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THE EFFECT OF BEE POLLEN ON MACROSCOPIC STRUCTURE OF FEMORA IN ADULT FEMALE RATS AFTER AN EXPERIMENTAL ADDITION IN DIET

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ABSTRACT

Bee pollen is considered a super food because it contains proteins and is rich in vitamins, minerals and phytochemicals. According to these benefits is bee pollen often used as a dietary additive, but its role on growth characteristics and bone metabolism is still poorly understood. Therefore the objective of this study was to determine the effect of diet supplementation with bee pollen on selected growth characteristics (body weight, femoral weight and femoral length) in adult female rats. One-month-old female Wistar rats were randomly divided into four groups of 5 animals each. In the control group (1), rats were fed a commercial diet throughout the experiment (90 days). Rats of three other experimental groups received standard diets with a different content of bee pollen: group 2 – 0.2%, group 3 – 0.5% and group 4 – 0.75% of bee pollen for 90 days. The statistical analysis of obtained data showed a statistically significant decrease of femoral weight in rats from experimental group 4 as compared to control one (1) and also relevant differences were found between rats from the experimental groups 2 and 4 ($P<0.05$). The results produced by the current study allow better understanding of the role of bee pollen on growth and bone metabolism in rats.

Keywords: Bee pollen. Body weight. Femoral weight. Femoral length. Rats.

INTRODUCTION

Bee pollen is composed of flower pollen mixed with nectar and bee secretions **(Silva *et al.*, 2006)**. Bee pollen is one of the widely used natural supplements. It contains many essential nutritional elements important for growth and development of animals and humans **(Bell *et al.*, 1983; Orzaez Villanueva *et al.*, 2002; Haščík *et al.*, 2011; Capcárová *et al.*, 2012; Petruška *et al.*, 2012)**. Bees use pollen as their nutritional source of proteins (25-30%), carbohydrates (30-55%), lipids, including fatty acids and sterols (1-20%), vitamins and minerals. Futhermore, bee pollen is rich in carotenoids, flavonoids, phytosterols, polyphenols and other beneficial compounds **(Baltrušaityté *et al.*, 2007; Moreira *et al.*, 2008)**. This natural product is recognized to be a valuable apitherapeutic product with potential for medical and nutritional applications **(Almeida-Muradian *et al.*, 2005)**. Bee pollen is promoted as a health food with a wide range of nutritional and therapeutic properties **(Yamaguchi *et al.*, 2006)**, triggering beneficial effects to human health and the prevention of prostate problems **(Shoskes, 2002)**, allergy desensitization **(Mizrahi and Lensky, 1997)**, arteriosclerosis **(Wojcicki *et al.*, 1986)** and

tumors **(Zhang *et al*., 1995)**. Its important physiological functions have already been widely praised. It has been reported that bee pollen accelerates mitotic rate, promotes tissue repair, enhances greater toxic elimination and reduces excessive cholesterol levels **(Morais *et al*., 2011)**. Its radical scavenging activity has already been reported **(Baltrušaityté *et al*., 2007)**. Moreover **Yamaguchi *et al*. (2004)** demonstrated that the water-solubilized extract of bee pollen (*Cistus ladaniferus*) has an anabolic effect on several bone components in rats. The extract of bee pollen has a stimulatory effect on bone formation and an inhibitory effect on bone resorption *in vitro* **(Yamaguchi *et al*., 2004; Hamamoto *et al*., 2006)** and also stimulates bone caltification **(Yamaguchi *et al*., 2004)**.

Growth and development of animals and humans is affected by numerous factors, including nutritional regime, genetic factors, sex, age, management conditions and production system. Recent years have witnessed an increasing interest in the use of various feed additives and dietary supplements believed to improve growth characteristics of animals. Therefore the aim of this study was to determine the effect of bee pollen as alternative feed additive on selected growth characteristics (body weight, femoral weight and femoral length) in adult female rats.

