

IMPROVEMENT OF THE COMPOSITION OF POLLEN SUBSTITUTE FOR HONEY BEE (*Apis mellifera* L.), THROUGH IMPLEMENTATION OF PROBIOTIC PREPARATIONS

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S u m m a r y

The study addressed a laboratory evaluation of the effect of probiotic preparations addition on the nutritional value of a pollen substitute for honey bee. Investigations were carried out on worker bees *Apis mellifera carnica*, at a laboratory of the Department of Functional Insects Breeding, Agricultural University of Poznań, in the years 2002 and 2003.

The substitute was made of the following constituents: potato protein, soybean meal, rapeseed meal, *Candida utilis* yeast, wheat flour, corn grits, soybean oil, lecithin, polfamix W, Vitazol AD3EC, and glucose with vitamin C. Individual constituents of the substitute were subjected to technological treatment: micronization, extrusion, and comminution to particles 150 µm in diameter. Protein content of the substitute mixed with powdered sugar (1:1, w:w) reached 20%. In the experiments, 2 probiotic preparations were applied, that were added to the substitute in three doses immediately before administration to bees, i.e. "Biogen-N" (a biological stimulant of immunity and growth for piglets, calves, foals and kids in their early life, containing 4 strains of the genus *Bifidobacterium bifidum* and *Enterococcus faecium*, *Lactobacillus acidophilus*, *Pediococcus acidilactiti*), and "Trilac" (a preparation restoring functions of gastrointestinal microflora in humans, containing, among others, *Lactobacillus acidophilus*, *L.delbrueckii subsp.bulgaricum* and *Bifidobacterium bifidum*).

Total bacteria count per 1 mg of Biogen-N accounted for 11×10^9 and was similar to bacteria number in 1.267 g of Trilac.

The statistical analysis demonstrated that the addition of probiotics had no significant influence on the increase in feed intake. Only in the first year of the study was the substitute supplemented with Biogen-N in a dose of 2 mg more readily consumed by the bees. The administration of probiotics decreased the number of fatal cases among bees. However, the decrease appeared to be statistically significant already after pollen substitute supplementation with the Trilac preparation in a dose of 1.267 g/100 g of substitute and with the Biogen-N preparation in a dose of 1mg. The addition of probiotic preparations to the substitute was found to stimulate the growth of the faucial gland and fat body.

Keywords: honey bee feeding, pollen substitute, probiotics.

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INTRODUCTION

Improvement of the composition of pollen substitutes applied in honey bee feeding is still necessary, and the substitutes them-

selves are an alternative to endangered and diminishing reserves of a natural source of feed for bees, namely pollen.

Although the nutritional value of sub-

stituents is high, compared to pollen, still they are no match for natural feed. The substituents do not contain specific microflora that provides the production of, among others, so important lactic acid having a positive impact on organisms, and do not enrich the gastrointestinal tract of a bee with microflora which, by colonizing the intestine, serves a key function in the local immunity of the gastrointestinal tract. The species composition of intestinal microflora is, to a great extent, subject to a modifying influence of the biochemical environment of bee midgut. The biochemical environment of the intestine is composed by feed and products of its enzymatic degradation, digestive enzymes, reaction and redox potential of the intestinal digesta, autochthonous and, sometimes, incidental microflora (Gliński and Jarosz 1991, Muszyńska and Leźnicka 1992, Matras et al. 1998).

Gastrointestinal microflora of worker bees is diversified qualitatively and quantitatively. The intestinal microflora of a healthy bee is predominated by gram-negative bacteria from the family *Enterobacteriaceae*, gram-positive bacteria of the genus *Bacillus* and micrococci, e.g. *Streptococcus faecium*, *S. faecalis*, *Micrococcus* sp., (El-Leithy and El-Sibael 1972, Gilliam and Valentine 1976, Gilliam and Morton 1978, Gilliam et al. 1990). The process of destroying bacteria that colonize the gastrointestinal tract contributes of the impairment of symbiosis and competition, which in turn creates favorable conditions for the proliferation of yeasts and moulds. Nevertheless, intestinal digesta of queens and workers are likely to contain (though in low numbers) microorganisms of the genus *Penicillium*, *Aspergillus*, *Alternaria*, *Hansenula*, and additionally in workers bees - *Rhizopus* and *Cladosporium* (Gliński and Chmielewski 1994).

