

NUTRITIONAL VALUE FOR BEES OF POLLEN SUBSTITUTE ENRICHED WITH SYNTHETIC AMINO ACIDS

Part I. CHEMICAL METHODS

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Received 05 May 2004; accepted 29 June 2004

S u m m a r y

Faithful reproduction of pollen, natural feed of all bees, is not easy because it contains a wealth of substances. Of these, protein is the basic component. It contains, along with endogenous amino acids, all exogenous amino acids essential for bees. The objective of the study was to raise the nutritional value of the protein contained in pollen substitute to be used in honeybee nutrition, through the optimization of its amino acid composition by supplementing it with the synthetic DL and L isomers of the lacking amino acids and thus upgrading it to the pollen protein standard. The surrogate was made up of the following components: potato protein 32%, soybean cake 18%, rapeseed cake 6%, yeast *Candida utilis* 6%, wheat flour 14.8%, maize grits 17.5%, soybean oil 3.3%, lecithin 0.5% polfamix W 1.4%, Vitazol AD3EC 0.2%, glucose with vitamin C 0.1%. The test was performed on the honeybee *Apis mellifera carnica*, in the years 1998 and 1999. Small nuclei were colonized with one day-old bees which were fed on varied protein diet over two weeks. The hives were kept in incubators at 31°C and at a relative humidity of 40%. The nutritional value of protein in the surrogate feeds was determined using chemical methods (Szymaś and Przybył 2000). Experiment feed protein and bee bodies were assessed. The chemical composition of pollen substitutes was close to that of pollen from pollen loads. The essential amino acids index in the feed EAAI reached a relatively high value (80.99 and 78.95 for substitutes A and B, respectively). In the chemical evaluation of the tested material it was found that supplementing pollen substitute with lacking amino acids to bring them up to the pollen level caused the nutritional value of the protein in the surrogate to equal that of pollen. When fed to bees, the substitute gave similar dry matter, protein and crude fat contents of bee bodies.

The study also confirmed that bees utilized the L isomer better than they did the DL isomer.

Keywords: honeybee nutrition, pollen substitute, surrogate nutritional value, amino acids, chemical methods.

INTRODUCTION

Pollen is the basic source of protein and amino acids for bees. It contains ca. 25% of the aforementioned substances (Herbert and Shimanuki 1978, Szczęsna et al. 1995). Pollen protein contains, along with endogenous amino acids, all amino acids essential for bees. The presence of 15 to 19 aminoacids was detected in pollen. Of these, the most abundant are proline,

leucine and lysine as well as arginine (McLellan 1977, Gilliam et al. 1980, Kauffeld 1980, McCaughey et al. 1980, Szczęsna et al. 1995). The nutritional value of pollen for bees may vary substantially depending on the source of origin (Maurizio 1954, Standifer 1967, Hayes 1984). Periodical deficit of pollen can be offset by feeding substitute feeds i.e. pollen substitute to the bees

(Maurizio 1954, Herbert and Shimanuki 1982, Peng et al. 1984, Pedersen and Omholt 1993, Watanabe 1993, Szymaś 1994, Łangowska et al. 2002).

The suitability of different protein feeds in the nutrition of honeybees has been tested over many years and their stimulating effect on the development of bee colonies, oviposition, and the condition of young queens was demonstrated. In order to provide bees with a bee bread substitute in the periods of high demand protein feeds or feed mixtures made up of many components and with improved ingestibility were fed to bees (Szymaś 1977, Szymaś and Torgowski 1979, Szymaś 1994). In multi-component pollen substitutes chemical composition and amino acid make up of the protein could be better balanced so that it was comparable to that of pollen (Szymaś 1994). Surrogate protein feed for the honeybee can also be made from synthetic amino acids. However, the price of such a formula puts a constraint on its widespread use in beekeeping. In order to improve the nutritional value of pollen substitutes and to improve the intake of complex proteins and sugars by bees individual components have to be upgraded by thermal and hydrothermal treatments and by enzymatic hydrolysis (Szymaś and Przybył 1989, 1999, 2002; Mościcki 1982).

The objective of the study is to improve the nutritional value of pollen substitutes to be used as honeybee feed through the optimization of its amino acids composition by upgrading their amino acids to the pollen protein standard.

