while maize was processed using a thermoplastic method (extrusion) in order to break down starch grains. The basic raw material composition of pollen substitutes was enriched using a mineral-vitamin mixture Polfamix W.

Chemical and amino acid compositions of the experimental pollen substitutes and bee bread are presented in Tables 2 and 3.

Table 2. Chemical composition of pollen substitutes and bee bread (%)
Tabela 2. Skład chemiczny namiastek pyłku kwiatowego i pierzgi (%)

Specification	Pollen substitute I	Pollen substitute II	Pollen substitute III	Bee bread
Crude protein	25.9	25.1	25.4	23.8
Crude fat	2.6	1.7	1.4	3.4
ZBAW – NFE	54.4	55.7	58.1	59.9
Crude fibre	1.1	1.4	1.3	0.8
Crude ash	3.5	4.9	4.6	3.1

Table 3. Content of exogenous amino acids in experimental feeds (g in 100 g of protein)
Tabela 3. Zawartość aminokwasów egzogennych w testowanych pokarmach (g w 100 g białka)

Amino acid	Pollen substitute I	Pollen substitute II	Pollen substitute III	Bee bread
Arginine	5.17	5.27	5.30	4.78
Hystidine	2.97	3.39	3.44	2.77
Lysine	7.48	7.13	7.21	7.48
Tryptophan	2.11	3.90	3.82	1.41
Phenylalanine, Tyrosine	9.41	7.77	7.84	7.78
Methionine, Cystine	3.39	2.74	3.11	3.09
Threonine	4.23	4.17	4.01	5.37
Leucine	8.78	8.29	8.32	8.88
Isoleucine	5.33	3.96	3.82	5.29
Valine	5.66	4.88	5.01	6.13

The experimental pollen substitutes were prepared on a uniform isonitrogenous level (from 25.1 to 25.95%). The crude protein content in the bee bread differed slightly from its content in the prepared pollen substitutes.

Securing bees for experiments

Experiments were conducted on worker honey bees *Apis mellifera carnica* L. Worker bees for the experiments were obtained by putting into the incubator with the temperature of 33°C and 80% relative air humidity frames with emerging brood derived from one mother. Next, one-day old bees were transferred into nuclei of SZYMAŚ and WÓJTOWSKI (1974) construction and fed for the period of two weeks different protein diets.

Methods

Feeding bees in laboratory conditions

The employed nuclei with 100 worker bees each were equipped in feeders and water fountains. In the course of the entire feeding period of two weeks, bees were kept in the incubator in which the temperature was maintained at the level of 31°C and relative air humidity of 40%. They had no possibility of making flights, did not take care of the brood and the surrounding temperature and air humidity corresponded to bee-hive conditions during summer period when the brood is reared. Both the feed which was fed *ad libitum* and water were changed daily. The experimental design is shown in Table 4.

Table 4. Experimental design
Tabela 4. Układ doświadczenia

Nutritional group	Feed	Number of replications
I (experimental)	Pollen substitute I	5 × 100 individuals
II (experimental)	Pollen substitute II	
III (experimental)	Pollen substitute III	
Kp (control)	Bee bread	
Kc (control)	Sugar (sugar dough)	

Assessment of the worker bees' conditions

Survivability

Dead bees were removed every day. Survivability was estimated on the basis of the number of worker bees left alive after the two-week period of feeding.

Size of pharyngeal glands and of the fat body

The condition of experimental worker bees was also assessed on the basis of the physiological condition of pharyngeal glands and the fat body employing the modified Maurizio method (SZYMAŚ 1994, SZYMAŚ et AL. 2002) where score 4 was used to refer to the maximal growth of the organ.

Chemical assessment of feeds (Table 5)

Organic amino acid index (Chemical score – CS)
Exogenous amino acid index (EAAI)
Gross energy (it was calculated by burning feed samples in an automatic calorimeter LK-10).