Machova et al. (1997) evaluated potential effects of intestinal microflora on

the survivability of maggot treated for calcareous mycosis by administration of bees (in sugar syrup) with selected bacterial strains (aerobic and anaerobic ones), commercial probiotic preparations "Laco" and "Microbion T" designed for farm animals, and dietetic additives. The assessment covered also the survivability of microorganisms in the gastrointestinal tract of the treated bees. In control bees administered with syrup containing bacteria of the genus *Bacillus subtilis*, the mean life span was unsatisfactory, presumably due to poor survivability of those bacteria in the gastrointestinal tract of the bees. A good coefficient of survivability was reported for bees that were receiving a combination of selected isolates and feed additives. The authors pointed out the necessity of everyday preparation of syrup with a vaccinal agent since such a procedure is prompted by environmental demands of the anaerobic bacteria species applied (a high sensitivity of those bacteria to oxygen). That fact hinders the application of such probiotic preparations whose composition is based exclusively on species and strains of obligate anaerobes (Ewing and Haresign 1989), including e.g. bacteria of the genus *Bifidobacterium* sp.

In addition, the production of B-group vitamins and own enzymes improving feed digestibility by probiotic bacteria is also worthy of notice (Irianto and Austin 2002).

The goal of the study was to evaluate, under laboratory conditions, the effect of probiotic preparations addition on the nutritional value of pollen substitute.

MATERIAL AND METHODS

Investigations were carried out at the Department of Functional Insects Breeding, Agricultural University in Poznań, in the years 2002 and 2003.

Pollen substitute was prepared in at the Research Station of Feed Production Technology and Aquaculture in Muchocin from the following raw materials: potato protein 32%, soybean meal 18%, rapeseed meal 6%, *Candida utilis* yeast 6%, wheat flour 14.8%, corn grits 17.5%, soybean oil 3.5%, lecithin 0.5%, polfamix W 1.4%, Vitazol AD3EC 0.2%, and glucose with vitamin C 0.1%.

In order to eliminate thermolabile antinutrients and improve digestible energy, the soybean and rapeseed meals were subjected to the extrusion process with the thermobaric method. *Candida utilis* yeast, wheat flour and corn grits were mixed and supplemented with 4% of water, then micronized for 40 seconds in a 1000 W power microwave. Next, a premix was prepared with the addition of soybean meal, polfamix W, Vitazol AD3EC and vitamin C with glucose. Thus prepared protein and carbohydrate components were mixed and then comminuted to particles 150 µm in diameter. Once the production process of the substitute has been finished, it was acidified with lactic acid up to pH=4.8, greased with soybean oil, and mixed with powdered sugar (1:1, w/w) to obtain protein content of 20%. Prior to administration to bees, the substitute was moistened with water so as to obtain consistency of a thick dough.

Two probiotic preparations were used in the study that were added to the substitute just before its administration to bees.

– “Biogen-N” (biological stimulant of immunity and growth for piglets, calves, foals and kids in the initial period of life), purchased in the Biotechnology and Genetic Engineering Implementations and Applications Plant BIO-GEN Ltd. (Przedsiębiorstwo Wdrożeń i Zastosowań Biotechnologii i Inżynierii Genetycznej BIO-GEN Sp. z o.o.) in Opole. The preparation contained the following microorganisms: 4 strains of the genus *Bifido-*

bacterium bifidum, and the following genera: *Enterococcus faecium*, *Lactobacillus acidophilus*, *Pediococcus acidilactiti*.

For experimental purposes the preparation's composition was modified, i.e. neither lactose nor micro- or macroelements were added.