MATERIAL AND METHODS

Pollen substitute was made at the Experiment Station of Feedstuffs Production Technology and Aquaculture, Agricultural University in Poznań, Muchocin Division.

The surrogate was made of the following components: potato protein 32%, soybean cake 18%, rapeseed cake 6%, yeast *Candida utilis* 6%, wheat flour 14.8%, maize grits 17.5%, soybean oil 3.3%, lecithin 0.5% polfamix W 1.4%, Vitazol AD3EC 0.2%, glucose with vitamin C 0.1%. The yeast *Candida utilis*, wheat flour, maize grits were mixed, 4% of water was added and the mixture was microwaved for 40 sec. in a 1,000 W microwave oven. In order to improve the digestive energy soybean flour, oilseed cake and maize grits were subjected to the thermobaric treatment or extrusion. When mixed, all components were ground in an impact grinder to particles 150 µm in diameter. Once ready the surrogate was acidified with lactic acid to pH 4.8 i.e. to the pH of pollen loads, Subsequently, it was greased with soybean oil. The chemical composition of the surrogate made over the experiment years and of pollen loads was shown in Table 1. Pollen substitute, prior to be fed to bees, was mixed with powder sugar in the weight ratio of 1 to 1 and was moistened with water to obtain a consistence of dense dough. Even though the composition of surrogates was the same they differed for their chemical (Table 1) and amino acid (Table 2) composition. It was due to the slightly different chemical composition of feed materials since the feedstuffs proceeded from different companies. To pollen loads some powder sugar was added to keep the protein level at 21% i.e. at the pollen substitute level.

The experiment was performed in the years 1998 and 1999 on the honeybee *Apis mellifera carnica*. The bees for the study were collected by placing frames with emerging brood from a single colony in incubators set to the temperature of 33°C and the relative humidity of 80%. Subsequently, small hives designed by Szymaś and Wójtowski (1974) were settled by

Table 1.

Percent composition of pollen loads and of pollen substitute

Specification	Pollen substitute A	Pollen loads (1998)	Pollen substitute B	Pollen loads (1999)
Dry matter	91.57	91.36	92.88	90.71
Total protein	42.01	27.62	42.08	24.66
Crude fat	5.43	4.18	5.85	4.07
Crude fibre	2.37	2.59	2.51	2.59
Crude ash	3.57	2.81	3.41	3.85
NFE(Nitrogen free extractives)	38.19	55.48	39.03	55.54

150 one day-old bees. The bees were subjected to different feeding treatments for two weeks. The nuclei were supplied with food and water feeders. Each year there were five replications of each treatment. Throughout the 2-week feeding period the bees were kept in incubators at 31°C and 40% relative humidity. The bees of each treatment were fed pollen substitutes to which lacking amino acids were added to make the composition equal to that of pollen. One kg of pollen substitute A was supplemented with: methionine 1.848 g;

lysine 3.612 g; histidine 2.058 g and threonine 0.420 g. Pollen substitute B was added 1.052 g of methionine; 2.819 g of lysine; 1.893 g histidine and 0.378 g of threonine. The amino acids were purchased at SIGMA-ALDRICH.

The food given to the bees *ad libitum* and water was changed daily. The experiment layout is shown in Table 3.

The nutritional value of the surrogate feedstuffs and of pollen was determined using chemical methods (Szymaś and Przybył 2000). To this end, the protein

Table 2.

Amino acid composition of feed formulations (g/100 g protein)

Amino acids	Pollen substitute A (1998)	Pollen loads (1998)	Pollen substitute B (1999)	Pollen loads (1999)
Arg	4.80	4.79	5.13	4.18
His	2.77	3.26	2.52	2.97
Lys	6.14	7.00	6.56	7.23
Phe+Tyr	9.41	9.30	8.94	8.82
Met+Cys	3.63	4.07	3.01	3.26
Thr	4.57	4.67	4.59	4.68
Leu	8.35	6.86	8.08	6.57
Ile	4.52	4.04	4.33	3.97
Val	5.39	4.80	5.12	4.51

Table 3.