Table 5. Chemical indices of protein nutritional value and gross energy level and energy-protein ratio
Tabela 5. Chemiczne wartości odżywcze białka i poziomu energii brutto oraz stosunek energetyczno-białkowy

Specification	Pollen substitute I	Pollen substitute II	Pollen substitute III	Bee bread	Sugar
CS					
I	Met+Cys 58.45	Met+Cys 47.24	Met+Cys 53.62	Met+Cys 53.28	
II	Phe 70.18	Isol 57.39	Isol 55.36	Arg 69.28	
III	Arg 74.93	Val 65.95	Val 67.70	Phe 70.18	
EAAI	84.46	78.58	79.33	83.67	
Gross energy (MJ/kg)	16.77	16.41	16.78	17.59	16.50
E/P (kJ/g crude protein)	64.52	65.39	66.08	73.57	

Consumption of feed and energy contained in it

Feed consumption was calculated from the gravimetric difference of the fed feed and feed left in the feeder after 24 h, taking into account the number of worker bees alive on a given day. The energy ingested in the feed was calculated from the quantity of the consumed feed.

Statistical elaboration of results

Weighted means of the fat body and pharyngeal glands were compared using for this purpose the Student t-test at $\alpha = 0.05$. Regression analysis was carried out for the energy in taken in the feed and for survivability.

Results and discussion

The results of the performed experiments are presented in Table 6. All experimental pollen substitutes examined in this study exerted a positive influence on the physiological condition of worker bees as expressed by the size of pharyngeal glands and the fat body. This was especially evident in the case of the pollen substitute I which consisted of the following raw materials: fish meal, hydrolysed casein and egg powder and which acted similarly to bee bread. Positive opinions about pollen substitutes which contained protein of high nutritional value were expressed, among others, by: SZYMAŚ (1994),

Table 6. Impact of gross energy contained in the feed and feed protein nutritional value on the survivability and physiological indices of worker bees following two-week period of feeding in laboratory conditions
Tabela 6. Wpływ energii brutto zawartej w paszy i wartości odżywczej białka paszy na przeżywalność pszczół robotnic oraz wskaźniki fizjologiczne po dwutygodniowym okresie żywienia w warunkach laboratoryjnych

Nutritional group	Gross energy in consumer feed per bee (kJ)	Survivability (%)	Pharyngeal glands – mean weighted valuation degree 1-4	Fat body – mean weighted valuation degree 1-4
I	2.70	63.0	3.28 a*	2.88 a*
II	2.48	53.5	2.80 b	2.41 b
III	4.35	76.2	2.88 ab	2.33 b
Kp	4.89	84.7	3.20 a	2.70 ab
Kc	4.60	91.0	1.00 c	1.25 c

*Student t-test results at $\alpha = 0.05$. Values in columns designated with different letters differ from one another significantly.

ROGALA and SZYMAŚ (2004 a, 2004 b), KAZNOWSKI et AL. (2005), STEEN (2007), DEGRANDI-HOFFMAN et AL. (2008), while KELLER et AL. (2005) emphasised the important role of pollen substitute protein for the development of pharyngeal glands which are involved in the process of brood rearing.

The protein-free feed employed in this study, i.e. sugar dough, was used by worker bees to manufacture energy needed for their survival. The laboratory conditions in which the experimental bees were kept corresponded to bee-hive conditions during brood rearing, except that in the laboratory bees did not have to use the produced energy to maintain the temperature of the surroundings or feeding the brood; they could utilise the entire energy to improve the physiological status of their organisms and their survivability. The supplied sugar feed did not affect the development of internal organs as confirmed by earlier investigations (SZYMAŚ et AL. 2003).

In the case of all experimental diets, methionine and cystine were the first limiting amino acids (CS), similarly to bee bread. In addition, values of the exogenous amino acid index (EAAI) were also similar in all diets. Pollen substitutes I, II and III can be considered as isocaloric. Levels of gross energy in the employed pollen substitutes was similar and ranged from 16.41 to 16.78 MJ/kg. Higher gross energy levels were found in the bee bread (17.59 MJ/kg) which was characterised by the highest E/P ratio of 73.57.

Despite the fact that bee bread contained less protein in its chemical composition than the prepared pollen substitute, it was found to exert the most favourable impact on the survivability of worker bees. However, its effect on the remaining physiological parameters (fat body and pharyngeal glands) was similar to that of the prepared pollen substitute. Therefore, the nutritional value of the prepared experimental pollen substitutes should be considered as high. The considerably higher survivability recorded in the group of bees consuming bee bread than in experimental groups (I, II and III) should be attributed to a higher gross energy level as well as to the highest energy – protein (E/P) ratio contained in bee bread.