MATERIAL AND METHODS

Our study was carried out on twenty one-month-old female Wistar rats. The animals were housed individually in plastic containers (Techniplast, Italy) under the same laboratory conditions of temperature (20-24°C) and relative humidity (55±10%) with acces to food (feed mixture M3, Bonagro, Czek Republic) and drinking water *ad libitum*. All experiments were provided in accordance with accepted standards of animal care in accredited laboratory (SK PC 50004) of the Slovak University of Agriculture in Nitra.

At the age of four weeks the young rats were divided into 4 groups, of 5 animals each. The control group (1) was fed with feed mixture without bee pollen additive. Experimental group 2 was fed with the bee pollen addition in concentration of 0.2%, group 3 with addition of bee pollen in concentration of 0.5% and group 4 with addition of bee pollen in concentration of 0.75% for 90 days. All procedures were approved by the Animal Experimental Committee of the Slovak Republic.

At the end of the experiment, all animals were killed, weighed and their femora were used for macroscopic analysis. Femora were weighed by analytical scales and their length was measured by a sliding instrument. Values for macroscopic analysis were expressed as mean ± standard deviation. Comparisons between experimental and control groups were assessed by the one-way analysis of variance (ANOVA) and Post Hoc Tukey's test. The significance level was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Our results demonstrate no significant effect of bee pollen application on body weight and femoral length in adult female rats. Statistically significant differences were found only for femoral weight in rats from experimental group 4 in comparison with those from the control one (1) and also relevant differences were observed between rats from the experimental groups 2 and 4 (Table 1).

In general, bee pollen contains a wide spectrum of amino acids, vitamins, hormones, and minerals, as well as enzymes and co-enzymes necessary for good digestion and growth. Our study revealed a non-significant effect of bee pollen administration (in concentrations of 0.2%, 0.5% and 0.75%) on body weight in adult female rats. In the contrary **Haro *et al*. (2000)** reported that male rats fed with multifloral bee pollen ($10 g.kg^{-1}$ of diet for 10 days) had increased body weight. Significantly increased body weight was also observed in chicken fed

Table 1 Average body weight, femoral weight and femoral length in the control (1) and experimental groups (2, 3, 4) of rats.

Rat's group	N	Body weight (g)	Femoral weight (g)	Femoral length (cm)
Control (1)	5	246±15.57	0.8185±0.05	3.3840±0.11
0.2% of bee pollen (2)	5	241±12.92	0.7780±0.04	3.4180±0.03
0.5% of bee pollen (3)	5	237±18.24	0.7535±0.09	3.4120±0.09
0.75% of bee pollen (4)	5	245±20.00	0.5832±0.05	3.4360±0.08
Tukey's test		NS	$1{:}4^{+}$; $2{:}4^{+}$	NS

N: number of rats, NS: non-significant changes, $P < 0.05$ (+)

with a basic diet supplemented with 1.5% of bee pollen over a period of 6 weeks **(Wang *et al*., 2007)** and also in male rabbits received bee pollen as water suspension at 100, 200 and 300 $mg.kg^{-1}$ of body weight for 10 weeks **(Attia *et al*., 2011)**. These differences, however, could be caused by different concentrations of bee pollen used in the mentioned studies. In addition, it is known that differences of bee pollen application on growing characteristics depend on animal species and also sex. The data observed by **Kolesárová *et al*. (2011, 212)** indicate a significant decrease in the secretion of insulin like growth factor I (IGF-I) and subsequent stimulation of reproductive female hormones after unifloral rape seed bee pollen addition in dose $5g.kg^{-1}$of diet for 90 days. In accordance with results obtained by **Haščík *et al.* (2012)** we suppose that some energy are therefore channelled to the reproductive system and don't affect growing of female rats supplemented by bee pollen.

Weights of femora were significantly decreased in female rats from experimental group 4 as compared to control group and also between rats from the experimental groups 2 and 4. In general, steroid hormones play the important role in bone cell development and in the maintenance of normal bone architecture. We assume, that changes in progesteron and estradiol production in female rats after bee pollen addition reported by **Kolesárová *et al*. (2011, 2012)**, could have an impact on activity of osteoblasts and bone mineralisation resulting in decreased femoral weight.

