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

Zuzana Hajková[1*], Róbert Toman[1], Svätoslav Hluchý[1], Branislav Gálik[2], Milan Šimko[2], Miroslav Juráček[2], Monika Martiniaková[3], Ivana Boboňová[3]

[1]Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Veterinary Disciplines, 949 76 Nitra, Tr. A. Hlinku 2, Slovakia
[2]Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Animal Nutrition, 949 76 Nitra, Tr. A. Hlinku 2, Slovakia
[3]Constantine the Philosopher University, Faculty of Natural Sciences, Department of Zoology and Antropology, 949 74 Nitra, Nábrežie mládeže 91, Slovakia

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

Pollen load is the main protein source for the bee colony, it may contain several different plants pollens mixed with plant nectar and bee saliva. The pollens are packed by the insects (worker bee) into small dust pellets [1]. Pollen has been described as “nature's perfect food” for centuries and is a highly concentrated food source, 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 chyrisin 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

* Corresponding author: Zuzana Hajková
Tel. +421 37 641 4111
E-mail: zuzana.hajkova01@gmail.com

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 mesured.

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 lenght 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.

In a similar study, the effects of bee pollen on the development of digestive organs in broiler chickens had been reported. 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 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 feed 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*

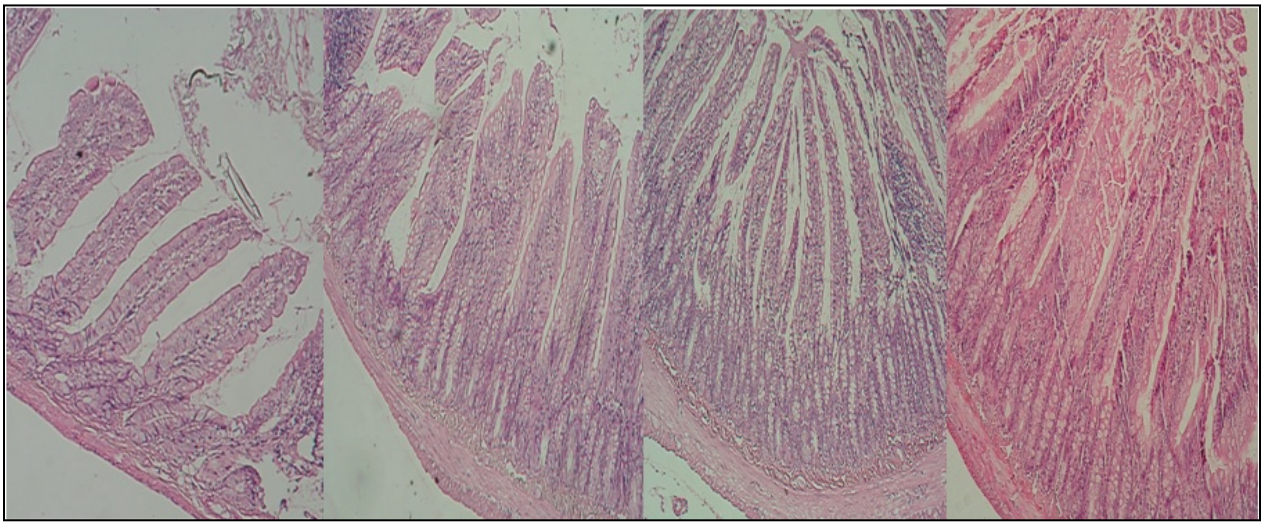
Group	N	X [µm]	SD	Minimum	Maximum	CV
Control	100	440.8962	159.0215	173.4037	858.4590	36.0678
a	100	**** 594.5687	156.3551	209.7866	1209.0702	26.2973
b	100	**** 621.4946	138.1072	230.1073	909.1675	22.2213
c	100	**** 645.1392	150.4786	370.8539	1034.2223	23.3250

**** $p<0.0001$ 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*

Group	N	X [µm]	SD	Minimum	Maximum	CV
Control	100	127.4508	47.2698	47.6781	258.8379	37.0886
a	100	**** 163.6492	73.8523	55.8630	454.3561	45.1284
b	100	**** 176.9955	44.2033	90.5625	288.4968	24.9742
c	100	**** 138.9074	56.9995	55.7561	319.6892	41.0342

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



Control group A (0.2%) group B (0.5%) group C (0.75%)

Figure 1. Visible differences between groups in the mucosa of the small intestine (*jejunum*), 200 x magnification

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

This work was supported by KEGA 025UKF-4/2012 (Ministry of Education, Slovak Republic) and VEGA 1/0662/2011 (Ministry of Education, Slovak Republic).

