BEE PRODUCTS EFFECT TO MICROBIAL COLONIZATION OF CHICKENS GASTROINTESTINAL TRACT

Miroslava Kačániová, Peter Haščík, Lukáš Hleba, Jaroslav Pochop, Martin Melich, Vladimíra Kňazovická, Jadža Lejková, Henrieta Arpášová

ABSTRACT

The general objective of this study was to examine the effect of bee pollen and propolis on the microbial colonization of chickens. In this experiment, quantitative counts of individual groups of microorganisms in ceca of 49-day-old chicken (Ross 308) were investigated. Microbiological characteristics were represented by CFU of coliforms bacteria, faecal Enterococci and Lactobacilli determined in 1 g of chyme. Counts of coliforms bacteria in CFU in 1 g of faecal chyme were determined on McConkey agar, counts of CFU of faecal Enterococci on Slanetz-Bartley agar and counts of Lactobacilli on MRS agar. Counts of CFU of coliforms bacteria, faecal Enterococci and Lactobacilli were compared in experimental and control treatments, respectively. Theoretical and empirical evidence suggest that the counts of coliforms bacteria CFU would be higher in control treatments and CFU counts of both faecal Enterococci and Lactobacilli as well in control treatments compared to the experimental ones. Similar results were also achieved in our experiments with chicken and turkey. In the trial with chickens after application of bee products in the number of coliforms bacteria, statistically significant differences ($P<0.05$) were found among first (200 mg propolis per 1 kg of feed mixture) and fifth experimental group (400 mg pollen to 1 kg of feed mixture).

Keywords: pollen, propolis, microorganisms, chickens, gastrointestinal tract

INTRODUCTION

Over the last ten years regarding some disadvantages of antibiotics, their substitution with prebiotics, probiotics and natural products have been considered more seriously. One of the regarded candidate group in natural products are flavonoids which are produced in plants **(Croft, 1998; Hassig et al., 1999)** and stored in different forms such as propolis **(Giurgea et al., 1981; Dobrowolski, et al., 1991).** Flavonoids have been used against bacterial, protozoan, and fungal infections for relatively long period **(Harborne et al., 1976; Bagaev, 1978; Lopes et al., 1998).** It is believed that flavonoids form strong ligand complexes with heavy metal atoms of metalloenzymes present in the prokaryotic cells such as phosphatases. Therefore, the bactericidal effect of flavonoids may well be the result of a metabolic perturbation. Pollen is a fine, powder-like material produced by flowering plants and gathered by bees **(Broadhurts et al., 1999; Kolesárová et al., 2010)**. It is considered as a valuable special food with varied enhancing effects on health **(Bogdanov, 2004).** This beehive product also has several useful pharmacological properties, such as antibiotic, antineoplastic, antidiarrhoeati and along with its nutritional composition, antioxidant and antiradical activities **(Fatrcová-Šrámková et al, 2008, 2010; Kolesárová et al., 2010).** Pollen contains nutritional compounds like carbohydrates, proteins, amino acids, lipids, vitamins, minerals and traces of micronutrients **(Sérra-Bonvehi and Esscola-Jorda, 1997).** In addition, pollen contains significant amounts of polyphenolic substances, mainly flavonoids **(Almeid A-Murandian et al., 2005).** Digestive system of broilers, particularly their cecum and ileum contain different species of bacteria **(Vaughan et al., 2000)** which is manipulated by live microbial feed supplements (probiotics) to improve intestinal microbial balance **(Fuller, 1989; La Ragione et al., 2003)**, or using antibiotics to limit their population **(Jones et al., 2003).** In this case decreasing the microbial load of digestive system makes the nutrients more available to the host and improves the weight gain and feed conversion ratio. But regarding the antibiotic side effects **(Roy, et al., 2002)** using the natural products and additives for these purposes seems more logical. Propolis is a concentrated complex of flavonoids and polyphenols which should be considered.

The general objective of this study was to examine the effect of bee pollen and propolis on the microbial colonization of chickens.