The physiological condition of worker bees kept in laboratory conditions was influenced by protein and other chemical compounds contained in the consumed feed. Survivability was positively correlated (p1 = 0.000305) with the in taken energy. This correlation can be presented in the form of simple regression equation y = 18.95x.

The survivability of worker bees in laboratory conditions was strongly influenced by gross energy contained in the feed consumed by them. The more gross energy they ingested, the higher was their survivability.

Conclusions

1. The physiological condition of worker bees kept in laboratory conditions was influenced by protein and other chemical compounds contained in the consumed feed.

2. The survivability of worker bees in laboratory conditions was strongly influenced by gross energy contained in the feed consumed by them. The more gross energy they ingested, the higher was their survivability.

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WPŁYW ENERGII BRUTTO ORAZ WARTOŚCI ODŻYWCZEJ BIAŁKA NAMIASTEK PYŁKU KWIATOWEGO NA WYBRANE WSKAŹNIKI FIZJOLOGICZNE I PRZEŻYWALNOŚĆ PSZCZÓŁ ROBOTNIC PRZETRZYMYWANYCH W WARUNKACH LABORATORYJNYCH

Streszczenie. Jednym z głównych celów żywienia pszczół, podobnie jak innych zwierząt, jest dostarczenie im energii. Głównym źródłem energii dla rodziny pszczelej jest miód, a w drugiej kolejności pyłek kwiatowy. Celem pracy było określenie wpływu energii brutto i wartości odżywczej białka namiastki pyłku podanej w warunkach laboratoryjnych na kondycję pszczół robotnic. Doświadczenie wykonano na robotnicach pszczoły miodnej *Apis mellifera carnica* L. Namiastki pyłku przygotowano na wyrównanym poziomie izoazotowym. Namiastki pyłku zostały wykonane z następujących surowców: I – mączka rybna 6%, kazeina hydrolizowana 9%, mleko w proszku 10%, jaja w proszku 5%, drożdże Torulla 8%, mąka sojowa 7% (autoklawowana w temperaturze 121°C), śruta kukurydziana ekstrudowana 18%, cukier 35%, olej sojowy 1%, mieszanka witaminowo-mineralna 1%; II – mączka rybna 8%, mączka z krwi 8%, mleko w proszku 10%, drożdże Torulla 10%, mąka sojowa 9% (autoklawowana w temperaturze 121°C), śruta kukurydziana ekstrudowana 19%, cukier 35%, mieszanka witaminowo-mineralna 1%; III – mączka rybna 9%, mączka z krwi 8%, mleko w proszku 10%, drożdże Torulla 10%, mąka sojowa 8% (autoklawowana w temperaturze 121°C), śruta pszenna hydrolizowana 19%, cukier 35%, mieszanka witaminowo-mineralna 1%. Zawartość białka w namiastkach wynosiła około 25%. Przeżywalność określono na podstawie liczby osobników pozostałych przyżyciowo po dwutygodniowym okresie żywienia. Kondycję pszczół określono również na podstawie stanu fizjologicznego gruczołów gardzielowych i ciała tłuszczowego, posługując się zmodyfikowaną metodą Maurizio, gdzie maksymalny rozwój narządu wyrażono w stopniu 4. Poziom energii brutto w namiastkach był podobny i wynosił, od 16,41 do 16,78 MJ/kg. Wyższy poziom energii brutto zawierała pierzga – 17,59 MJ/kg; charakteryzowała się ona najwyższym stosunkiem E/P – 73,57. Na stan fizjologiczny robotnic przetrzymywanych w warunkach laboratoryjnych miały wpływ białko i inne związki chemiczne zawarte w spożytej paszy. Na przeżywalność pszczół w warunkach laboratoryjnych zdecydowany wpływ miała energia brutto zawarta w pobranej przez pszczoły paszy. Przeżywalność była dodatnio skorelowana ($p1 = 0,000305$) z energią pobraną z paszy. Związek ten można przedstawić za pomocą równania prostej regresji $y = 18,95x$. Im więcej energii brutto pobrały pszczoły, tym większa była ich przeżywalność.

Słowa kluczowe: pszczoła miodna, żywienie, namiastka pyłku, energia brutto, przeżywalność

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