The femoral lengths did not differ between rats from all groups. The results by **Kleczek *et al*. (2012)** revealed that bee pollen (0.5% concentration) in the diet did not cause significant changes in the weight and length of tibial bones in broilers. However, the addition of bee pollen (*Cistus ladaniferus*) water-solubilized extract in the diet has shown to increase bone formation and decrease bone resorption **(Hamamoto *et al*., 2006; Yamaguchi *et al*., 2006)**, and had a preventive effect on the decrease of mineral content and mineral density in the femora of ovariectomized rats **(Yamaguchi *et al*., 2007)**. We suppose that a positive impact of bee pollen on bone metabolism could have concentration-dependent manner and further research is needed to determine the optimal dosage of bee pollen in the diet of rats with a stimulative effect on bones.

CONCLUSION

Out results revealed a significant effect of bee pollen administration on femoral weight in adult female rats. Statistically significant differences were found between rats receiving 0.75% of bee pollen in their diet and those from the control group. Also, relevant differences were observed in rats with 0.2% addition of bee pollen in the diet as compared to those with 0.75% of bee pollen supplementation. On the other hand, body weight and femoral length were non-significantly affected by the administration of different concentrations of bee pollen in female rats.

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REFERENCES

ALMEIDA-MURADIAN, L.B., PAMPLONA, L.C., COIMBRA, S., BARTH, O.M. 2005. Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of Food Composition and Analysis,* 18, 105-111.

ATTIA, Y.A., ALL-HANOUN, A., BOVERA, F. 2011. Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months. *Journal of Animal Physiology and Animal Nutrition,* 95, 17-26.

BALTRUŠAITYTÉ, V., VENSKUTONIS, P.R., ČKSTARYTÉ, V. 2007. Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry,* 101, 502-514.

BELL, R.R., THRONBER, E.J., SEET, J.L., GROVES, M.T., HO, N.P., BELL, D.T. 1983. Composition and protein quality of honeybee-collected pollen of Eucalyptus marginata and Eucalyptus calophylla. *Journal of Nutrition,* 113, 2479-2484.

CAPCÁROVÁ, M., SLAMEČKA, J., ABBAS, K., KOLESÁROVÁ, A., KALAFOVÁ, A., VÁLENT, M., FILIPEJOVÁ, T., CHRASTINOVÁ, L., ONDRUŠKA, L., MASSANYI, P. 2012. Effect of diatery inclusion of Rhuscoriaria on internal milieu of rabbits. *Journal of Animal Physiology and Animal Nutrition,* 96, 459-465.

HAMAMOTO, R., ISHIYAMA, K., YAMAGUCHI, M. 2006. Inhibitory effects of bee pollen cistus ladaniferus extract on bone resorption in femoral tissues and osteoclast-like cell formation in bone marrow cellsin vitro. *Journal of Health Science,* 52, 268-275.

HARO, A., LOPEZ-ALLIAGA, I., LISBONA, F., BARRIONUEVO, M., ALFEREZ, M.J.M., CAMPOS, M.S. 2000. Beneficial effect of pollen nad/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. *Journal of Agricultural and Food Chemistry,* 48, 5715-5722.

HAŠČÍK, P., ELIMAN, I.E., BOBKO, M., KAČÁNIOVÁ, M., POCHOP, J., GARLIK, J., KROČKO, M., ČUBOŇ, J., VAVRIŠÍNOVÁ, K., ARPÁŠOVÁ, H., CAPCÁROVÁ, M., BENCZOVÁ, M. 2011. Oxidative stability of chicken meat after pollen extract application in their diet. *Journal of Microbiology, Biotechnology and Food Sciences,* 1, 70-82.

HAŠČÍK, P., ELIMAN, I.E., GARLIK, J., KAČÁNIOVÁ, M., ČUBOŇ, J., BOBKO, M., ABDULLA, H. 2012. Impact of bee pollen as feed suplements on the body weight of broiler Ross 308. *African Journal of Biotechnology,* 11, 15596-15599.

KOLESÁROVÁ, A., BAKOVÁ, Z., CAPCÁROVÁ, M., GARLIK, J., JURACEK, M., SIMKO, M., TOMAN, R., SIROTKIN, A.V. 2012. Consumption of bee pollen affects rat ovarian functions. *Journal of Animal Physiology and Animal Nutrition,* 9, 1-7.