Experiment layout

Group	Experiment I (1998)	Experiment II (1999)	Number of individuals in groups
	Food		
Control treatment Kp	Pollen loads	Pollen loads	5x150
Experimental treatment D1	Pollen substitute A	Pollen substitute B	
Experimental treatment D2	Pollen substitute A + DL-Met+DL-Lys	Pollen substitute B + DL-Met+DL-Lys	
Experimental treatment D3	Pollen substitute A + L-Met+L-Lys	Pollen substitute B + L-Met+L-Lys	
Experimental treatment D4	Pollen substitute A + DL-Met+DL-Lys+ DL-His+DL-Thr	Pollen substitute B + DL-Met+DL-Lys +DL-His+DL-Thr	
Experimental treatment D5	Pollen substitute A + L-Met+ L-Lys+ L-His+ L-Thr	Pollen substitute B + L-Met+L-Lys+ L-His+L-Thr	

from experimental feeds and bee bodies were evaluated.

Based on the amino acids composition analysis of the feedstuffs and of that of chicken egg white the limiting amino acid index (Chemical score – CS) after Block and Mitchell (1946), Essential Amino Acids Index-EAAI after Oser (1952) and the gross energy by combusting feed samples in the automatic colorimeter KL-10 were determined.

After the feeding period the bee bodies were analyzed for dry matter, total protein and crude fat. The analyses were run on two samples collected each year from each group, each sample made up of 100 bees (Muszyńska and Konopacka 1974). Prior to analysis, alimentary canals were dissected from bee bodies, and then dried, ground and homogenized.

The chemical and amino acid composition was determined in the chemical laboratory of the Dept. of Animal Nutrition and Feed Management, Agricultural

University, Poznań. Crude protein was determined using Kjeldahl method by means of the apparatus manufactured by the Danish company Foss Electric as the multiplication of the determined nitrogen by 6.25. The amino acid composition was determined using the analyzer AAA339 manufactured by the Czech company Mikrotechna after hydrolysis in 6N HCl at 105°C for 23 hrs. Sulfur amino acids were determined after being oxidized and fixed with formic acid. Due to technical reasons, tryptophane determinations were not made since the amino acid undergoes decomposition during acid hydrolysis of the protein.

The results were subjected to statistical analysis. ANOVA and Fisher's F test at $\alpha=0.05$ was performed on the means of dry matter, crude protein and fat of bee bodies.

RESULTS

The gross energy (Table 4) in surrogates A and B and in pollen loads was at a

Table 4.

Gross energy, Essential Amino Acids Index – EAAI,
Chemical Score CS of pollen substitutes and pollen loads

Specifi- cation	Pollen substitute A (1998)	Pollen loads (1998)	Pollen substitute B (1999)	Pollen loads (1999)
Total energy kJ/kg	18922.97	19029.30	19254.70	17289.00
EAAI	80.99	79.09	78.95	75.86
CS	I. Ile 65.51 II Arg 69.57 III. Met+Cys70.17	I. Ile 58.55 II. Val 64.86 III. Arg 69.47	I. Met+Cys 56.21 II. Ile 62.75 III. Val 69.19	I. Met+Cys 56.21 II. Ile 57.54 III. Val 60.95

similar level (from 17289.00 to 19254.70 kJ/kg). The essential amino acid index (EAAI) reached a high value in the surrogates (A-80.99; B-78.95) and in pollen loads (1998– 79.09; 1999 –75.86) in both experiments. For surrogate A and for pollen loads in 1998 isoleucine was the primary limiting amino acid. Methionine and cystine were the primary limiting amino acids for pollen loads in 1999 and for surrogate B. Arginine was the secondary limiting amino acid in surrogate A and valine was the secondary limiting amino acid in pollen loads of 1998. Isoleucine was the secondary limiting amino acid in surrogate

B and in pollen loads of 1999.

The contents of dry matter, protein and fat of worker bees fed different diets upon termination of the experiment is shown in Table 5. It appears from the data that in the 1998 experiment the lowest value of dry matter content was obtained in group D2 which received surrogate A supplemented with DL-methionine and DL-lysine (29.48). After surrogate A was supplemented with L-methionine and L-lysine the dry matter content of bee bodies was 31.89. The value was not statistically different from the mean value in the control group (Kp). The highest dry matter content of bee bodies

Table 5.