References

1. Leblanc, B. W., Davis, O. K., Boue, S., Delucca, A., Deeby, T., Antioxidant activity of Sonoran Desert bee pollen, Food Chemistry, 2009, 115, 1299-1305

2. Haro, A., Lopez-Aliaga, I., Lisbona, F., Barrionuevo, M., Alférez, M. J., Campos, M. S., Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia, Journal of Agricultural and Food Chemistry, 2000, 48, 5715-5722

3. Human, H., Nicolson, S. W., Nutritional content of fresh, bee-collected and stored pollen of *Aloe greatheadii* var. davyana (Asphodelaceae). In Phytochemistry, 2006, 67, 1486-1492

4. Tyler, V. E., The Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies, 3-rd edition, New York, NY, In Pharmaceutical Products Press, 1992, pp. 35

5. Meurer, B., Wiermann, R., Strack, D., Phenylpropanoid Patterns in Fagales Pollen and Theirphylogenetic Relevance, Phytochemistry, 1988, 27, 823-828

6. Serra Bonvehí, J., Soliva Torrentó, M., Centelles Lorente, E., Evaluation of Polyphenolic and Flavonoid Compounds in Honeybee-Collected Pollen Produced in Spain, Journal of Agricultural and Food Chemistry, 2001, 49, 1848-1853

7. Pichichero, E., Cicconi, R., Mattei, M., Canini, A, Chrysin-induced apoptosis is mediated through p38 and Bax activation in B16-F1 and A375 melanoma cells, International Journal of Oncology, 2010, 10, 473-483

8. Nakajima, Y., Tsuruma, K., Shimazawa, M., Mishima, S., Hara, H., Comparison of bee products based on assays of antioxidant capacities, BMC Complemented Alternative Medicine, 2009, 9, 4

9. Han, X., Shen, T., Lou, H., Dietary Polyphenols and Their Biological Significance, International Journal of Molecular Science, 2007, 8, 950-988

10. Kroyer, G., Hegedus, N., Evaluation of bioactive properties of pollen extracts as functional dietary food supplement, Innovative Food Science and Emerging Technologies, 2001, 2, 171-174

11. Manning, R., Fatty acids in pollen: a review of their importance for honeybees, In Bee World, 2001, 82, 60-75
12. Liu, X., Li, L., Morphological observation of effect of bee pollen on intracellular lipofuscin in NIH mice, Zhongguo Zhong Yao Za Zhi, 1990, 15, 561-563
13. Iarosh, A. A., Macheret, E. L., Iarosh, A. A., Zapadniuk, B. V., Changes in the immunological reactivity of patients with disseminated sclerosis treated by prednisolone and the preparation Proper-Myl, Vrachebnoe Delo, 1990, 2, 83-86
14. Campos, M., Markham, K. R., Mittchel, K. A., Cunha, A. P., An approach to the characterization of bee pollens via their flavonoid/phenolic profiles, Phytochemical Analysis, 1997, 8, 181-185
15. Dubtsova, E., Komisarenko, I., Kasyanenko, V., Bee pollen and bee bread: biological action and use in aged people, Clinical Gerontology (Russia), 2007, 13, 50-52
16. Wang, J., Li, S., Wang, Q., Xin B., Wang, H., Trophic effect of bee pollen on small intestine in broiler chickens, Journal of Medicinal Food, 2007, 10, 276-280
17. Zhang, G. F., Diao, Q. Y., 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, 2010, 41, 981-987
18. Xie, Y., Wan, B., Li, W., Effect of bee pollen on maternal nutrition and fetal growth, Hua Xi Yi Ke Da Xue Xue Bao (chinese), 1994, 25, 434-437
19. Slijepcevic, M., Nadyija, M., Blazij-Poljak, M., Boranic, M., Impact of the pollen contained in food on the reproduction of mice IIIème Symposium international d'apithérapie, Apimondia, Bukarest, 1978, pp. 251-253
20. Šaric, A., Balog, T., Sobočanec, S., Kušić, B.,Šverko, V., Rusak, G., Likić, S., Bubalo, D., Pinto, B., Reali, D., Marotti, T., Antioxidant effects of flavonoid from Croatian *Cystus incanus* L. rich bee pollen, Food and chemical toxicology an international journal, 2009, 47, 547-554
21. Wu, Y. D., Lou, Y. J., A steroid fraction of chloroform extract from bee pollen of *Brassica campestris* induces apoptosis in human prostate cancer PC-3 cells 194, Phytotherapy Research, 2007, 21, 1087-1091
22. Fan, M. Z., Matthews, J. C., Etienne, N. M., Stoll, B., Lackeyram, D., Burrin, D. G., Expression of apical membrane L-glutamate transporters in neonatal porcine epithelial cells along the small intestinal crypt-villus axis, American Journal of Physiology, Gastrointestinal and Liver Physiology, 2004, 287, 385–398
23. Mace, O. J., Lister, N., Morgan, E., Shepherd, E., Affleck, E., Helliwell, P., Bronk, J. R., Kellett, G. L., Meredith, D., Boyd, R., Pieri, M., Bailey, R., Pettcrew, D., Foley, D., An energy supply network of nutrient absorption coordinated by calcium and T1R taste receptors in rat small intestine, The Journal of Physiology, 2009, 587, 195–210
24. Mace, O. J., Marshall, F., Gut chemo sensing and the regulation of nutrient absorption and energy supply, Journal of Animal Science, 2013, 91, 1932-1945
25. Shi, H., Kong, M., Chen, G. Zhaoa, J., Shia, H. L., Chena, Y., Rowan, F. G., Compound Pollen Protein Nutrient Increases Serum Albumin in Cirrhotic Rats, Gastroenterology Research, 2010, 3, 253-261