The Effect of Pollen on the Structure of the Small Intestine in Rats after an Experimental Addition in Diet

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Abstract
In this study, the effects of pollen addition in diet on the small intestine structure in rats were investigated. The microscopic changes in the small intestine after administration of the pollen addition were evaluated. The experimental animals were divided into two experimental and one control group of ten rats (5 males and 5 females). Experimental group A was given the addition of pollen in concentration of 0.2% and group B was fed diet containing 0.5% of pollen for 90 days. Using quantitative morphometrical methods, we have found statistically significant increase in the relative volume of epithelial tissue (P<0.0001) and decrease in the mucosa tissue volume (P<0.0001) of the small intestine in experimental group B as compared to control. The results of our work show that the addition of pollen in diet had demonstrable concentration-dependent effects on the mucosa of small intestine and could have a positive impact on improving the absorptive mucosal surface and therefore could affect the usability for the received nutrients in food.

Keywords: nutrition, pollen, rats, small intestine

1. Introduction
Pollen load is a fine powder-like material produced by flowering plants pollen, mixed with bee secretions [1]. A honey bee moistens the forelegs with a protruding tongue and brushes the pollen that has collected on head, body and forward appendages to the hind legs. The pollen is transferred to the pollen comb on the hind legs and then combed, pressed, compacted, and transferred to the corbicula on the outside surface of the tibia of the hind legs [2]. Pollen is bee’s primary food source, containing concentrations of phytochemicals and nutrients and rich in secondary metabolites. It is known that it contains lipids, sugars, proteins, amino acids, vitamins, carotenoids, polyphenolics such as flavonoids and carbohydrates [3]. The phenolic composition of pollen principally consists of flavonol glycosides and other phenolic components [4]. Primarily rutin, quercetin, myricetin and trans-cinnamic acid [5-7]. The flavonoid/phenolic components play a significant role in the free radical scavenging capacity of a floral or bee pollen [8]. Pollens which exhibit the highest scavenging capacity and antioxidant activity tend to be those that contain the highest levels of flavonoids and phenolic acid derivatives [9]. This composition tends to be species-specific and has been related to the therapeutic properties (antibiotic, antineoplastic, antidiarrhoeic and antioxidant) of pollen [10]. The optimal amount of pollen load is used for direct consumption, for the preparation of

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functional foods, to feed livestock, as a raw material for the pharmacological industry and the subject of research, discovery and isolation of new, previously unknown biologically active components [11, 12].

The small intestine is classified as a vitally important organ of the human body. Its primary functions include digestion of food and absorption of nutrients. It is characterized by intense metabolism and contributes to the optimal activity of the immune system [13]. There are very limited information about how the pollen load could influence the structure, digestion and absorption of substances from the small intestine. The object of our study was to extend these findings and to find the structural changes in the small intestine of rats after a peroral intake of bee pollen in food.

2. Materials and methods

In the experiment, Wistar rat line was used. Experiment was carried out in experimental facility of the Department of Veterinary Sciences of Slovak University of Agriculture in Nitra (SK 50004 PC). The animals were housed individually in plastic containers (TECNIPLAST, Italy) on bedding of wood shavings. Animals were housed under basic requirements for living conditions (temperature 20-22°C, humidity 55±10%, 12 h light regime). Animals were fed with water and complete feed mixture for laboratory mice and rats M3 (Machal, Czech Republic) ad libitum.

At the age of 4 weeks the young rats were divided into 3 groups (control, A, B). Each group consisted of 5 males and 5 females. The control group was fed with feed mixture without pollen additive. Experimental group A was fed wit pollen in concentration of 0.2% and group B was fed with addition of pollen in concentration of 0.5%. Feeding continued during 90 days.

After 90 days of the experiment, the animals were humanely killed in accordance to Government regulation no. 23/2009 coll. The animals were weighed and the samples of the small intestine (jejenum) for histological processing were taken immediately after sacrifice. Histological preparations of the small intestine was assessed by light microscope (Olympus AX 70 Provis, Japan). The structure of the small intestine wall, especially the mucosa, forming of villi and the different types of epithelial cells were evaluated. The structure and any visible changes in the tissue of the small intestine were described. The changes in the small intestine were also evaluated using the quantitative morphometrical methods [14]. The pictures were taken using the digital camera (Olympus C5050-Z) and light microscope (Nikon Eclipse E600). Ten different visual fields (approximately from 2 or 3 samples) from each experimental rat, together 300 microphotographs of the small intestine were recorded. The quantitative analysis was realized using the test grid containing 494 test points and the relative volume of the epithelium and soft tissue in small intestine were evaluated. Morphometric measurements were based on computerized techniques with PC morphometric software M.I.S. Quick Photo and using light microscope Olympus AX 70 Provis (Japan). The basic statistical indicators, the simple arithmetic mean, standard deviation, coefficient of variation, minimum and maximum, simple, linear, correlation analysis were calculated using Pearson's correlation coefficient and one-way analysis of variance to determine the statistical significance of differences between groups. Statistical analysis of the results was performed by the statistical program - SAS Enterprise Guide 9.1 (USA).

3. Results and discussion

After 90 days of feeding the pollen in concentration of 0.2%, lengthening of villi in the evaluation of microscopic preparations of jejenum (Figure 2) compared to the control (Figure 1) was observed. Similarly, after administration of 0.5% of pollen concentration in the feed, higher villi and densier arrangement of these villi per unit area were noted (Figure 3).

Using a quantitative morphometric methods we found that the percentage of epithelial volume in jejenum in the group A compared to the control was slightly increased by 1.78% and also a slight decline in tissue volume, identically by 1.78% was counted. These differences were statistically insignificant.
In group B, we found statistically significant (P<0.0001) increase in the epithelium volume by 4.22% and statistically significant (P<0.0001) decrease in the tissue just the same 4.22% when compared to the control. The differences are shown in Table 1 and Table 2.