Mean dry matter content of bee bodies, total protein and crude fat contents of dried bee bodies upon termination of the experiment (%) and the results of Fisher's F test, $\alpha=0.05$

Group	Dry matter		Total protein		Crude fat	
	(1998)	(1999)	(1998)	(1999)	(1998)	(1999)
Kp	34.26 ab	38.28 a*	24.47 a	34.53 a	2.81 a	4.18 a
D1	31.13 c	31.63 c	22.09 a	21.57 c	2.23 b	2.53 b
D2	29.48 c	31.60 c	20.87 a	24.98 bc	2.27 b	2.83 b
D3	31.89 bc	32.50 bc	23.08 a	26.14 b	2.38 ab	2.94 b
D4	29.81 c	34.45 abc	22.16 a	31.64 a	2.43 ab	3.23 b
D5	35.17 a	36.70 ab	25.25 a	34.31 a	2.87 a	4.10 a

* abc – the means in columns are not significantly different when followed by at least one same character

was obtained in group D5 which received surrogate A with L-methionine, L-lysine, L-histidine and L-threonine (35.17). The result was even better than that obtained in the control group (34.26). Instead, in the bees which received surrogate B in 1999, like with surrogate A in 1998, the lowest dry matter content was found in group D1 which received the non-enriched pollen substitute (31.60) and pollen substitute enriched with methionine and lysine (DL form). Supplementing pollen substitute with all lacking amino acids as DL and L isomers (groups D4 and D5) raised the nutritional value of the protein in pollen substitute when compared to protein value in pollen (Kp).

The highest protein content of bee bodies (on dry weight basis) was found in group D5 (25.24) in 1998. However, statistical analysis failed to demonstrate significant differences among nutritional groups. In 1999, the lowest value of that parameter was found in bees of group D1. When supplemented with all lacking amino acids as DL and L isomers (groups D4 and D5), pollen substitute had the nutritional value of its protein increased to equal that of pollen (Kp).

The lowest crude fat content of bee bodies (on dry weight basis) was found in the insects of experiment group D1, 2.23 and 2.53 in the respective study years. The highest crude fat content was found in bees fed pollen substitute supplemented with L-methionine, L-lysine, L-histidine and L-threonine (group D5). In both years the value approximated that obtained in the control group in bees fed pollen from pollen loads (Kp).

DISCUSSION

Supplementing feed protein with indispensable amounts of lacking amino acids is a commonly used approach to the nutrition of higher animals since elevated

protein value allows better production results to be obtained. To date, the above method has not become widespread in the upgrading of protein feeds to be used in the nutrition of the honeybee. The honeybee is known to have high requirement for essential amino acids. Likewise, it is known to prefer pollen that is of higher nutritive value to it and to prefer nectar of higher amino acid content (de Groot 1953, Maurizio 1954, Alm et al. 1990). Szymaś (1994) found that the bees utilize food protein better when, through the handling of its composition, the amino acid content of pollen substitute is balanced to be brought up to the level in natural pollen. The results of this study bear out the above statement as through the supplementation of the amino acid content of pollen substitute formulas with synthetic amino acids and bringing them up to the level in pollen loads nutritional value of the protein in pollen substitute formulas was improved. Szymaś and Maliszewska (1999) improved a pollen substitute formula by adding methionine and lysine – the amino acids that were in short supply relative to those in natural pollen, the reference feed of the study. In that group, increased addition of methionine and lysine to the feed further improved the performance.

In the study chemical tests were employed to determine protein value of the feeds obtained. The tests showed that the chemical composition of experiment feeds was close to that of pollen loads. The essential amino acid index EAAI reached a relatively high value. A similarly high EAAI (from 74.49 to 96.83) was found in feeds used to feed bee colonies by Szymaś (1994), the feeds being qualified as of high nutritive value to the honeybee by the investigator.

