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MIDGUT HISTOLOGICAL PICTURE OF THE HONEY BEE (*APIS MELLIFERA* L.) FOLLOWING CONSUMPTION OF SUBSTITUTE FEEDS SUPPLEMENTED WITH FEED ADDITIVES

Summary. The histological picture of the honey bee ventriculus undergoes changes as a result of action of such factors as, among others: toxic substances, incorrect nutrition, bacterial infections or age of the insect. The objective of this research project was to assess morphological changes in the midgut of the honey bee with a special emphasis on the degree of the peritrophic membrane production following the consumption of sugar syrup or pollen substitute to which either lactic acid or probiotic preparation were added. The performed investigations failed to reveal significant levels of bee deaths and the recorded high percentage proportion of live insects indicates their good physical condition. The histological analysis of the morphological changes of the midgut epithelial cells of bees fed substitute feeds confirmed slight changes in the cell structures. Differences were recorded in the quantities of the developed peritrophic membranes. The microscopic pictures of midguts of worker bees fed carbohydrate or protein pollen substitute revealed that the highest numbers of peritrophic membranes could be found when the sugar syrup or pollen substitute was supplemented with the probiotic preparation.

Key words: honey bee, midgut, histological picture, perytrophic membranes, feeding

Introduction

The histological picture of the honey bee ventriculus undergoes changes in the result of action of such factors as, among others: toxic substances (BIELENIN and IBEK 1980), incorrect nutrition (SZYMAŚ 1976, 1994), bacterial infections (GREGORC and BOWEN 2000) or age of the insect (JIMENEZ and GILLIAM 1990). The production of excessive quantities of peritrophic membranes by the ventricular epithelium of the honey bee may be associated either with the flaking off of the epithelium and diarrhoea, a mechanism

isolating the epithelium from the noxious substance or with the length of intake and better utilization of nutrients (BIELENIN and IBEK 1980, CRAILSHEIM 1988).

The objective of this research project was to assess morphological changes in the midgut of the honey bee with a special emphasis on the degree of the peritrophic membrane production following the consumption of sugar syrup or pollen substitute to which either lactic acid or probiotic preparation were added.

Material and methods

One-day old bees were obtained by placing frames with breaking brood deriving from one mother in an incubator at the temperature of 33°C and 80% air humidity. Each of the 30 small hives designed by SZYMAŚ and WÓJTOWSKI (1974) was settled with 100 one-day old worker bees which, for 11 days, were kept in an incubator at the temperature of 31°C and 40% air humidity. One of the feeds listed below was fed to bees from five small hives:

- sugar syrup at 50% sugar concentration (sugar to water ratio 1:1),
- sugar syrup at 50% sugar concentration acidified with lactic acid to pH = 4.1,
- sugar syrup at 50% sugar concentration + 'Trilac' (Pharmacia & Upjohn Allergon AB, Sweden) probiotic preparation; diet composition – 0.905 g of the preparation per 100 g of the syrup,
- pollen substitute (crude protein content – 10%),
- pollen substitute (crude protein content – 10%) acidified with lactic acid to pH = 4.1,
- pollen substitute (crude protein content – 10%) + 'Trilac' probiotic preparation; diet composition – 0.905 g of the preparation per 100 g of the pollen substitute.

Deaths of bees were recorded during the entire period of the experiment.

After the termination of the feeding experiment, histological investigations were carried out. For this purpose, bees were anaesthetized and the midgut was removed from two bees randomly selected from each small hive which were then fixed in 4% formalin. The total of 60 midguts was obtained for histological examinations. Next the tissue material was dehydrated in ethyl alcohol and embedded in paraffin. Paraffin slices 4-5 µm thick were stained with hematoxylin and eosine (BAGIŃSKI 1969).

The experimental pollen substitute was manufactured in Muchocin in the Production Experimental Plant of Feed and Aquaculture Technology according to recommendations suggested by SZYMAŚ (1994). The following raw materials were used to prepare the substitute: potato proteins – 32%, soybean meal – 18%, rapeseed meal – 6%, *Candida utilis* yeasts – 6%, wheat flour – 14.8%, maize 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%. The pollen substitute was comminuted to particles of ø 150 µm using for this purpose an impact grinder.

