Changes in the Intestinal Mucosa Structure of Rats Caused by Pollen Administration in Diet

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Abstract
The aim of this work was to evaluate the microscopic changes in the small intestine of rats after administration of the pollen addition in diet. Experimental groups A, B and C (10 rats each) were given the addition of pollen in concentration of 0.2%, 0.5% and 0.75%, respectively for 90 days in food. Using the quantitative morphometrical methods, statistically significant increase of intestinal villi length (p<0.0001) and Lieberkühn crypts depth (p<0.0001) in jejunum in the experimental groups A and B were found when compared to control without the pollen addition. In experimental group C we have found only statistically significant increase of intestinal villi length (p<0.0001) but statistically significant decrease of Lieberkühn crypts depth (p<0.0001) compared to control group. The results of our work show that the addition of pollen in the diet affects the structure of the small intestine mucosa of rats. The increased length of intestinal villi could modify the absorption rate in the digestive system.

Keywords: jejunum, Lieberkühn crypts, pollen, rat, villi

1. Introduction
Pollens are one of the most important components of bee colonies, they are a source of food for bees, containing a complex of quality nutrients. It is rich in vitamins, minerals, trace elements, enzymes and amino acids [2-4]. The main biological components of bee pollen are the phenolic acid derivatives and polyphenolic compounds, mostly flavonoid glycosides. Wide range of flavonoid types has been determined from pollen samples of different geographic origin among, mainly flavonols [5], flavones, dihydroflavonols, benzoic acid derivatives and hydroxycinnamic acids [6]. Pollen flavonoids quercetin, rutin and chrysin have been shown to have a chemopreventive activity [7]. Pollen antioxidant effects may be attributed to these polyphenol substances [8, 9]. Pollen varies in the relative proportions of fatty acids as well, in general, the dominant fatty acids present in pollens are: palmitic, oleic, linoleic and linolenic acid [10]. Certain fatty acids, such as linoleic, linolenic, myristic and lauric acids, have bactericidal and antifungal properties [11]. Bee pollen administered to rats was also found to possibly display antiaging effects [12]. Bee pollen has been reported to immunologically strengthen

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multiple sclerosis patients, this effect was attributed to the antioxidant potential [13]. One of the most important uses of pollen in human medicine is its prophylactic and curative activity in prostate disorders [14]. Pollen load have been also successfully applied in geriatrics and against chronic fatigue [15].

The optimal amount of pollen load is used for direct consumption, for the preparation of 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. The object of our study was to extend the information about how the pollen load could influence the small intestine structure.

2. Materials and methods

Experiment was carried out in experimental facility of the Department of Veterinary Sciences of Slovak University of Agriculture in Nitra (SK 50004 PC). In the experiment, Wistar rat were used. The animals were housed individually in plastic containers (TECNIPLAST, Italy), under basic needs for living conditions (temperature 20 to 22°C, humidity 55±10%, 12 h light regime). Animals were fed with water and basic diet for laboratory mice and rats M3 (Machal, Czech Republic) ad libitum. At the age of four weeks, they were divided into four groups (control, A, B, C) and included into the experiment.

The control group was fed with feed mixture without additive of pollen. The pollen-exposed group A was fed with the standard basic diet supplemented with 0.2% concentration of pollen of Brassica napus L., group B was fed with addition of pollen in concentration of 0.5% and group C in concentration of 0.75%. Feed mixture with added pollen was administered to experimental groups for 90 days.

After 90 days, the animals were killed humanely in accordance to Government regulation no. 23/2009 coll. Then the animals were weighed and immediately after sacrifice the samples of the small intestine (jejunum) were taken for histological processing.

Histological preparations of the small intestine were assessed by light microscopy using the Nikon Eclipse E600 microscope (Japan). We evaluated the construction of the wall of the small intestine, especially the mucosa, forming of villi and the different types of epithelial cells. We have described the structure of the tissue of the small intestine and evaluated the tissue by the quantitative morphometric methods using the computer software M.I.S Quick Photo (Germany) and analysis software for microphotography measurements Nikon Instruments Inc. (Japan).

The pictures were taken using the digital camera (Olympus C5050-Z) and light microscope (Nikon Eclipse E600). Ten intestinal villi length and ten Lieberkühn crypts depth for each experimental rat, together 400 measurements for intestinal villi length and 400 measurements for Lieberkühn crypts depth approximately from 2 or 3 different visual fields were measured.

All measurements were expressed as mean and standard deviation values. The data was assessed by analysis of variance followed by Student T–test using the statistical software SAS Enterprise Guide 9.1 (USA). The significance level was accepted at p<0.05.