The Biogen-N preparation was administered in 3 doses per 100 g of pollen substitute (mixed with powdered sugar at a ratio of 1:1, w/w):

A: 0.5 mg/100 g substitute with protein content of 20%

B: 1.0 mg/100 g substitute with protein content of 20%

C: 2.0 mg/100 g substitute with protein content of 20%

– “Trilac”- containing, among others, 2 species of lactic acid bacteria, including: *Lactobacillus acidophilus*, *L. delbrueckii subsp. Bulgaricus* and *Bifidobacterium bifidum*, that enhance and restore functions of gastrointestinal microflora in humans. (Producer: Pharmacia&Upjohn Allergon AB Ängelholm, Switzerland. Made by: Apiculture and Pharmaceutical Plant APIPOL-Farma Ltd. (Przedsiębiorstwo Pszczelarsko-Farmaceutyczne APIPOL-Farma Sp. z o.o.)). The preparation was administered in 3 doses per 100 g of pollen substitute (mixed with powdered sugar at a ratio of 1:1, w/w).

A: 0.724 g (4 capsules)/100 g substitute with protein content of 20%

B: 1.267 g (7 capsules)/100 g substitute with protein content of 20%

C: 2.534 g (14 capsules)/100 g substitute with protein content of 20%

Total bacteria count per 1 mg of Biogen-N accounted for 11×10^9 was similar to the number of bacteria in 1.267 g of Trilac, (Fuller 1992).

The experiment was carried out on a honey bee *Apis mellifera carnica* L. Worker bees gnawed out from maggot originating from one queen, in an incubator

Table 1.

Scheme of the feeding experiment.

Group	Experiment 1 – Year 2002		Experiment 2 – Year 2003		Number of bees in each group
	Feed	Dose of probiotic per 100 g substitute	Feed	Dose of probiotic per 100 g substitute	
Control D1	Substitute	–	Substitute	–	5 x 150 workers
D2	Substitute+Biogen-N	A-0.5 mg	Substitute+Biogen-N	A-0.5 mg	
D3	Substitute+Biogen-N	B-1.0 mg	Substitute+Biogen-N	B-1.0 mg	
D4	Substitute+Biogen-N	C-2.0 mg	Substitute+Biogen-N	C-2.0 mg	
D5	Substitute+Trilac	A-0.724 g	Substitute+Trilac	A-0.724 g	
D6	Substitute+Trilac	B-1.267 g	Substitute+Trilac	B-1.267 g	
D7	Substitute+Trilac	C-2.534 g	Substitute+Trilac	C-2534 g	

at a temperature of 33°C and relative humidity of 80%. In both years investigated, 7 experimental groups were formed – a control one, in which bees were receiving a pure substitute, and 6 groups including: 3 in which the bees were receiving the substitute enriched with three different doses of Biogen-N and the other 3 in which the bees were administered with the substitute supplemented with different doses of Trilac. In each group, 5 little hives as designed by Szymaś and Wójtowski (1974), were colonized with one-day-old bees that were fed for two weeks the substitute with protein content of 20% and the substitute enriched with probiotics (Table 1). The feed was administered in tray drills and weighed and supplemented each day. Everyday practices included also the removal of dead bees and supplementation of water in drinkers.

The nutritional value of the feed administered was evaluated with the biological method. A measure of the nutritional value were: survivability of insects and morphological picture of faucial glands and fat body according to the classification

method of Maurizio (1954), in 10% of bees selected at random on completion of the experiment. A 4-degree scale was used for the faucial glands and fat body. The maximal development was expressed in the 4th grade, whereas the minimal one – in the 1st grade.

The survivability of bees was determined based on the number of dead bees in consecutive days of the experiment.

The assessment of feed intake was carried out with the gravimetric method. Feed intake in a given day was converted into the number of intravitally remained bees.

The statistical analysis, including an analysis of variance and the LSD Fisher's test (At a significance level of $\alpha=0.05$), was carried out for feed intake, number of dead bees, and for weighted means of faucial glands and fat body development. For discrete variables, transformation was carried out according to Bliss degrees (Bliss 1934).

RESULTS

The statistical analysis of results obtained in the second experimental year as

Table 2.

Mean daily intake of feed in particular feeding groups (in mg/bee) and results of Fisher's LSD test, $\alpha=0.05$.

