

# Changes in Intestinal Morphology of Rats Fed with Different Levels of Bee Pollen

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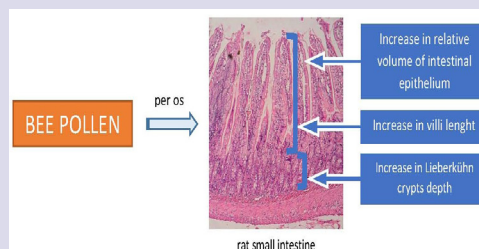
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## ABSTRACT

**Context:** Bee pollen has been related to therapeutic properties including antibiotic, antineoplastic, antiarrhoeic and antioxidant activities. However, little is known about the effect of pollen feeding on the intestinal structures and functions. **Aims:** The objective of this study was to evaluate the small intestine morphology in rats fed with three different pollen levels. **Materials and Methods:** Forty rats were randomly separated into 4 groups of 10 rats each. Control group (C) was fed with a basic diet, group L received a diet supplemented with 0.2% (w/w), group M with 0.5% (w/w) and group H with 0.75% (w/w) bee pollen for 90 days. The relative volume of intestinal mucosa structures, length of villi and Lieberkühn crypts formation were evaluated. **Results:** Quantitative morphometry and histology methods revealed significant increase in the relative volume of epithelium ( $P < 0.0001$ ) and decrease in the connective tissue volume ( $P < 0.0001$ ) of jejunum in groups M and H as compared to the control. The intestinal villi length significantly ( $P < 0.0001$ ) increased in all experimental groups. On the other hand, the Lieberkühn crypts depth significantly ( $P < 0.0001$ ) increased only in groups L and M but decreased ( $P < 0.0001$ ) in the highest pollen-exposed group H. **Conclusion:** The most significant results were recorded in the medium pollen exposed group. We conclude that the bee pollen concentration of 0.5% is the most effective in the increase of relative volume of the intestinal epithelium and villi length. The results demonstrated that bee pollen affects the small intestine development in a concentration-dependent manner and could be beneficial for intestinal function.

**Key words:** Bee pollen, Histology, morphometry, Rat, Small intestine.

## PICTORIAL ABSTRACT



## SUMMARY

- Bee pollen enhances the development of the small intestine in rats from 4 weeks to 4 months of age
- Intestinal epithelium volume, villi length and crypts depth increase.
- Bee pollen acts in a concentration-dependent manner.

**Abbreviations used:** w/w: Percent Concentration Weight/Weight.

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## INTRODUCTION

Bee pollen is the result of the agglutination of flower pollens, made by worker honey bees, with nectar and/or honey and salivary substances, and is collected at the hive entrance.<sup>1</sup> Pollen is bee's primary food source, containing various nutrients and metabolites. It is composed of proteins, lipids, sugars, fibre, mineral salts, amino acids, phenolic compounds and vitamins. Bee pollen contains high concentration of reducing sugars, essential amino acids, unsaturated and saturated fatty acids, Zn, Cu, Fe, and a high K/Na ratio.<sup>2-4</sup> The phenolic composition of pollen consists of flavonol glycosides and other species-specific phenolic compounds, primarily rutin, quercetin, myricetin and trans-cinnamic acid.<sup>5-7</sup> The composition of bee pollen strongly depends on other factors such as region, climate conditions for flowering and soil type.<sup>8</sup> Antioxidant properties of the flavonoid and phenolic compounds are important in the free radical scavenging activity. Pollen flavonoids quercetin, rutin and chrysin have been shown to have a chemopreventive activity.<sup>9</sup> Kaempferol and quercetin can be used as markers for determining the quality of bee pollen, suggesting their potential role in prevention of free radical-implicated diseases such as cancer, cardiovascular diseases and also diabetes, among others.<sup>10,11</sup> Bee pollen has been shown to possess antimicrobial,<sup>12,13</sup> antifungal,<sup>12,13</sup> antioxidant,<sup>11,13,14</sup> anti-radiation,<sup>15</sup> hepatoprotective,<sup>16,17</sup> anti-estrogenic,<sup>18,19</sup> antigenotoxic/antimutagenic,<sup>13,18</sup> anticancerogenic,<sup>18</sup> anti-inflammatory,<sup>13</sup> and various anti-histopathologic capacities.<sup>20</sup>