The applied wheat flour and maize grits, after the addition of 4% water, were subjected to micronization lasting 40 seconds in a microwave of 1000 W power. Prior to feeding it to bees, the pollen substitute was mixed with icing sugar so that the protein level, reached 10%.

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Results

Figure 1 shows the percentage proportion of live bees after the termination of the experiment.

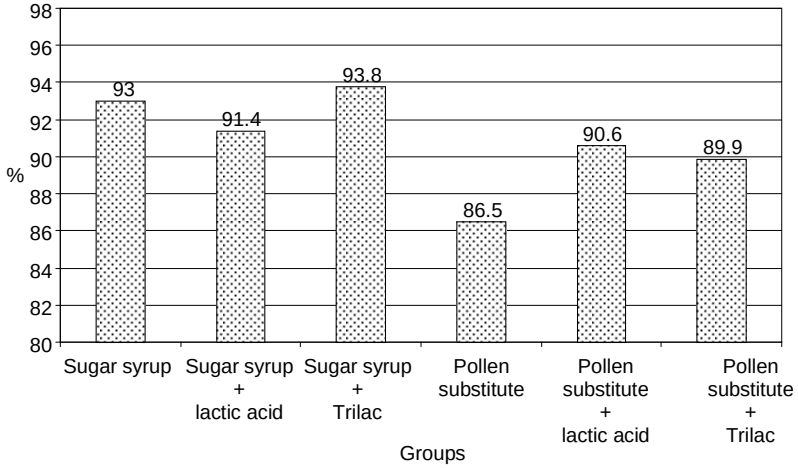

Fig. 1. Percentage proportion of live bees after the termination of the experiment
Rys. 1. Odsetek pszczół pozostałych przyżyciowo po zakończeniu doświadczenia

It was found that after 11 days of feeding, the number of live bees was higher in the experimental group which was fed the sugar syrup than in the one which was fed the pollen substitute.

In groups which were fed the same type of feed, i.e. feed based on either sugar syrup or pollen substitute, the number of bees after 11 days was similar. The highest number of deaths was recorded in the group of bees which were fed the pollen substitute without feed additives as the number of live bees after 11 days of feeding was the lowest. No diarrhoea was observed in worker bees in any of the feeding groups.

Figures 2 to 7 present the histological pictures of the midgut of bees fed different carbohydrate and protein substitute diets. The performed assessment of the morphological changes of the midgut epithelium of worker bees fed sugar syrup revealed distinct hyperchromatic cell nuclei as well as marked rabdorium and the absence of peritrophic membranes (Fig. 2). The number of peritrophic membranes increased considerably when the sugar syrup was supplemented with lactic acid (Fig. 3) and the experimental probiotic preparation (Fig. 4).

The microscopic picture of the midgut epithelium of worker bees fed the pollen substitute showed preserved rabdorium as well as distinct peritrophic membranes, cytoplasm vacuolized at the basement membrane (Fig. 5). The number of peritrophic membranes in the inside diameter of the midgut increased considerably after the addition to the pollen substitute of lactic acid (Fig. 6) and probiotic preparation (Fig. 7).

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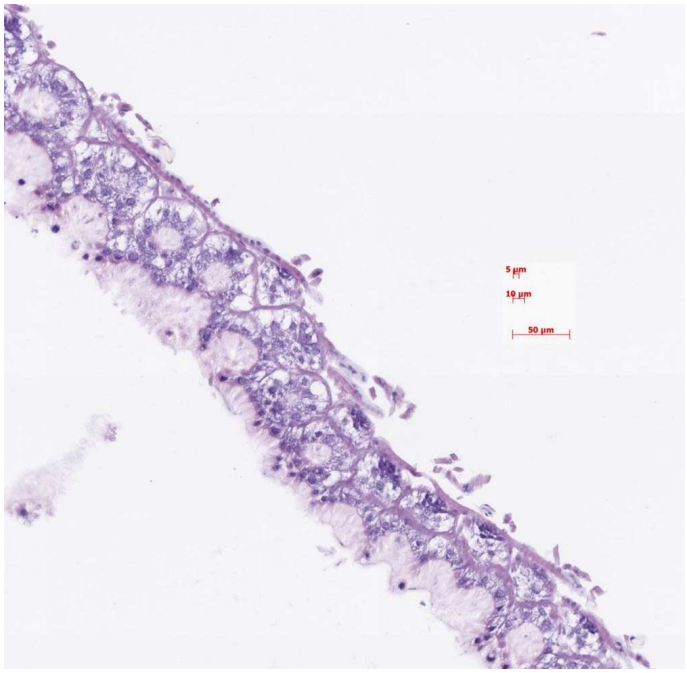