Group	Feed	Dose of probiotic	Experiment 1	Experiment 2	Two experimental years
D1	Substitute	–	0.006839 b*	0.010750 a	0.008794 a
D2	Substitute+Biogen-N	A	0.009922 ab	0.012669 a	0.011295 a
D3	Substitute+Biogen-N	B	0.009162 ab	0.009550 a	0.009356 a
D4	Substitute+Biogen-N	C	0.011569 a	0.013999 a	0.012784 a
D5	Substitute+Trilac	A	0.006894 ab	0.013008 a	0.009951 a
D6	Substitute+Trilac	B	0.009770 ab	0.008515 a	0.009143 a
D7	Substitute+Trilac	C	0.006987 ab	0.015556 a	0.011272 a

Table 3.

Death rate of bees in particular feeding groups (in %), for the entire experimental period, and results of Fisher's LSD test, $\alpha=0.05$.

Group	Feed	Dose of probiotic	Experiment 1	Experiment 2	Two experimental years
D1	Substitute	–	32.20 a*	28.80 a	30.5 a
D2	Substitute+Biogen-N	A	27.00 ab	27.00 a	27.0 ab
D3	Substitute+Biogen-N	B	29.00 ab	18.40 b	23.7 ab
D4	Substitute+Biogen-N	C	23.60 ab	27.67 a	25.6 ab
D5	Substitute+Trilac	A	21.00 ab	21.00 ab	21.0 ab
D6	Substitute+Trilac	B	11.20 b	21.20 ab	16.2 b
D7	Substitute+Trilac	C	19.67 ab	27.00 a	23.3 ab

* explanations as in Table 2.

well as of results from both years of the study demonstrated that the addition of probiotics had no statistically significant effect on the increase in feed intake (Table 2). Only in the first year of the study were the bees more eager to consume the substitute enriched with Biogen-N in a dose of 2 mg/100 g feed (D4), compared to the "pure" substitute (D1).

The number of fatal cases among bees in particular feeding groups after termination

of the experiment (Table 3) indicates that the addition of probiotic preparation decreased the death rate of bees. However, the decrease appeared to be statistically significant only after administration of substitute with Trilac preparation (1.267 g) in the first year, and Biogen-N preparation in a dose of 1.0 mg in the second year of the study. The synthesis of results from the two experimental years demonstrated that the decrease in death rate of bees was statisti-

Table 4.

Development of faucial glands of worker bees expressed in weighted means of classification degrees, and results of Fisher's LSD test, $\alpha=0.05$.

Group	Feed	Dose of probiotic	Experiment 1	Experiment 2	Two experimental years
D1	Substitute	–	2.87 b*	2.99 c	2.90 d
D2	Substitute+Biogen-N	A	3.11 b	2.93 c	3.00 cd
D3	Substitute+Biogen-N	B	3.04 b	3.32 ab	3.20 cd
D4	Substitute+Biogen-N	C	3.56 a	3.38 a	3.50 a
D5	Substitute+Trilac	A	3.39 a	3.10 abc	3.20 bc
D6	Substitute+Trilac	B	3.47 a	3.43 a	3.50 a
D7	Substitute+Trilac	C	3.46 a	3.33 ab	3.40 ab

* a, b, c – mean values in columns denoted with different letters are significantly different.

Table 5.

Development of fat body of worker bees expressed in weighted means of classification degrees, and results of Fisher's LSD test, $\alpha=0.05$.

Group	Feed	Dose of probiotic	Experiment 1	Experiment 2	Two experimental years
D1	Substitute	–	2.92 c*	2.32 c	2.62 e
D2	Substitute+Biogen-N	A	3.02 c	2.86 ab	2.94 cd
D3	Substitute+Biogen-N	B	3.09 bc	2.54 b	2.82 d
D4	Substitute+Biogen-N	C	3.31 ab	3.18 a	3.24 a
D5	Substitute+Trilac	A	3.01 c	2.86 ab	2.96 bcd
D6	Substitute+Trilac	B	3.11 bc	2.74 b	2.93 cd
D7	Substitute+Trilac	C	3.40 a	2.69 b	3.04 bcd

* a, b, c, d, e – mean values in columns denoted with different letters are significantly different.

cally significantly affected by Trilac in a dose of 1.267 g.