MATERIAL AND METHODOLOGY

In this experiment, quantitative counts of individual groups of microorganisms in ceca of 49-day-old chicken were investigated. The trial was carried out on an experimental basis of the Department of Poultry and Small Farm Animals at Slovak Agricultural University in Nitra. The experiment was realized in three-etage cage from the company SALMET. Cage technology has been divided into 6 parts: each cage (11 pcs chicken), i. e. one group of experiments (3 cages), i.e. a total of 33 chickens. Each cage had parameters 70x100 cm.

Experiment of monitoring the impact of pollen and propolis in the form of the extract applied as a feed additive through the feed mixture was realized in half-operating conditions in the experimental operation. Fattening itself went on from 1 to 49 days of chicken age. One-day-old chickens of Ross 308 breed were randomly distributed to 6 groups. Chickens were fed *ad libitum* with standard mixture in two phases of feeding:

HYD-01 starter (powder mixture) Norm-type within 21 days of feeding

HYD-02 growth (powder mixture) Norm-type from 21th day of feeding to the end of feeding (42 days)

Bee products were extracted with ethanol (80%), under reflux condenser at 80 °C during 1 hour. After chilling the mixture was centrifugated and supernatant was evaporated in the vacuum rotary evaporator at temperatures 40-45 °C.

The evaporation residue was dissolved. Residue of bee products was applied to feed mixture.

Dosing of feed additives

Propolis and pollen was administered to both feed mixtures in various amounts in addition to the control group. 1st experiment

Control group: the feed mixture without the addition of propolis and pollen.

1st Experimental group: feed mixture with the addition of 200 mg propolis per 1 kg of compound,

2nd Experimental group: feed mixture with the addition of 300 mg propolis per 1 kg of compound,

3rd Experimental group: feed mixture with the addition of 400 mg propolis per 1 kg of compound,

4th Experimental group: feed mixture with the addition of 400 mg pollen to 1 kg of compound,

5th Experimental group: feed mixture with the addition of 800 mg pollen to 1 kg of compound.

Quantitative microbiological analysis

Applied methods:

Plate diluting method

Determination of CFU counts: Plate diluting method was applied for quantitative CFU counts determination of respective groups of microorganisms in 1 g of substrate.

Gelatinous nutritive substrate in Petri dishes was inoculated with 1 ml of chyme samples pour plate method (*Lactobacillus sp.*) and on surface (*colforms bacteria*, faecal Enterococci) in three replications. Homogenized samples of faecal chyme (chyme was taken to sterile Petri dishes) were prepared in advance by sequential diluting based on decimal dilution system application.

Isolated species, genera and groups of microorganisms and their fundamental identification signs **(Holt et al, 1994).**

The basic statistical values and P value, we evaluated by STATGRAPHIC software.

RESULTS AND DISCUSION

The microbial populations in the gastrointestinal tracts of poultry play a key role in normal digestive processes and in maintaining animal health. Disease and stress induced changes in the physicochemical environment in the gastrointestinal tract, or simple changes in feed management practices can significantly influence the microbial populations and their effects on animal performance and health. In the last five decades, increased knowledge of the factors that influence the activities of microorganisms in the alimentary tract has helped to define the critical role of these symbiotic organisms **(Kačániová et al. 2006).**

Honey and propolis are bee products that have been used for centuries in folk medicine **(Zumla and Lulat, 1989; Gonsales et al., 2006).** Several studies have been conducted to authenticate this 'forklore' on medicinal properties of honey and there has been a renaissance in the use of honey and propolis as medicine in more recent times **(Molan, 1992; Bogdanov, 1997; Fearnlei, 2001).**

The application of bee products influenced faecal Enterococci of chickens showed table 1. The statistically differences of feacal Enterococci between groups showed table 2. In the trial with chickens after application of bee products, no statistically significant differences were found. The highest count of feacal Enterococci was found in the forth group where 400 mg of pollen to 1 kg was added to feed mixture. The lower count of faecal Enterococci was found in the control group.