Fig. 2. Microscopic picture of midgut of a bee fed on sugar syrup. In some places, cell nuclei are hyperchromatic. Rabdorium distinct. Not numerous peritrophic membranes. H&E stain
Rys. 2. Obraz mikroskopowy pszczoły żywionej syropem cukrowym. Jądra komórkowe miejscami hyperchromatyczne. Rabdorium wydatne. Nieliczne błony perytroficzne. Barwienie H&E

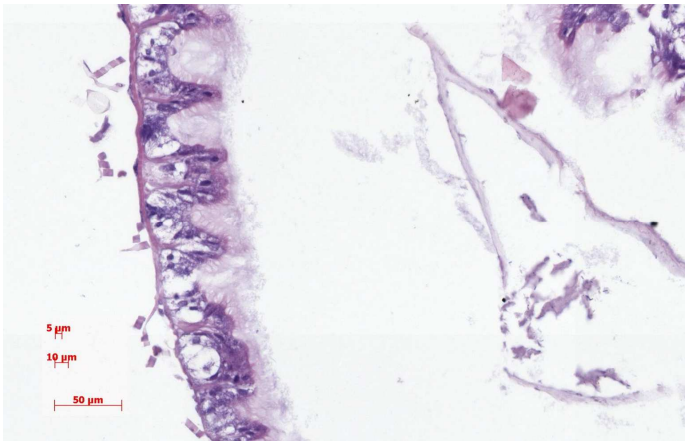

Fig. 3. Epithelium of the midgut of a bee fed on sugar syrup with lactic acid. Cell cytoplasm is granular, slightly vacuolized. Cell nuclei and rabdorium are distinctly preserved. H&E stain
Rys. 3. Nabłonek żołądka właściwego pszczoły żywionej syropem cukrowym z kwasem mlekowym. Cytoplazma komórek ziarnista, nieco zwakuolizowana. Jądra komórkowe oraz rabdorium są zachowane. Barwienie H&E

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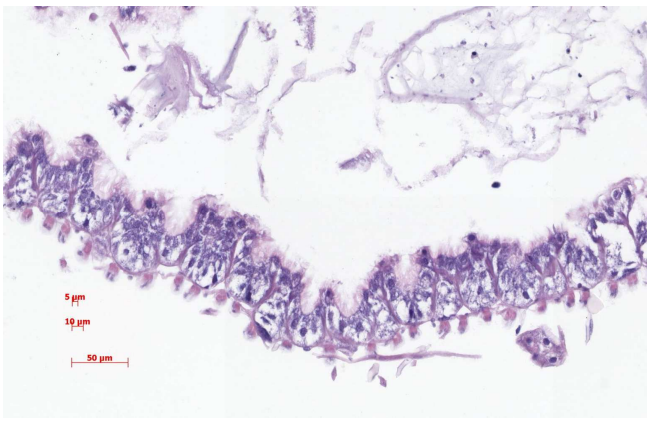

Fig. 4. Microscopic picture of the ventriculus of a bee fed on sugar syrup with probiotic "Trilac". In some places, cytoplasm vacuolization is visible. Visible reproduction centers and the separation of peritrophic membranes. H&E stain

Rys. 4. Obraz mikroskopowy jelita środkowego pszczoły żywionej syropem cukrowym z preparatem probiotycznym „Trilac". Miejscami cytoplazma wyraźnie zwakuolizowana. Widoczne centra namnażania i odzielanie się błon perytroficznych. Barwienie H&E

