The effect of bee pollen consumption on functional morphology of small intestine of rats

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Abstract: In our experiment, the effects of bee pollen addition in diet on the functional morphology of small intestine 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 one control and three experimental groups of ten rats in each group. Feed given to animals in group A contained addition of pollen in concentration of 0.2%, in group B the addition of pollen was 0.5% and in group C 0.75%. Animals of the control group were fed without the pollen addition. Using the quantitative morphometrical methods, we have found statistically significant increase in the relative composition of epithelial tissue ($P < 0.0001$) and decrease in the connective tissue volume ($P < 0.0001$) of the small intestine in experimental groups B and C as compared to the control. The results of our work show that the addition of pollen in diet had concentration-dependent effects on the mucosa of small intestine and thus it could be used as a proof of another beneficial use of pollen as a feed supplement.

Key-Words: pollen, rat, small intestine

Introduction

Pollen grains are carriers of higher plants gender (sperm cells) containing genetic information for future sporophyte [1]. Pollen load represents a large number of pollen grains of different plant species. Pollen grains of each plant species have specific characteristics and properties [2].

Bee pollen has a complex chemical composition; it provides valuable nutrients such as carbohydrates, essential amino acids, proteins, fatty acids, lipids [3, 4, 5]. The significant components of bee pollen are pro-vitamins and vitamins in particular: thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, folic acid, beta-carotene (pro-vitamin of vitamin A), vitamin C, tocopherol and ergocalciferol (vitamin precursor of vitamin D2) [6, 7]. The most particular minerals contained in pollen grains are: phosphorus, potassium, magnesium, copper, iron, manganese and zinc [8]. Pollen is also rich in polyphenolic substances, flavonoids, phytosterols and other health-promoting substances [9, 10, 11]. Another biologically active and beneficial pollen load components are organic acids, terpentines, nucleic acids, purine compounds, essential oils, plant growth regulators and more than 100 enzymes [12].

From pharmacological and health effects which were tested on animals, bee pollen is best known for: antioxidant, anticancerogenic, antibacterial, anti-allergic, immune-stimulating and antianabolic effects, as well as the effect of specific enzyme inhibitors [13, 14, 15, 16, 17, 18]. As to its use against various diseases, pollen is used to treat: prostatitis, some serious skin diseases, chronic liver damage and acute chronic hepatitis, stomach ache and various intestinal disorders, it regulates bowel function, chronic constipation and chronic diarrhea [19, 20, 21]. Bee pollen also supports detoxification functions of the liver [22]. It has been shown to inhibit histamine reactions that cause inflammation and allergy symptoms. This feature is probably

duesss to the presence of quercetin in the bee pollen [23].

These features allowed using bee pollen as a widely known food supplement [24, 25].

Our scientific aim was to describe the changes in the structure of the small intestine using morphometric methods.

Material 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). Wistar rats were used as experimental animals. Animals were fed with water and complete feed mixture for laboratory mice and rats M3 (Machal, Czech Republic) *ad libitum*. They were housed individually in plastic containers (Tecniplast, Italy) on bedding of wood shavings under basic requirements for living conditions (temperature 20 - 22° C, humidity 55 ± 10%, 12 h light regime).

At the age of 4 weeks, young rats were divided into 4 groups (control, A, B, C) for 10 animals in each group. The control group was fed with feed mixture without pollen additive from *Brassica napus var. Napus*. Experimental group A was fed with the pollen addition in concentration of 0.2 %, group B was fed with addition of pollen in concentration of 0.5% and group C with pollen addition in 0.75% concentration. The duration of the experiment was 90 days.

After 90 days of pollen intake, the animals were humanely killed in accordance to Government regulation no. 23/2009 coll. The samples of small intestine (*jejunum*) were taken immediately after sacrifice, they were treated by the special method, to ensure the elimination of autolytic processes in cells, so they could captured the actual state of the tissue. The samples were fixed in 10% formaldehyde solution and paraffin - watered. For the microscopic detection of any changes in the tissue of the *jejunum* the sections were stained with hematoxylin and eosin. Samples of experimental and control animals were treated in the same manner.

Histological preparation of the small intestine (*jejunum*) was assessed by light microscope (Olympus AX 70 Provis, Japan). The structure of the small intestine wall, especially the mucosa and the different types of epithelial cells were observed. 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. 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, totally 400 microphotographs of the small intestine were recorded and analyzed. The quantitative analysis was realized using the test grid containing 494 test points and the relative composition of the epithelium and connective tissue (*lamina propria mucosae*) 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, minimum and maximum were calculated and by one-way analysis of variance we determined the statistical significance of differences between groups using Scheffe test. All statistical analysis of the results was performed by the statistical program - SAS Enterprise Guide 9.1 (USA).