Pollen has been described as "nature's perfect food" for centuries and is a highly concentrated food source. Therefore, this natural product is

used as widely known food supplement in alternative medicine. Pollen extracts also have been found to inhibit or counteract the tissue damage after toxic compounds intake. Apitherapy diets reduce the carbon tetrachloride toxicity in liver, spleen and pancreas in Wistar rats.<sup>21</sup> The positive effect on the intestinal regeneration were found in rats after honey and bee pollen peroral intake. These effects were thought to be a result of the anti-inflammatory and antioxidant properties of bee products.<sup>22</sup>

The information how, if at all, the bee pollen could influence the morphology, structure, and function of the small intestine are very limited. Therefore, we aimed to find and quantify the structural changes in the small intestine of rats after a peroral intake of bee pollen in food. We also tried to find the most effective dose which could affect the intestinal structural and functional parameters.

## MATERIALS AND METHODS

### Experimental design

Wistar albino rats of both sexes (4 weeks old) were used in the study. Animals were housed in individual polypropylene cages (Tecniplast, Italy) in an environment maintained under standard laboratory conditions, at a temperature of 20-24°C, relative humidity of 55 ± 10% with 12/12 hour light/dark regime. The animals were allowed free access to water and standard food (feed mixture M3, Machal, Czech Republic) *ad libitum*. Experiments were designed and conducted in accordance with

ethical standards approved by Ethical Committee of Slovak University of Agriculture (EC SAU) and State Veterinary and Food Administration of the Slovak Republic in accredited laboratory (SK PC 6012, SUA Nitra).

Forty, four weeks old rats were randomly divided into four groups of ten animals (5 males and 5 females in each group). The control group (C) was fed with a basic diet without pollen addition. Experimental group L received diet supplemented with 0.2% (w/w), group M with 0.5% (w/w) and group H with 0.75% (w/w) bee pollen *Brassica napus*L. for 90 days.

## Histology and morphometry

The small intestines of all animals were sampled after 90 days of the experiment. The rats were humanely sacrificed and the small intestine was immediately removed, rinsed carefully and the *jejunum* was sectioned for histological processing. The samples were fixed in 10% neutral buffered formalin and after processing tissues were embedded in paraffin, sectioned on a microtome into 5 µm sections and stained with haematoxylin-eosine. The sections were viewed and photographed by using an Olympus light microscope (Olympus AX-70 Provis, Japan) with attached camera (Olympus Camedia C-5050, Japan) under 100x magnification. The structure of the small intestine wall, especially the mucosa with epithelium and *lamina propria mucosae*, length of villi and Lieberkühn crypts formation were evaluated.

Morphometric measurements were based on computerized techniques with morphometric software M.I.S. Quick Photo according to micromorphological criteria.<sup>23,24</sup> Ten different visual fields from each animal, altogether 400 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 *lamina propria* in small intestine mucosa were evaluated. Moreover, ten intestinal villi and Lieberkühn crypts were randomly selected and measured in length and depth, respectively, for each experimental rat (400 measurements for intestinal villi length and 400 measurements for Lieberkühn crypts depth).

## Statistical analysis

The values of control and experimental animal analyses were expressed as arithmetical mean ± standard deviation (SD). The results were analyzed by one-way Analysis of Variance (ANOVA) followed by Scheffe's test for *post hoc* comparisons using statistical software SAS 9.2 Enterprise Guide 4.3 (SAS Institute Inc., USA). Differences were considered significant at  $p < 0.05$ .