The condition of bees is, among others, dependent on fresh and dry matter content of bee bodies. The protein level of the hemolymph is influenced by the nutritional

value of the feed ingested by the insect (Konopacka 1974, Konopacka and Muszyńska 1981, Cremonez et al. 1998). It was found in this study that the protein of pollen substitute, with better balanced amino acid makeup than that of natural pollen, rises the dry matter content of bee bodies. It was indicative of the improved condition of the bees. Total nitrogen and crude fat content is, on the other hand, one of the criteria for the assessment of bee quality (Konopacka et al. 1975). In this study, the better balanced the protein fed to the bees was the more protein and crude fat the bee bodies contained.

The L isomer of the tested amino acids was better utilized than the DL isomer. Protein and fat levels of bee bodies that were fed pollen substitute and the amino acids of which were supplemented with L isomers were comparable to those in bee bodies that were fed natural pollen. The results confirm the observations by de Groot (1953) that the honey bee is a poor utilizer of amino acids as DL isomers.

CONCLUSIONS

Supplementing pollen surrogate with the lacking synthetic amino acids to the level equal to that in natural pollen caused the nutritional value of the protein in the feed approximate that of natural pollen as judged by the similar contents of dry weight, protein and crude fat in bee bodies.

The study also confirmed a better utilization by bees of amino acids fed as L isomers so any amino acid deficits of pollen surrogates should be supplemented with L-amino acids.

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WARTOŚĆ ODŻYWCZA DLA PSZCZOŁY MIODNEJ SUROGATU PYŁKU KWIATOWEGO WZBOGACONEGO AMINOKWASAMI SYNTETYCZNYMI Część I - METODY CHEMICZNE

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S t r e s z c z e n i e

Wierne odtworzenie pyłku kwiatowego, naturalnego pokarmu wszystkich pszczoł nie jest łatwe z uwagi na występowanie w nim bardzo wielu substancji. Podstawowym składnikiem pyłku kwiatowego jest białko, które posiada obok aminokwasów endogennych wszystkie potrzebne dla pszczoły aminokwasy egzogenne. Celem pracy było podniesienie wartości odżywczej białka wytworzonej namiastki pyłku kwiatowego, przydatnej w żywieniu pszczoły miodnej, poprzez optymalizację składu aminokwasowego, przez uzupełnienie brakującymi aminokwasami syntetycznymi w formie izomeru DL i L do standardu, jakim jest białko pyłku kwiatowego. W skład namiastki weszły następujące komponenty: białko ziemniaka 32%, śruta sojowa 18%, śruta rzepakowa 6%, drożdże *Candida utilis* 6%, mąka pszenna 14,8%, grys kukurydziany 17,5%, olej sojowy 3,3%, lecytyna 0,5% polfamix W 1,4%, Vitazol AD3EC 0,2%, glukoza z wit.C 0,1%. Doświadczenie wykonano na pszczole miodnej *Apis mellifera carnica*, w latach: 1998 i 1999. Małe nukleusy zasiedlono jednodniowymi pszczołami, które przez okres dwu tygodni żywiono zróżnicowaną dietą białkową. Uliki przetrzymywano w ciepłarkach w temperaturze 31°C i wilgotności względnej powietrza 40%. Wartość odżywczą białka wytworzonych pasz zastępczych oraz pyłku kwiatowego określono metodami chemicznymi (Szymaś i Przybył 2000). Ocenie poddano białko pasz doświadczalnych oraz ciało pszczoł. Skład chemiczny namiastki pyłku kwiatowego był zbliżony do pyłku kwiatowego z obnóży. Wskaźnik aminokwasów egzogennych w paszy EAAI osiągnął stosunkowo wysoką wartość (odpowiednio dla namiastki A i B, 80.99 i 78.95). W ocenie chemicznej przedstawionych badań własnych stwierdzono, że uzupełnienie surogatu pyłku kwiatowego o brakujące aminokwasy syntetyczne do poziomu jaki jest w pyłku kwiatowym spowodowało podniesienie wartości odżywczej białka wytworzonej paszy do wartości pyłku kwiatowego, uzyskując podobną zawartość suchej masy, białka i tłuszczu surowego w ciałach pszczoł.

Badania potwierdziły również, że pszczoły lepiej wykorzystywały aminokwasy w formie izomeru L niż DL.

Słowa kluczowe: żywienie pszczoły miodnej, namiastka pyłku kwiatowego, wartość odżywcza, białka namiastki, aminokwasy, metody chemiczne.