Table 1 Summary statistical values for *Enterococcus* spp.

Values/Groups	K	P1	P2	P3	P4	P5
Average	6.87	7.71	7.50	7.60	7.85	7.74
Standard deviation	0.09	0.17	0.24	0.36	0.14	0.21
Coeff. of variation (%)	1.24	2.16	3.22	4.76	1.84	2.72
Minimum	6.78	7,56	7.23	7.26	7.69	7.56
Maximum	6.95	7,89	7.69	7.98	7.97	7.97

Table2 P-values and T-test for *Enterococcus* spp.

Groups	P1	P2	P3	P4	P5
K	0.5244 (-)	0.2654 (-)	0.6631 (-)	0.5769 (-)	0.4524 (-)
P1	N	0.7899 (-)	0.1387 (-)	0.8987 (-)	0.072 (-)
P2	N	N	0,9285 (-)	0,3114 (-)	0,7179 (-)
P3	N	N	N	0.76 (-)	0.2107 (-)
P4	N	N	N	N	0.9707 (-)

The main mechanism regulating the microbial ecology in the gut of chickens and the importance that changes in the intestinal microflora play in birds are still poorly understood **(Rubio et al., 1998).** There has been an upsurge in interest in the role that the normal intestinal flora, both aerobic and anaerobic plays in protecting against *Salmonella* infection **(Barnes et al., 1979; Corrier et al., 1995; Holista et al., 1999).** Paired caeca are situated at the junction of the small and large intestine and they normally contain a stable population of bacteria of very many different types **(Barnes 1982; Fuller, 1984).** The flow rate is low in these regions which would allow for greater microbial multiplication in the lumen **(Savage 1983).** It is impossible to make detail microbial analysis of this heterogenous composite because the estimation of the most representative bakteria will do satisfactory notion for gastrointestinal microflora **(Simon and Gorbach, 1984).**

The application of bee products influenced Lactobacilli number of chickens showed table 3. The statistically differences of Lactobacilli number between groups showed table 4. In the trial with chickens after application of bee products, no statistically significant differences were found. The lowest count was detected in the control experimental group. The highest count was detected in the first experimental group where was 200 mg of Propolis added to 1 kg of feed mixture.

It was also around this time that the *Lactobacillus* spp. and Bifidobacteria were established in low concentrations. The mechanism for this changes in bacteria has not been defined.

Lactobacilli and Bifidobacteria are predominant in the caecal contents in the healthy chickens and may be their presence is considered clinical for maintaining the ecological balance of the caecal microflora **(Kokosharov, 2001).**

Table 3 Summary statistical values for *Lactobacillus* spp.

Values/Groups	K	P1	P2	P3	P4	P5
Average	7.12	8.70	8.48	8.40	8.45	8.51
Standard deviation	0.38	0.20	0.22	0.25	0.22	0.26
Coeff. of variation (%)	5.31	2.30	2.61	2.99	2.61	3.07
Minimum	6.89	8.51	8.23	8.25	8.26	8.21
Maximum	7.56	8.91	8.65	8.69	8.69	8.69

Table 4 P-values and T-test for *Lactobacillus* spp.

Groups	P1	P2	P3	P4	P5
K	0.3953 (-)	0.1557 (-)	0.6288 (-)	0.5014 (-)	0.7651 (-)
P1	N	0.2396 (-)	0.976 (-)	0.1061 (-)	0.3698 (-)
P2	N	N	0.7845 (-)	0.3457 (-)	0.6094 (-)
P3	N	N	N	0.8698 (-)	0.6062 (-)
P4	N	N	N	N	0.2637 (-)