The statistical analysis of result from the first year of the study demonstrated a statistically significant effect of the addition of probiotics to the substitute, especially 2 mg of Biogen-N and Trilac at doses of: 0.724 g, 1.267 g and 2.534 g, per 100 g substitute, on the development of faucial glands. In the second year, both prepara-

tions in the following doses: 1 mg of Biogen-N and 2.534 g of Trilac, stimulated the development of the above-mentioned glands, which appeared to be significantly statistically better as compared to the bees fed the "pure" substitute. The synthesis of two-year results indicated the least development of the faucial glands in bees receiving the substitute supplemented with probiotic preparations, (Table 4).

Probiotics added to the substitute were also found to stimulate the growth of fat body, (Table 5). The highest degree of its development was gained in individuals receiving the substitute supplemented with 2.534 g of the Trilac preparation (experiment 1) and the substitute with 2 mg of Biogen-N (experiment 2). In bees which received the "pure" substitute the fat body was developed to the least extent and probiotics addition had a statistically significant effect on its better development.

DISCUSSION

Undisputed and positive properties of probiotic preparations, corroborated in humans and animals, have prompted an attempt of using them as an additive increasing the nutritional value of protein of pollen substitutes for honey bee and, simultaneously, contributing to the creation of stable enough bacterial environment of bee intestine, which was emphasized by Kaznowski et al. (2005).

The pollen substitute presented in this study was prepared on the basis of multiple feed components, hence it could be better balanced in terms of amino acid concentration. Precise amino acid and chemical composition as well as the chemical evaluation of feed have been described in details in a paper by Rogala and Szymaś (2004a). The authors have claimed that the chemical composition was similar to that of pollen from propolis, and the essential amino acid index (EAAI) reached a relatively high value.

The probiotic preparations applied in the study failed to significantly contribute to the increase in feed intake, but affected the decrease in death rate of bees, especially the Trilac preparation administered in a dose B (7 capsules per 100 g substitute). Alike results were reported by Kaznowski et al. (2005), who proved that single supplementation of feed for honey bee with

probiotics, in a dose of 1mg of Biogen and 7 capsules of Trilac, had a positive impact on the nutritional value of feed, a decrease in death rate, and colonization of bacteria in the midgut.

The addition of probiotic preparations, especially Biogen-N (in the highest dose) and Trilac (in all 3 doses), influenced significantly the development of faucial glands and in all the three doses – that of fat body. Slightly worse results, compared to those presented here, were obtained by Łangowska et al. (2003) upon the application of a "Lakcid" preparation containing lactic acid bacteria. Rogala and Szymaś (2004b), having supplemented the level of amino acids in the substitute with synthetic amino acids to values reported from pollen, obtained similar values of degrees of faucial glands and fat body development to those noted after the administration of probiotics presented in the reported study.

Based on the results obtained in this study it can be concluded that the controlled administration of probiotic preparations, containing specified and properly-selected species and strains of lactic acid-producing bacteria, in the pollen substituent affects better feed protein utilization by the body of bee.

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DOSKONALENIE SKŁADU SUROGATU PYŁKU KWIATOWEGO DLA PSZCZOŁY MIODNEJ (*Apis mellifera* L.), POPRZEZ WPROWADZENIE PREPARATÓW PROBIOTYCZNYCH

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

Namiastki nie zawierają specyficznej mikroflory, zapewniającej produkcję m. in. tak istotnego kwasu mlekowego, działającego pozytywnie na organizm oraz nie wzbogacają przewodu pokarmowego pszczoły w mikroflorę, która zasiedlając jelito owada odgrywa kluczową rolę w miejscowej odporności przewodu pokarmowego. Poza tym bakterie probiotyczne produkują witaminy z grupy B oraz enzymy poprawiające strawność pasz.

Celem badań była laboratoryjna ocena wartości odżywczej namiastki pyłku kwiatowego wzbogaconej preparatami probiotycznymi Biogen-N i Trilac, wyrażonej przeżywalnością doś-

wiadczalnych pszczoł oraz rozwojem gruczołów gardzielowych i ciała tłuszczowego robotnic. Dla ciała tłuszczowego i gruczołów gardzielowych zastosowano 4 stopniową skalę bonitacyjną. Maksymalny rozwój wyrażono w stopniu 4, a minimalny w 1. Przeżywalność pszczoł określano liczbą martwych osobników w kolejnych dniach doświadczenia, a wielkości spożycia paszy – metodą wagową. Spożycie paszy w danym dniu przeliczano na liczbę pszczoł pozostałych przyżyciowo.