The above results show that the pollen in the diet given orally to experimental animals, during 90 days, at a concentration of 0.2% caused a slight, but inconclusive increase of epithelial mucosa of the small intestine. The pollen concentration of 0.5% significantly (P<0.0001) increased the percentage of epithelium volume and decreased the percentage of tissue volume.

In a similar study, the effects of bee pollen on the development of digestive organs were evaluated in broiler chickens. The control group was fed with a basic diet, while the pollen group was fed with a basic diet supplemented with 1.5% bee pollen over a period of 6 weeks. The results demonstrated that compared to the control group, the small intestine villi from the duodenum, jejunum, and ileum were longer and thicker in the pollen group. These findings suggest that bee pollen could promote the early development of the

<table>
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<th>Epithelium</th>
<th>X [%]</th>
<th>SD</th>
<th>minimum</th>
<th>maximum</th>
<th>CV</th>
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<tr>
<td>Group B</td>
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<td>44.49</td>
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**** P<0.0001, X-arithmetic mean, SD-standard deviation, CV-coefficient of variation

<table>
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<tr>
<th>Tissue</th>
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<td>18.03</td>
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<td>6.22</td>
<td>16.67</td>
<td>55.52</td>
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development of digestive organs were evaluated in broiler chickens. The control group was fed with a basic diet, while the pollen group was fed with a basic diet supplemented with 1.5% bee pollen over a period of 6 weeks. The results demonstrated that compared to the control group, the small intestine villi from the duodenum, jejunum, and ileum were longer and thicker in the pollen group. These findings suggest that bee pollen could promote the early development of the digestive system and therefore is potentially beneficial food supplement for certain conditions, such as short bowel syndrome [15].

Supplement of bee pollen and polysaccharides in calves’ diet could improve the growth performance of calves. Bee pollen additive in 25 g·d⁻¹ and polysaccharides in 5 g·d⁻¹ in milk replacer could get better performance and higher apparent digestibility in calves [16].

Supplementation of bee pollen-based product-Dynamic Trio 50/50 increased the feed intake and thus nutrient retention of arabian horses and that may have a positive effect on their performance [17].

In weanling piglets the addition of dietary fibre differing in lignin content from Pinus massoniana pollen reduced apparent (faecal) digestibility of dry matter and crude protein [18].

Pollen show different types of walls. In humans after 24 h of treatment, only 26% of carbohydrates and 48% of proteins were digested in Papaver rhoeas and only 3% and 59% in Corylus avellana pollen grains. This is probably due to the difficulty of enzymes to penetrate the intine of pollen grains [19]. For example glossophagine bats Glossophaga longirostris, Leptonycteris curasoae digested 64.2% and 71.3% of all the pollens fed, respectively [20].

In another study the effect of bee pollen on growing rabbit’s performance was studied on 40 New Zealand White rabbits from 4 to 12 week of age. Bee pollen at 200 mg significantly (P<0.01) increased body weight, conception rate, milk yield, litter size; improved biochemical profiles of blood and helps outstanding during both seasons. The same dose of bee pollen significantly increased growth and their survival rate until weaning [22].

Other results indicate that bee pollen possess a noticeable source of compounds with health protective potential and antioxidant activity. Schisandra chinensis pollen extract has strong antioxidant activities and significant protective effect against acute hepatotoxicity induced by carbon tetrachloride CCl₄, and has been supported by the evaluation of liver histopathology in mice. The hepatoprotective effect may be related to its free radical scavenging effect, increasing antioxidant activity and inhibiting lipid peroxidation [23].

Oxidant and antioxidant status, estrogenic and antiestrogenic activity and gene expression profile were studied in mice fed with Cystus incanus L. (Cistaceae) reach bee pollen. Bee pollen as a food supplement (100 mg.kg⁻¹ bw mixed with commercial food pellets) compared to control (commercial food pellets) modulated antioxidant enzymes in the mice liver, brain and lysate of erythrocytes and reduced hepatic lipid peroxidation [24].

Positive, protective and restorative effects of pollen addition in feed have been reported in other studies. The effect of the addition of pollen and protein in rat’s diet artificially induced liver cirrhosis, overall malnutrition, and pathological changes in liver. The results showed that in cirrhotic rats treated with the addition of pollen proteins improve the state of malnutrition, increased levels of serum albumin and recorded regeneration of liver tissue, compared to the untreated group of cirrhotic rats [25].

The estrogenic and antiestrogenic activity and the genotoxicity and antigenotoxicity of bee pollen from Salix alba L. and Cystus incanus L. and its derivative extracts in yeast And human cells was investigated. Bee pollens were found to be neither genotoxic nor estrogenic as well as effective estrogen inhibitors, and able to reduce the chromosome damage induced by the three cancer drugs used, thus supporting their use as a chemoprotective agent [25].

4. Conclusions

After administration of 0.2% pollen concentration, lengthening of villi appeared in the small intestine of rats. Similarly, after administration of a 0.5% pollen concentration in the feed, higher villi and their denser arrangement per unit area were observed in jejunum.

Using morphometric techniques, we found that oral doses of pollen in the diet during 90 days at a concentration of 0.2% caused a slight but
inconclusive increase in epithelial layer of the small intestine and in a concentration of 0.5% significantly (P <0.0001) increased the epithelium volume and decreased the soft tissue volume. The addition of pollen in the diet has proven effects on the mucosa of the small intestine in a concentration-dependent manner and could have a positive impact on the absorption and mucosal surfaces for better utilization of nutrient from the food.

Acknowledgements

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References

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