Thus population of bacteria within the microflora of the caecum, appear to undergo significant changes fluctuation in number before a dynamic equilibrium is established between the species (14-21days). The demonstration of the clinical symptoms in the infected birds highly correlated with decreased concentration of Lactobacilli and Bifidobacteria and reverse-the number of aerobic and anaerobic bacteria returned to normal levels in correlation with clinical resolution of the disease. It is known that Lactobacilli and Bifidobacteria **(Qin et al., 1995; Robertfroid et al., 1998)** protect against potentially harmful bacteria such as *Salmonella*. Therefore, an increase in the number of these strains will improve the status of microbial ecology in the chicken's gut making it less sensitive to colonization by pathogens. A practical example of this hypothesis can be seen from studies on the therapeutic possibilities of supplementing diets with these bacterial species. The use of native gut microflora **(Silva et al., 1981)** and competitive exclusion culture **(Nisbet et al., 1995)**, which have been contained these bacterial species, partially protect against *Salmonella gallinarum* and it was recommended in geographic areas where poultry production is adversely affected by fowl typhoid newly hatched chicks to be threated with such bacterial cultures.

The application of bee products influenced coliforms bacteria of chickens showed table 5. The statistically differences of coliforms bacteria between groups showed table 6. In the trial with chickens after application of bee products, statistically significant differences were found among first and fifth experimental group. The lowest count was detected in the fourth experimental group where 400 mg of pollen in the feed mixture was applied. The highest counts were achieved in control group.

Table 5 Summary statistical values for coliforms bacteria

Values/Groups	K	P1	P2	P3	P4	P5
Average	7.52	6.95	6.72	6.77	6.49	6.81
Standard deviation	0.46	0,33	0.20	0.10	0.28	0.08
Coeff. of variation (%)	6.16	4.78	2.93	1.41	4.36	1.10
Minimum	7.13	6.74	6.54	6.71	6.25	6.77
Maximum	8.03	7.33	6.93	6.88	6.80	6.90

Table 6 P-values and T-test for coliforms bacteria

Groups	P1	P2	P3	P4	P5
K	0.8765 (-)	0.7346 (-)	0.1477 (-)	0.303 (-)	0.8478 (-)
P1	N	0.3889 (-)	0.7288 (-)	0.8204 (-)	0.0287 (+)
P2	N	N	0.8823 (-)	0.4315 (-)	0.4177 (-)
P3	N	N	N	0.4508 (-)	0.70 (-)
P4	N	N	N	N	0.8492 (-)

The microbial population of chicken ileum increases with age, and at the first week lactobacillus is predominant, but gradually the *E. coli* and clostridium level increases in control group. Comparing the total population shows propolis significantly ($P<0.05$) and dose dependently controls the microbial load, particularly the *E. coli* and clostridium rates. These findings confirmed our previous studies that propolis improves the performance and immunity in broilers **(Rahmani et al., 2005).**

It also supports the antibacterial effect of propolis as a flavonoid complex (**Harborne et al., 1976; Bagaev, 1978; Lopes et al., 1998)**, *in vivo* circumstances of broilers which contain different kinds of microorganisms **(Vaughan et al., 2000).** In this relation propolis as a natural additive might be a candidate for controlling the microbial content of broilers GIT instead of probiotics **(Fuller, 1989; La Ragione et al., 2003)** or antibiotics **(Jones et al., 2003)**, but further researches are essential to evaluate propolis fractions in this relation.

CONCLUSION

In general, literature seems little focused on this topic. Instead, more attention had been devoted to the investigation of zootechnical characteristics as, for example, growth ability of animals, feed conversion, general well-being of animals. Hence, a selection of bee products is important. The present testing of a wide spectrum of randomly chosen natural and sometimes even collection bee products suplements is time-consuming and does not solve the problem sufficiently quickly. Apparently, a proper way of solving the problem seems to be primary laboratory testing of the bee products and their mutual antagonisms of the bacteria. It is obvious that commercial preparations have to be standardized, produced in an appropriate applicable form

and containing a declared number of exactly defined bee products.

REFERENCES

ALMEID, A. - MURANDIAN, L. B. - PAMPLONA, L. C. - COIMBRA, S. - BARTH, O. M. 2005. Chemical composition and botanical evaluation of dried bee pollen pellets. In *J. Food Compos. An.*, vol. 18, 2005, no. 1, p. 105-111.