KOLESÁROVÁ, A., CAPCÁROVÁ, M., BAKOVÁ, Z., GÁLIK, B., JURÁČEK, M., ŠIMKO, M., SIROTKIN, A. 2011. The effect of bee pollen on secretion activity, markers of proliferation and apoptosis of porcine ovarian granulosa cells *in vitro*. *Journal of Environmental Science and Health, Part B. Pesticides, Food Contaminants, and Agricultural Wastes,* 46, 207-212.

MIZRAHI, A., LENSKY, Y. 1997. Bee Products: Properties, Aplications and Apitherapy. Springer-Verlag: New York, USA.

MORAIS, M., MOREIRA, L., FEÁS, X., ESTEVINHO, L.M. 2011. Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. *Food and Chemical Toxicology,* 39, 1096-1101.

MOREIRA, L., DIAS, G., PEREIRA, E. 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugam honey. *Food and Chemical Toxicology,* 46, 3774-3779.

ORZAEZ VILLANUEVA, M.T., DIAZ MARQUINA, A., BRAVO SERRANO, R., BLAZQUEZ ABELIAN, G. 2002. The importance of bee-collected pollen in the diet: a study of its composition. *International Journal of Food Sciences Nutrition,* 53, 217-224.

PETRUŠKA, P., TUŠIMOVÁ, E., KALAFOVÁ, A., HAŠČÍK, P., KOLESÁROVÁ, A., CAPCÁROVÁ, M. 2012. Effect of propolis in chicken diet on selected parameters of mineral profile. *Journal of Microbiology, Biotechnology and Food Sciences,* 1, 593-600.

SHOSKES, D.A. 2002. Phytotherapy in chronic prostatitis. *Urology,* 60, 35-37.

SILVA, T.M.S., CAMARA, C.A., LINS, A.C.S., BARBOSAFILHO, J.M., SILVA, E.M., FREITAS, B.M., SANTOS, F.A.R. 2006. Chemical composition and free radical scavenging activity of pollen loads from stingless bee Melipona subnitida Ducke. *Journal of Food Composition and Analysis,* 19, 507-511.

WANG, J., LI, S., WANG, Q., XIN, B., WANG, H. 2007. Trophic effect of bee pollen on small intestine in broiler chickens. *Journal of Medicinal Food,* 10, 276-280.

WOJCICKI, J., SAMACHOWIEC, L., BARTLOMOWICZ, B., HINEK, A., JAWORSKA, M., GAWRONSKA-SZKLARZ, B. 1986. Effect of pollen extract on the development of experimental atherosclerosis in rabbits. *Atherosclerosis,* 62, 39-45.

YAMAGUCHI, M., HAMAMOTO, R., UCHIYAMA, S., ISHIYAMA, K., HASHIMOTO, K. 2006. Anabolic effects of bee pollen *Cistus ladaniferus* extract on bone components in the femoral diaphyseal and metaphyseal tissues of rats *in vitro* and *in vivo*. *Journal of Health Science,* 52, 43-49.

YAMAGUCHI, M., IGARASHI, A., UCHIYAMA, S., MORITA, S., SUGAWARA, K., SUMIDA, T. 2004. Prolonged intake of juice (*Citrus unshiu*) reinforced with β-cryptoxanthin has an affect on circulating bone biochemical markers in normal individuals. *Journal of Health Science,* 50, 619-624.

YAMAGUCHI, M., UCHIYAMA, S., NAKAGAWA, T. 2007. Preventive effects of bee pollen *cistus ladaniferus* extract on bone loss in ovariectomized rats *in vivo*. *Journal of Health Science,* 53, 571-575.

ZHANG, X., HABIB, F.K., ROSS, M., BURGER, U., LEWENSTEIN, A., ROSE, K., JATON,J. 1995. Isolation and characterization of a cyclic hydroxamic acid from a pollen extract, which inhibits cancerous cell growth *in vitro*. *Journal of Medicinal Chemistry*, 38, 735-738.