## RESULTS

Three concentrations of bee pollen were used to investigate the changes in the *jejunum* of rats. Microscopic evaluation revealed after 90 days of feeding low concentration of bee pollen (0.2% - group L) an increase in length of villi in the *jejunum*. Similarly, after administration of 0.5% and 0.75% of bee pollen concentrations in the feed (group M), higher and more densely packed villi than that of the control were found. The changes were more evident than in group L.

These findings were confirmed by morphometrical analysis (Table 1 and Table 2). Measurement of small intestine villi showed significant increase

**Table 2: Measurements of intestinal villi and crypts in jejunum of rats**

Group	Villi length (µm)	Crypts depth (µm)
Control	440.90 ± 159.02	127.45 ± 47.27
Group L	594.57 ± 156.35 ****	163.65 ± 73.85 ****
Group M	621.49 ± 138.11 ****	176.99 ± 44.20 ****
Group H	645.14 ± 150.48 ****	138.91 ± 56.99 ****

Data provided as mean ± SD (n=100), \*\*\*\*  $p < 0.0001$  treated group vs. control

( $p < 0.0001$ ) in the length of villi in *jejunum* of all three experimental groups exposed to bee pollen for 90 days. The length of villi increased by 34.9% in group L, 40.9% in group M and 46.6% in group H. The changes were also recorded for Lieberkühn crypts. The crypts in small intestine were significantly affected during the experiment. Depth of crypts in *jejunum* significantly ( $p < 0.0001$ ) increased in all experimental groups. Bee pollen increased the depth of crypts by 28.4% in group L, 38.9% in group M and 9% in group H with the highest pollen concentration.

Quantitative morphometric analysis also showed statistically significant ( $p < 0.0001$ ) increases in the epithelium relative volume in groups with medium (group L) and high (group H) concentrations of bee pollen. On the contrary, the relative volume of lamina propria in the intestinal mucosa significantly ( $p < 0.0001$ ) decreased in both groups when compared to the control. Other changes in the relative volumes were insignificant.

## DISCUSSION

The interest in the physiological functionality of natural compounds in human health is increasing in the past decades. Among natural products, honey bee derived apicultural products such as pollen and propolis may be regarded as effective natural and functional dietary food supplement due to their remarkable content of polyphenol substances and significant radical scavenging capacity with special regard to their nutritional-physiological implications and their health promoting effect.<sup>25</sup> The positive effect of bee pollen and its compounds on the various biological activities is known. The aim of our study was to find the trophic effect of bee pollen during the experiment in rats. The small intestine and its longest part, jejunum, is an important organ responsible for digestion and absorption of nutrients from food. Any changes in its function affect the function of other organs and systems in the organism. In our study, the small intestine development was enhanced in the young rats from 4 weeks to 4 months of their age. Similar results were previously reported in birds. Bee pollen promoted the gut development in broiler chicken. These effects were beneficial for the next growth of the broilers.<sup>26</sup> Another study comparing the effect of bee pollen and propolis revealed that they were effective in increasing growth performance of broiler chickens.<sup>27</sup> Honey bee pollen also significantly increased the body weight, length, average daily gain, specific growth rate, feed efficiency ratio, and immunological, hematological and biochemical parameters in fish (*Oreochromis niloticus*)<sup>28</sup> and rabbits.<sup>29</sup> Supplements of bee pollen and polysaccharides in calves diet could improve the growth performance of calves, apparent digestibility rate of dry matter and crude protein.<sup>30</sup> The increase in villi length in group with medium (0.5%) and high (0.75%) concentration of bee pollen were recorded in our study. These findings are consistent with results of the experiment with broilers. Authors have found longer and thicker villi in the small intestine of the pollen groups of chickens than that of the control.<sup>26</sup> Our microscopic observations revealed higher and denser villi in the jejunum of all pollen-exposed groups. We confirmed that the higher the concentration of pollen, the more evident changes visible in the small intestine mucosa. The quantification of the changes by morphometric methods confirmed the enhanced intestine development. As well as the significant increase in villi length, the glandular