BAGAEV, IUN. 1978. Flavonoids in the complex treatment of patients with pulmonary tuberculosis. (Russian). In *Probl Tuberk*, 1978, no. 12, p. 74-75.

BARNES, E. 1982. *Salmonella* colonisation of the chick. In *Poultry World*, 1982, no. 135, p. 21-21.

BARNES, E.M. – IMPEY, C.S. – COOPER, D.M. 1980. Competitive exclusion of *Salmonella* from the newly hatched chick. In *Vet. Record.*, 1980, no. 160, p. 61-66.

BOGDANOV S. 1997. Nature and origin of the antibacterial substances in Honey. In *Lebensm Wiss. Technol.*, vol. 30, 1997, p. 748 - 753.

BOGDANOV, S. 2004. Quality and standards of pollen and beeswax. Apia. 2004, no. 38, p. 334-341.

BROADHURTS, C. L. 1999. Bee Products: Medicine from the Hive. In *Nut. Sci. New.* 1999, no.4, p. 366-368.

CROFT, K.D. 1998. The chemistry and biological effects of flavonoids and phenolic acids. In *Ann N Y Acad Sci*, 1998, no. 854, p. 435-442.

DOBROWOLSKI, J.W. - VOHORA, S.B. - SHARMA, K. - SHAH, S.A. – NAGVI, S.A. - DANDIYA, P.C. 1991. Antibacterial, antifungal, antiamoebic, anti-inflammatory and antipyretic studies on propolis bee products. In *J. Ethnopharmacology*, 1991, no. 35, p. 72-82.

FATRCOVÁ-ŠRÁMKOVÁ, K. - NÔŽKOVÁ, J. - KAČÁNIOVÁ, M. - MARIASSYOVÁ, M. - KROPKOVÁ, Z. 2010. Microbial properties, nutritional composition and antioxidant activity.. In. *Ecol. Chem.*, vol. 17, 1 2010, no. 1, p. 45-54.

FATRCOVÁ-ŠRÁMKOVÁ, K. - NÔŽKOVÁ, J. - MARIASSYOVÁ, M. - KAČÁNIOVÁ, M. - DUDRÍKOVÁ, E. 2008. Content of polyphenols and antiradical activity of bee pollen. In *Chem. Lis.* 2008, no.102, p. 265-1309.

FEARNLEY J. 2001. *Bee Propolis: Natural Healing from the Hive*. Souvenir Press Ltd. Great Russell Street : London WC1B 3PD, 2001, p. 24-59.

FULLER, R. 1984. Microbail activity in the alimentary tract of birds. In *Proc.Nutr. Soc.*, 1984, no. 43, p. 55-61.

FULLER, R. 1989. Probiotics in man and animals. In *J. Appl. Bacteriol.*, 1989, no. 66, p. 365-378.

GIURGEA, R. - TOMA, V. - POPESCU, H. - POLINICENCU, C. 1981. Effects of standardized propolis extract on certain blood constituents. In *Clujul Medical*, vol. 54, 1981, no. 2, p. 33-36.

GONSALES, G.Z. - ORSI R.O. - FERNANDES, A. - RODRIGUES, P. – FUNARI, S.R.C. 2006. Antibacterial activity of propolis collected in different regions of Brazil. In J. *Venom. Anim. Toxins incl. Trop. Dis.*, vol. 12, 2006, no. 2, p. 276 – 284.

HARBORNE, J.B. - INGHAUS, J.L. - KING, I. - PAYNE, M. 1976. The isopentenyl isoflavone luteone as a proinfectional antifungal agent in the genus lupines. In *Phytochemistry*, 1976, no. 15, 1485 p.

HASSIG, A. - LIANG, W.X. - SCHWABL, H. - STAMPFLI, K. 1999. Flavonoids and tannins: plant-based antioxidants with vitamin character. In *Med Hypotheses*, 1999, no. 52, p. 479-481.