3. Results and discussion

Using morphometric techniques, we found that oral doses of pollen of Brassica napus L., in the diet to experimental animals during 90 days at a concentration of 0.2% caused statistically significant (p<0.0001) increase of intestinal villi length by 153.67 µm and statistically significant (p<0.0001) increase of Lieberkühn crypts depth by 36.20 µm in the jejunum when compared to control group (Table 1). Similarly, after administration of a 0.5% concentration of pollen in the feed, we observed significantly (p<0.0001) higher villi by 180.60 µm and their denser arrangement per unit area. The depth of the Lieberkühn crypts were also significantly (p<0.0001) increased by 49.54 µm compared to control group (Table 2). The addition of pollen in concentration of 0.75% caused statistically significant (p<0.0001) increase in villi length by 204.24 µm and significant (p<0.0001) increase in the depth of the Lieberkühn crypts by 11.46 µm compared to control group. The visible differences are shown in Figure 1.
fed with a basic diet supplemented with 1.5% bee pollen over a period of 6 weeks. The results demonstrated that the small intestine villi from the duodenum, jejunum, and ileum were longer and thicker in the pollen group than in the control one. Addition of bee pollen to the diet stimulates the early development of the small intestine of experimental broilers and subsequently promotes their digestion and absorption functions, body growth, and development [16]. Supplement of bee pollen and polysaccharides in calves’ diet could improve the growth performance of calves, apparent digestibility rate of dry matter and crude protein. 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 [17]. Positive, protective and restorative effects of pollen addition in feed have been reported in other studies. Ingestion of bee pollen by rats improves their maternal nutrition without affecting the normal fetal development and thus might be a favorable nutrient during pregnancy [18]. Mice fed with bee pollen from different plants for 6 months showed an increase of the reproduction rates [19]. Oxidant/antioxidant status, estrogenic/anti-estrogenic 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 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. [20]. A chloroform extract of Brassica bee pollen showed anticancer activity by increasing apoptosis of human prostate cancer PC-3 cells [21]. Improving feed consumption, gut development, and health is important for growth efficiency [22, 23]. Increasing the amount of animal protein produced per unit of feed consumed is a major issue impacting the economic and environmental sustainability of animal-protein production [24, 25].

Table 1. Effects of pollen load on the length of villus in small intestine: jejunum

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>X [µm]</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>440.8962</td>
<td>159.0215</td>
<td>173.4037</td>
<td>858.4590</td>
<td>36.0678</td>
</tr>
<tr>
<td>a</td>
<td>100</td>
<td>594.5687</td>
<td>156.3551</td>
<td>209.7866</td>
<td>1209.0702</td>
<td>26.2973</td>
</tr>
<tr>
<td>b</td>
<td>100</td>
<td>621.4946</td>
<td>138.1072</td>
<td>230.1073</td>
<td>909.1675</td>
<td>22.2213</td>
</tr>
<tr>
<td>c</td>
<td>100</td>
<td>645.1392</td>
<td>150.4786</td>
<td>370.8539</td>
<td>1034.2223</td>
<td>23.3250</td>
</tr>
</tbody>
</table>

**** p<0.0001 N–number of observation, X-arithmetic mean, SD-standard deviation CV-coefficient of variation

Table 2. Effects of pollen load on increasing of the depth of Lieberkühn crypts in small intestine: jejunum

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>X [µm]</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>127.4508</td>
<td>47.2698</td>
<td>47.6781</td>
<td>258.8379</td>
<td>37.0886</td>
</tr>
<tr>
<td>a</td>
<td>100</td>
<td>163.6492</td>
<td>73.8523</td>
<td>55.8630</td>
<td>454.3561</td>
<td>45.1284</td>
</tr>
<tr>
<td>b</td>
<td>100</td>
<td>176.9955</td>
<td>44.2033</td>
<td>90.5625</td>
<td>288.4968</td>
<td>24.9742</td>
</tr>
<tr>
<td>c</td>
<td>100</td>
<td>138.9074</td>
<td>56.9995</td>
<td>55.7561</td>
<td>319.6892</td>
<td>41.0342</td>
</tr>
</tbody>
</table>

**** p<0.0001 N–number of observation, X-arithmetic mean, SD-standard deviation CV-coefficient of variation
4. Conclusions

After addition of 0.2% concentration of pollen, lengthening of villi and increasing of the Lieberkühn crypts depth in the jejunum compared to the control group was observed. Similarly, after administration of a 0.5% and 0.75% concentration of pollen in the feed, significantly higher and thicker villi and denser arrangement of these villi per unit area, also the depth of the Lieberkühn crypts were observed. 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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