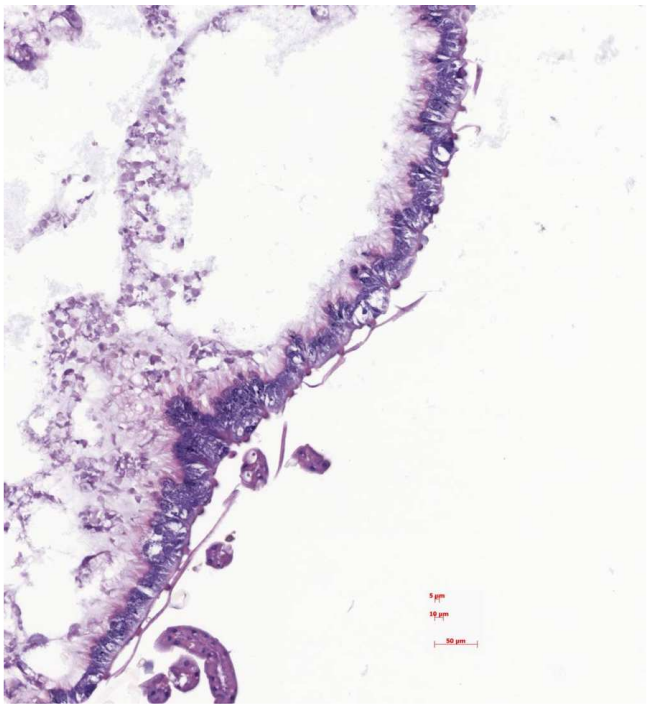

Fig. 5. Cross section of ventriculus of a bee fed on pollen substitute. Strong merocrine type secretion. H&S stain

Rys. 5. Przekrój poprzeczny przez jelito środkowe pszczoły żywionej namiastką pyłku. Silna sekrecja typu merokrynowego. Barwienie H&E

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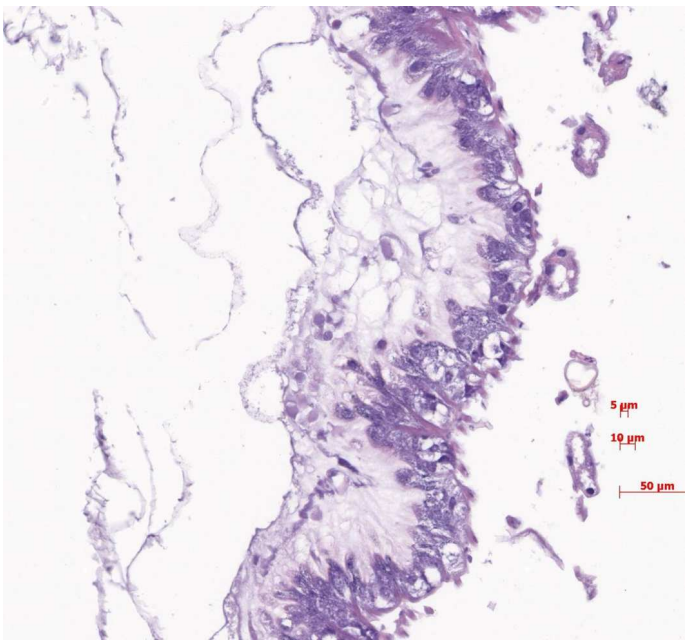

Fig. 6. Microscopic picture of the ventriculus of a bee fed on pollen substitute with lactic acid. Granular cytoplasm of cells. Numerous peritrophic membranes. H&E stain
Rys. 6. Obraz mikroskopowy jelita środkowego pszczoły żywionej namiastką pyłku z kwasem mlekowym. Cytoplazma komórek ziarnista. Liczne błony perytroficzne. Barwienie H&E

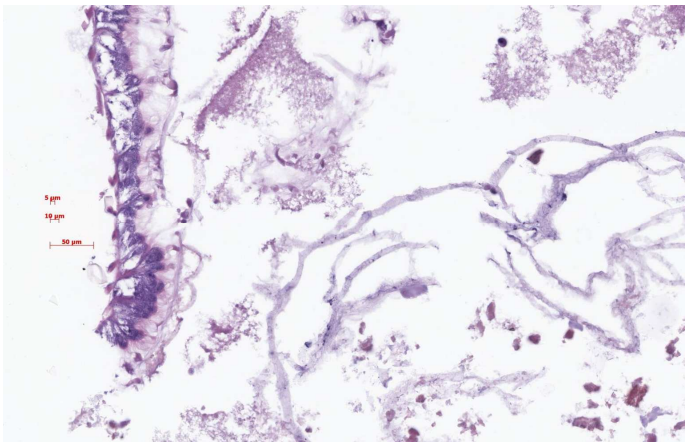

Fig. 7. Microscopic picture of bee midgut fed on pollen substitute with probiotic preparation "Trilac". Very numerous peritrophic membranes. H&E stain
Rys. 7. Obraz mikroskopowy jelita środkowego pszczoły żywionej namiastką pyłku z preparatem probiotycznym „Trilac". Dużo błon perytroficznych. Barwienie H&E