HOLLISTER, A.G. – CORRIER, D.E. - NISBET D.J. - DELOACH J.R. 1999. Effects of chickens-derived caecal microorganisms maintained in continuous culture of caecal colonization by *Salmonella Typhimurium* in turkey poults. In *Poultry Sci*., 1999, no. 78, p. 546-549.

HOLT, G. J. - KRIEG, N. R. - SNEATH, P. H. A. - STALEY, J. T. - WILLIAMS, S. T. 1994. *Bergey' s Manual of Determinative Bacteriology*. 1994. Williams and Wilkins: Baltimore, 1994, 787 p.

JONES, F.T. - RICKET, S.C. 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. In *Poult. Sci*., 2000, no. 82, p. 613-617.

KAČÁNIOVÁ, M. - KMEŤ, V. – ČUBOŇ, J. 2006. Effect of Enterococcus faecium the Digestive Tract of Poultry as a Probiotic Turk. In *J. Vet. Anim. Sci*., vol. 30, 2006, p. 291-298.

KOKOSHAROV, T. 2001. Some observations on the caecal microflora of the chickens during experimental acute fial typhoid. In *Revue Méd. Vét.,* vol. 7, 2001, no.152, p. 531-534.

KOLESÁROVÁ, A. - CAPCAROVÁ, M. - BAKOVÁ, Z. - GALÍK, B. - JURAČEK, M. - ŠIMKO, M. – SIROTKIN, A.V. 2010. The effect of bee pollen on secretion activity, markers of proliferation and apoptosis of porcine ovarian granulosa cells *in vitro.* In *Journal and Enviromental and Health Science*, 2010, in press.

LA RAGIONE, R.M. - WOODWARD, M.J. 2003. Competitive exclusion by Bacillus subtilis spores of Salmonella enterica serotype enteritidis and Clostridium perfringens in young chickens. In *Vet. Microbiol*., 2003, no. 94, p. 245-256.

LOPES, N.P. - CHICARO, P. - KATO, M.J. - ALBUQUERQUE, S. - YOSHIDA, M. 1998. Flavonoids and lignans from Virola surinamensis twigs and their in vitro activity against Trypanosoma cruzi. In *Planta Med*., 1998, no. 64, p. 667-668.

MOLAN, P.C. 1992. The antibacterial activity of honey: the nature of the antibacterial activity. In *Bee World*, vol. 73, 1992, p. 5 – 28.

MULI, E.M – MAINGI, J.M. – MACHARIA, J. 2008. Antimicrobial Properties of Propolis and Honey from the Kenyan Stingless bee, Dactylurina Schimidti. In *Apiacta*, vol. 43, 2008, p. 49 – 61.

NISBET, D.J. – TELLER, G.I. – LOWRY, V.K. – ANDERSON, R.C. – CARSIA, G. – NAVA, G. – KOGUT, M.H. – CORRIER, D.E. – TANKER, L.H. 1998. Effect of commercial competitive exclusion culture (Preempt) on mortality and horizontal transmission of *Salmonella Gallinarum* in broiler chickens. In *Avian Dis*., 1998, no. 42, p. 651-656.

ORRIER, D.E. – NISBET, D.J. – SCANLAN, C.M. - HOLLISTER, A.G. - DELOACH J.R. 1995. Control of *Salmonella Typhimurium* colonization in broiler chicks with continuous-flow characterized mixed culture of caecal bacteria. In *Poultry Sci*., 1995, no. 74, p. 916-924.

QIN, Z.R. – FUKATA, T. – BABA, E. – ARAKAWA, A. 1995. Effect of laktose and *Lactobacillus acidophilus* on the colonization of *Salmonella Enteritidis* in chicks currently infected with *Eimeria tenella.* In *Avian Dis*., 1995, no. 39, p. 548-553.