Results and Discussion

Assessing the small intestine mucosa under the light microscope we observed in all experimental histological samples: normal mucosa configuration, covered with single-layer columnar epithelium, enterocytes and goblet cells. Among villi were well observable Lieberkühn crypts and there were no signs of inflammatory process in the mucosa.

Using the quantitative morphometric methods we found that the percentage of epithelial volume in *jejunum* in the group A compared to the control was slightly increased by 1.02% and also a slight decline in connective tissue volume, identically by 1.02% was counted. These differences were not statistically insignificant.

In group B, we found statistically significant ($P < 0.0001$) increase in the epithelium volume by 5.20% and statistically significant ($P < 0.0001$) decrease in the connective tissue just the same by 5.20% when compared to the control. In group C we found also statistically significant ($P < 0.0001$) increase in the epithelium volume by 3.74% and statistically significant ($P < 0.0001$) decrease in the connective tissue 3.74% when compared to the control group. All differences between the groups are shown in Table 1 and Table 2.

The 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 increase of epithelium of the small intestine and the pollen concentration of 0.5% and 0.75% significantly ($P < 0.0001$) increased the percentage of epithelium volume and decreased the percentage of connective tissue volume (*lamina propria mucosae*).

Table 1 Morphometric analysis of epithelium relative volume in small intestine of rats

	X [%]	Significance	SD	Minimum	Maximum
Control	**64.18**		8.54	31.52	83.47
Group A	**65.20**		6.85	34.12	82.36
Group B	**69.38**	****	7.86	30.39	87.85
Group C	**67.92**	****	7.09	47.18	79.94

**** *P < 0,0001, X - arithmetic mean, SD - standard deviation*

Table 2 Morphometric analysis of soft tissue relative volume in small intestine of rats

	X [%]	Significance	SD	Minimum	Maximum
Control	**35.82**		8,54	16.53	68.48
Group A	**34.80**		6.85	17.64	65.88
Group B	**30.62**	****	7.86	12.15	69.61
Group C	**32.08**	****	7.09	20.06	52.82

**** *P < 0,0001, X - arithmetic mean, SD - standard deviation*

In weanling piglets the adition of dietary fibre differing in lignin content from *Pinus massoniana* pollen reduced apparent (faecal) digestibility of dry matter and crude protein [26]. Zhang, Diao and Tu (2010) had also concluded that supplement of bee pollen and polysaccharides in calves diet could improve the growth performance of calves. Bee pollen additive of 25 $g.d^{-1}$ and polysaccharides in dose of 5 $g.d^{-1}$ in milk replacer could get better performance and higher apparent digestibility in calves.

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 [28]. 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 CCl_4, and it 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 inhibition of the lipid peroxidation [29].

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 [30]. 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 $mg.kg^{-1}$ b.w. 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 [31].

In another 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 digestive system and therefore is potentially beneficial food supplement for certain conditions, such as short bowel syndrome [32].

Conclusion

Using the morphometric techniques, we found that oral doses of pollen in the diet during 90 days at a concentration of 0.2% caused a slight increase in epithelial layer of the small intestine and in a concentration of 0.5% and 0.75% significantly increased the epithelium volume and decreased the connective 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 be used as a proof of another beneficial use of pollen as a feed additive.

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References:

[1] Ostrolucká MG, Charakteristika kvetového peľu, *Formovanie obnôžkového peľu*, Nitra: SPU, 2010, pp. 69-106.
[2] Nôžková J, et al., Hodnotenie kvantitatívnych a kvalitatívnych znakov včelieho peľu a ich klasifikácia podľa navrhnutých deskriptorov, *Potravinárstvo*, Vol. 4, 2010, pp. 204-216.
[3] Cocan O, et al., Composition and biological activities of bee pollen: review: *Bulletin of the University of Agricultural Science and Veterinary Medicine*, Vol. 61, 2005, pp. 221-226.
[4] Hamamoto R, Ishiyama K, Yamaguchi M, Inhibitory effects of bee pollen Cistus ladaniferus extract on bone resorption in femoral tissues and osteoclast-like cell formation in bone marrow cells *in vitro*, *Journal of Health Science*, Vol. 52, 2006, pp. 268- 275.
[5] Carpes ST, et al., Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil. *Brazilian Journal of Food Technology*, Vol. 12, No. 3, 2009, pp. 220-229.
[6] Villanueva MTO, et al., The importance of bee-collected pollen in the diet: a study of its composition. *International Journal of Food Sciences and Nutrition*, Vol. 53, 2002, pp. 217-224.
[7] Bastos DHM, et al., Fatty acid composition and palynological analysis of bee (Apis) pollen loads in the states of Sao Paulo and Minas Gerais, Brazil, *Journal of apicultural research*, Vol. 43, 2004, pp. 35-39.
[8] Human H, Nicolson, SW, Digestion of maize and sunflower pollen by the spotted maize beetle *Astylus atromaculatus* (*Melyridae*): Is there a role for osmotic shock, *Journal of Insect Physiology*, Vol. 49, 2003, pp. 633-643.
[9] Serra-Bonvehi J, Gonell Galindo J, Gomez Pajuelo A, Estudio de la composicion y caracteristicas fisico-quimicas del polen de abejas, *Alimentaria*, Vol. 23, No. 176, 1986, pp. 63-67.
[10] Almedia - Muradian LB, et al., Chemical composition and botanical evaluation of dried bee pollen pellets, *Journal Food Composition and Analysis*, Vol. 18, 2005, pp. 105-111.
[11] Moreira L, et al., Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal, *Food Chemistry and Toxicology*, Vol. 46, 2008, pp. 3482-3485.
[12] Crane E, *Bees and beekeeping*: Science, Practice and World Resources Ithaca: Cornstock Publisher, 1990. 593 pp. ISBN 0-8014-2429-1.
[13] Santos F, De AR, Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke, *Journal of Food Composition and Analysis*, Vol. 19, 2006, pp. 507-511.
[14] Yang X, et al., Characterization and anti-tumor activity of pollen polysaccharide, *International Immunopharmacology*, Vol. 7, 2007, pp. 401-408.
[15] Ikeno K, et al., Antioxidative activity of honeybee pollen in comparison with other bee products, *Honeybee-Science*, Vol. 25, No. 3, 2004, pp.113-118.
[16] Basim E, Basim H, Özcan M, Antibacterial activities of turkish pollen and propolis extracts against plant bacterial pathogens., *Journal of Food Engineering*, Vol. 77, 2006, pp. 992-996.
[17] Özcan M, Antifungal effect of pollen and propolis extracts collected from different regions of Turkey, *Uludag Bee Journal*, Vol. 3, 2003, pp. 27-34.
[18] Stocker A, Isolation and characterisation of substances from Royal Jelly. France, Orléans, 2003, 213 pp. PhD Thesis, Université d'Orléans.
[19] Handl B, *Včelí produkty ve výživě člověka a v lékařství.* ZO ČSV v Kunštátu na Moravě, 1991, 24 pp.
[20] Richter J, *Léčení včelími produkty*. 1. vyd. Bratislava : Eko-konzult, 1999. 104 pp. ISBN 80-88809-01-0.
[21] Přidal A, *Včelí produkty*. Dotisk. Brno : MZLU, 200, 102 pp. ISBN 80-7157-717-0.
[22] Ceglecka M, Effect of pollen extracts on prolonged poisoning of rats with organic solvents, *Phytotheraphy Research,* Vol. 5, 1991, pp. 245-249.
[23] Elkins RMA, *Bee Pollen, Royal Jelly, Popolis and Honey*, Pleasant Grove, UT : Woodland Health Publishing, 1996, 32 pp. ISBN 1885670125.
[24] Schmidt JO, *Chemical composition and application*, Mizrahi A. and Lensky Y. Bee products, New York : Pleum Press, 1996, pp.15-26.

[25] Silva TMS, et al., Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke, *Journal of Food Composition and Analysis*, Vol. 19, 2006, pp. 507-511.
[26] Schedle K, et al., Effects of insoluble dietary fibre differing in lignin on performance, gut microbiology, and digestibility in weanling piglets, *Archives of animal nutrition*, Vol. 62, No. 2, 2008, pp. 141-151.
[27] Zhang GF, Diao QY, Tu Y, Effects of Bee Pollen and Its Polysaccharides on Growth Performance,Nutrient Digestibility and Serum Biochemical Indexes in Calves, *Journal of acta veterinaria et zootechnica sinica*, Vol. 41, 2010, pp. 981-987.
[28] Turner KK, et al., Bee pollen product supplementation to horses in training seems to improve feed intake: a pilot study, *Journal of animal physiology and animal nutrition*, Vol. 90, 2006, pp. 414-420.
[29] Cheng N, et al., Antioxidant and hepatoprotective effects of Schisandra chinensis pollen extract on CCl(4)-induced acute liver damage in mice, *Food and Chemical Toxicology*, Vol. 50, 2006, pp. 10.
[30] Attia YA, Al- Hanoun A, El-Din AE, Effect of bee pollen levels on productive, reproductive and blood traits of NZW rabbits, *Journal of Animal Physiology and Animal nutrition*, Vol. 95, No. 3, 2011, pp. 294-303.
[31] Saric A, Balog T, Sobokanec S, Antioxidant effects of flavonoid from Croatian Cystus incanus L. rich bee pollen, *Food and chemical toxicology an international journal*, Vol. 47, 2009, pp. 547-554.
[32] Wang J, et al., Trophic effect of bee pollen on small intestine in broiler chickens, *Journal of Medicinal Food*, Vol. 10, No. 2, 2007, pp. 276-280.