Discussion

The performed investigations failed to reveal significant levels of bee deaths and the recorded high percentage proportion of live insects indicates their good physical condition. The complete elimination of the lethal factor in this type of investigations is complicated due to difficulties in carrying out cleaning flights by worker bees. SZYMAŚ (1994) maintains that a lower percentage of deaths of bees is recorded when they are kept in laboratory conditions and fed carbohydrate feed than when protein feed is provided.

KAZNOWSKI et AL. (2005) reported that when the pollen substitute containing 20% protein was supplemented with a probiotic preparation, it exerted a positive impact on the death rate reduction. The results of these investigations did not corroborate the findings of the above-mentioned researchers but this may have been connected with the lower protein content (10%) in the applied pollen substitute (HERBERT et AL. 1977).

The investigations performed in this study failed to record significant changes or complete degeneration of epithelial structures of the ventriculus. Cell proliferation centres, cell nuclei and radborium were all preserved. The most extensive changes were found in the pictures of nuclei or cytoplasm which were vacuolized, most frequently at the basement membrane. Cytoplasm was not found to be vacualized completely, which was the case in the experiment conducted by BIELENIN and IBEK (1980), when the insects were fed strong poisons. In this study, the histological picture of the midgut exhibited the presence of large quantities of the peritrophic membrane when the applied feed was supplemented with the probiotic preparation. According to CRAILSHEIM (1988), their plentiful presence is associated with the length the feed stays in the digestive tract; when it remains there longer, its nutrients may be utilized better.

In the majority of bees fed the pollen substitute, strong merocrine secretion was observed in the epithelial cells of the ventriculus. In the merocrine type of secretion, there is no cell degradation; cells can secrete enzymes in the form of small bubbles through the radborium layer. This type of secretion occurs most frequently in insects and was confirmed in this study.

Conclusions

1. The histological analysis of the morphological changes of the midgut epithelial cells of bees fed substitute feeds confirmed slight changes in the cell structures. Differences were recorded in the quantities of the developed peritrophic membranes.

2. The microscopic pictures of midguts of worker bees fed carbohydrate or protein pollen substitute revealed that the highest numbers of peritrophic membranes could be found when the sugar syrup or pollen substitute was supplemented with the probiotic preparation.

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OBRAZ HISTOLOGICZNY JELITA ŚRODKOWEGO PSZCZOŁY MIODNEJ (*APIS MELLIFERA* L.) PO SPOŻYCIU ZASTĘPCZYCH POKARMÓW WZBOGACONYCH DODATKAMI PASZOWYMI

Streszczenie. Obraz histologiczny żołądka właściwego pszczoły miodnej zmienia się w wyniku działania między innymi takich czynników, jak: substancje toksyczne, niewłaściwy pokarm, infekcje bakteryjne, wiek owada. Celem pracy była ocena zmian morfologicznych w nabłonku jelita środkowego robotnicy pszczoły miodnej, ze szczególnym uwzględnieniem stopnia wytworzenia błon perytroficznych, po spożyciu syropu cukrowego lub namiastki pyłku kwiatowego, do których dodano kwas mlekowy lub preparat probiotyczny. W przedstawionych badaniach nie wykazano znacznych upadków pszczół, duży odsetek owadów pozostałych przyżyciowo świadczy o dobrej ich kondycji. Histologiczna analiza zmian morfologicznych komórek nabłonka jelita środkowego pszczół żywionych zastępczymi pokarmami potwierdziła nieznaczne zmiany w strukturach komórkowych. Różnice odnotowano w ilości powstałych błon perytroficznych. W obrazach mikroskopowych żołądków właściwych pszczół robotnic, otrzymujących zastępczy pokarm węglowodanowy lub białkowy, najwięcej błon perytroficznych stwierdzono, gdy syrop cukrowy lub namiastkę pyłku kwiatowego wzbogacono preparatem probiotycznym.

Słowa kluczowe: pszczoła miodna, jelito środkowe, obraz histologiczny, błony perytroficzne, żywienie

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