RAHMANI, H.R. - RAHMANI1, A. - TABATABAEI, Z. – EDRISS, M.A . 2005. *World Poultry Science Association, 15th European Symposium on Poultry Nutrition*, Balatonfüred, Hungary , 25-29 September, 2005. http://www.cabi.org/animalscience/Uploads /File/AnimalScience/additionalFiles/WPSAbalatonfured/1-18Opening%20pages.pdf

ROBERFROID, M.B. - VAN LOO, J.E. – GIBSON, G.R. 1998. The bifidogenic nature of chicory inulin and its hydrolisys products. In *J. Nutr.*, 1998, no. 128, p. 11-17.

ROY, P. - DHILLON, A.S. - LAUERMAN, L.H. - SCHABERG, D.M. - BANDLI, D. - JOHNSON, S. 2002. Results of salmonella isolation from poultry products, poultry, poultry environment, and other characteristics. In *Avian Dis*., 2002, no. 46, p. 17-24.

RUBIO, L.A. – BRENES, A. – SETIEN, I. – ASUNSION, G. – DURAN, N. – CUTULI, M.T. 1998. Lactobacilli count in crop, ileum and caecum of growing broilers chickens fed on practical diets containing whole or dehulled sweet lupin (*Lupinus angustifolius*) seed meal. In *British Poultry Sci*., 1998, no. 39, p. 354-359.

SAVAGE, D.C. 1983. Mechanisms by which indigenous microorganisms colonize gastrointestinal epithelial surfaces. In *Prog. Fd. Nutr. Sci*., 1983, no.7, p. 65-74.

SERRA-BONVEHÍ, J. - ESCOLA-JORDA, R. 1997. Nutrient composition and microbiological quality of honeybee-collected pollen in Spain. In *J. Agri. Food Chem.* Vol. 45, 1997, no. 3, p. 725-732.

SILVA, E.N. – SNOENBOS, G.N. – WEINACK, O.M. – SMYSER, C.F. 1981. The influence of native gut microflora on the colonization and infection of *Salmonella Gallinarum* in chickens. In *Avian Dis*., 1981, no. 25, p. 68-74.

SIMON, G.L. – GORBACH, S.L. 1984. Intestinal flora in health and disease. In *Gastroenterology*, 1984, no. 86, p. 174-193.

VAUGHAN, E.E. - SCHUT, F. - HEILING, H.G.H.J. - ZOETENDAL, E.G. - DE VOS, W.M. - AKKERMANS, A.D. 2000. A molecular view of the intestinal ecosystem. In *Curr. Issues in Intest. Microbiol*., 2000, no. 1, p. 1-12.

ZUMLA, A. - LULAT A. 1989. HONEY – a remedy rediscovered. In *Journ. of Royal Soc. Of Medicine*, vol. 82 , 1989, p. 384 – 385.

Acknowledgments:
This work was funded by Grant Agency VEGA 1/0372/09 and KEGA. 430-014SPU-4/2010.

Contact address:
doc. Ing. Miroslava Kačániová, PhD., Department of Microbiology, FBFS, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 641 4494, e-mail: miroslava.kacaniova@uniag.sk

doc. Ing. Peter Haščík, Department of Animal Products Evaluation and Processing, FBFS, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 641 4708, e-mail: peter.hascik@uniag.sk

Ing. Jaroslav Pochop, Department of Microbiology, FBFS, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 641 5814, e-mail: pochop.jaroslav@gmail.com

Ing. Lukáš Hleba, Department of Microbiology, FBFS, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 641 5814, e-mail: lukas.hleba@gmail.com

Ing. Martin Melich, Department of Microbiology, FBFS, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 641 5820 , e-mail: matko7903@gmail.com

Ing. Vladimíra Kňazovická, Department of Microbiology, FBFS, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 641 5812, e-mail: vladimira.knazovicka@uniag.sk

Ing. Jadža Lejková, Department of Microbiology, FBF, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 6415820, e-mail: j.lejkova@gmail.com

Ing. Henrieta Arpášová, PhD. Department of Poultry Science and Small Animal Husbandry, FAFR, SUA in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Tel.: 037 6414314, e-mail: henrieta.arpasova@uniag.sk