**Table 1: Morphometric analysis of mucosa in jejunum of rats**

Group	Epithelium (%)	Lamina propria (%)
Control	64.18 ± 8.54	38.82 ± 8.54
Group L	65.20 ± 6.85	34.80 ± 6.85
Group M	69.38 ± 7.86****	30.62 ± 7.86****
Group H	67.92 ± 7.09****	32.08 ± 7.09****

Data provided as mean ± SD (n=100), \*\*\*\*  $p < 0.0001$  treated group vs. control

part of the intestinal mucosa was also enlarged. The Lieberkühn crypts were deeper and wider than that of the control. The enhancement in the glands development was most effective in the group with medium pollen concentration (0.5%) in the feed mixture. The glandular section of the jejunum increased by 38.9% in this group. The higher concentration of bee pollen (0.75%) was not as effective in glands development promotion as the medium concentration. In fact, the crypts depth increase in the high pollen group H was only by 9%, it is lower by 30% than in the group M. All three concentrations of bee pollen were able to promote the small intestine glands development significantly. It is interesting that in broilers fed crude propolis, only crypts in ileum were affected and authors did not recommend the crude propolis supplementation in broiler diets from 1 to 21 days of age.<sup>31</sup>

Quantitative morphometric analysis showed also significant increase in the intestinal epithelium relative volume in group with medium (group L) and high (group H) concentrations of bee pollen. The relative volume of *lamina propria* decreased in both groups when compared to the control. The results of morphometric analysis of relative volume of intestinal mucosa main structures support the previously described changes. Increased villi and consequently the mucosa surface and volume of epithelium in jejunum may be helpful in the absorption processes in the digestive system. Moreover, the antimicrobial and antifungal activity of bee pollen is also important<sup>12</sup> and may help to keep the intestine healthy and fully functional. Bee pollen contains a substantial amount of biologically active substances which are potent antioxidants. It is recognized to improve and prevent functions of cells and organs.<sup>14,16,20,21,32</sup> The alleviation of the histopathological aspects of toxic compounds by bee products is known in liver, spleen, kidney, and pancreas but not in testis.<sup>21</sup> Bee pollen constitutes a good source of healthy compounds, namely, phenolics, and it might be useful in prevention of diseases in which free radicals are implicated.<sup>14</sup> Pollen intake can improve gut, gastroenterological and liver health.<sup>14</sup> Supplementation with fresh bee pollen increased the muscle protein synthesis rate and mitochondrial function in old malnourished rats. Alterations in muscle protein and energy metabolism were reversible in old rats receiving renutrition via fresh bee pollen-enriched diet. Moreover, a short-term refeeding period was able to restore body weight.<sup>32</sup> The changes in the development of the small intestine mucosa which were found in our experiments are important from the functional point of view. Longer and denser villi in jejunum allow more surface area to digest and absorb the nutrients at extended mucosa surface. Also the digestibility of the compounds from food can be improved when more glands are present in the intestinal mucosa. All these changes may be beneficial for the feed and nutrient efficiency and also in refeeding period.

## CONCLUSION

In this study, we provide evidence that bee pollen at concentration of 0.2%, 0.5% and 0.75% in feed mixture was able to enhance the development of the small intestine (*jejunum*) in rats from 4 weeks to 4 months of age. The most significant results were recorded in the medium pollen-exposed group. We conclude that the bee pollen concentration of 0.5% is the most effective in the increase of relative volume of the intestinal epithelium and villi length. These parameters are important in the absorption rate in the digestive system. The crypts were deeper indicating that the glandular part of the small intestine mucosa was also enhanced. The results demonstrated that the bee pollen affects the small intestine development in a concentration-dependent manner and could be beneficial for intestinal function.

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## CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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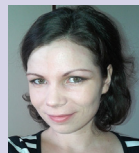
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