W skład namiastki wchodziły następujące surowce: białko ziemniaka, śruta sojowa, śruta rzepakowa, drożdże *Candida utilis*, mąka pszenna, grys kukurydziany, olej sojowy, lecytyna, polfamix W, Vitazol AD3EC oraz glukoza z witaminą C. Poszczególne składniki namiastki poddano procesom technologicznym: mikronizacji, ekstruzji i rozdrobnieniu, do cząsteczek o średnicy 150 µm. Poziom białka w namiastce, po wymieszaniu jej z cukrem pudrem w stosunku wagowym 1:1, wynosił 20%. W badaniach wykorzystano 2 preparaty probiotyczne, które dodawano do namiastki w trzech dawkach, zaraz przed podaniem pszczołom, tj. Biogenu-N (biologiczny stymulator odporności i wzrostu dla prosiąt, cieląt, źrebiąt i koźląt w początkowym okresie ich życia, zawierający 4 szczepy gatunku *Bifidobacterium bifidum* oraz *Enterococcus faecium*, *Lactobacillus acidophilus*, *Pediococcus acidilactiti*) i Trilac (preparat, który wzmacnia przywraca funkcje mikroflory przewodu pokarmowego u ludzi, zawierający m.in. *Lactobacillus acidophilus*, *L. delbrueckii subsp. bulgaricum* i *Bifidobacterium bifidum*).

Ogólna liczba bakterii w 1 mg Biogenu-N wynosiła 11×10^9 i była zbliżona do zawartości bakterii w 1,267 g Trilacu.

Doświadczenie przeprowadzono na pszczołach miodnej *Apis mellifera carnica* L. Pszczoły robotnice wygryzały się z czerwiu pochodzącego od jednej matki, w cieplarni w temperaturze 33°C i wilgotności względnej 80%. W obu latach badań utworzono 7 grup doświadczalnych – jedną kontrolną, w której pszczoły otrzymywały czystą namiastkę i po 6 grup, z których trzy otrzymywały namiastkę wzbogaconą trzema różnymi dawkami Biogenu-N i trzy, w których pszczoły otrzymywały namiastkę z różnym dodatkiem Trilacu.

Dodatek probiotyków nie wpłynął statystycznie istotnie na wzrost spożycia paszy. Jedynie w pierwszym roku badań, chętniej spożywały pszczoły namiastkę wzbogaconą Biogenem w ilości 2 mg/100 g paszy od „czystej” namiastki.

Synteza wyników z dwóch lat pokazała, iż podanie do diety pszczoł obniża istotnie ich śmiertelność tylko w wypadku podania Trilacu w dawce 1,267 g/100 g paszy.

Na rozwój gruczołów gardzielowych i ciała tłuszczowego pszczoł z poszczególnych grup żywieniowych, po zakończeniu doświadczenia, wyrażony w średnich ważonych stopni bonitacyjnych, wywarły stymulujący wpływ oba preparaty probiotyczne. Gruczoły gardzielowe szczególnie dobrze rozwinięte były u pszczoł spożywających namiastkę zawierającą 2 mg Biogenu-N lub wszystkie trzy dawki Trilacu.

Najwyższy stopień rozwoju ciała tłuszczowego osiągnięto u osobników otrzymujących namiastkę z dodatkiem 2,534 g preparatu Trilac i namiastkę z Biogenem-N w dawce 2 mg. Pszczoły otrzymujące samą namiastkę, miały najslabiej rozwinięte badane narządy. Na podstawie przeprowadzonych badań stwierdzono, że podanie preparatów probiotycznych do namiastki wpływa na lepsze wykorzystanie białka paszy przez organizm pszczeli.

Słowa kluczowe: żywienie pszczoły miodnej, namiastka pyłku, probiotyki.