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Original Paper

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Influence of propolis extract in Hubbard JV chickens nutrition on oxidative stabilty of meat

Peter Haščík*, Jozef Garlík, Ibrahim Omer Elamin Elimam, Vladimíra Kňazovická, Marek Bobko, Miroslava Kačániová

Slovak University of Agriculture in Nitra, Slovakia

In the experiment we evaluated the influence of propolis extract in nutrition of chickens on stability of meat in the most valuable parts of carcass that were stored by freezing at -18 °C. A total of 300 chicks in one day old which were divided into 3 groups (n = 100). The hybrid combination of tested chickens was Hubbard JV. Propolis extract was added to experimental groups at a dose of 600 mg kg^{-1} (group E1) and 800 mg kg^{-1} (group E2). Fattening lasted 42 days. Oxidative stability of breast and thigh muscles was evaluated from the 1st day to 6th month of storage in regular month intervals. In the breast muscle, values of malondialdehyde (MDA) were from 0.212 to 0.313 mg kg^{-1} in control group, from 0.186 to 0.264 mg kg^{-1} in E1 group and from 0.175 to 0.259 mg kg^{-1} in E2 group. In the thigh muscle, MDA values were from 0.255 to 0.339 mg kg^{-1} (control group), from 0.196 to 0.279 mg kg^{-1} (E1 group) and from 0.194 to 0.268 mg kg^{-1} (E2 group). Application of propolis extract in Hubbard JV chickens nutrition has significant influence ($P \leq 0.05$) on decreasing of oxidative processes in breast and thigh muscles during the whole period of storage.

Keywords: chickens, nutrition, feed supplements, propolis, chicken meat, oxidative stability, malondialdehyde

1. Introduction

In recent years, poultry industry has grown very fast because of relatively low production costs, shortening of fattening period in particular poultry species, high nutritional value of meat and the large number of products offered to consumers (Barbut, 2002). Broiler chickens are the most abundant animal species in breeding and meat of broiler chicken is used for poultry products and foodstuffs (Perry et al., 2002; Moreki et al., 2010). In recent period, after the ban of antibiotics and coccidiostats in poultry nutrition in EU, different alternative supplements, e. g. probiotics, prebiotic preparations, plant essential oils and their extracts, enzymatic preparations and bee products (pollen, propolis or their extracts), have begun to use for their positive influence on health state, feed utilization, nutritional and sensory quality of product as well as economics of poultry industry production (Prytzyk et al., 2003; Wang et al., 2004; Haščík et al., 2005ab, 2007; Shalmany and Shivazad, 2006; Seven et al., 2008).

Vennat et al. (1995) stated, that propolis contains large amount of flavonoids and shows wide range of activities. Banková et al. (2002) and Moura et al. (2009) warned that propolis samples differ in content of these substances and result in different biological effects. Some research studies confirm that propolis has always antimicrobial properties regardless to its source and composition, although different chemical substances are mostly responsible for antimicrobial activity (Trusheva et al., 2006). In many experiments, different effects to immune system were discovered, e. g. propolis affects increasing of macrophages activity (Dimov et al., 1991). Nagei et al. (2003) and Kumazawa et al. (2004) have described antioxidant and anti-inflammatory properties of propolis. Namgoong et al. (2004) warn, that effects of propolis are related to inhibition of prostaglandins synthesis.

In term of nutritional composition, sensory properties and antioxidant stability, it is necessary to evaluate the carcass product, not only the production of poultry meat (Garlík et al., 2011). Antioxidant stability is important for storage and durability of meat, because the poultry meat and mainly the poultry fat contain higher amount of polyunsaturated fatty acids in compare with fat of other slaughtered animals (Bystrický and Dičáková, 1998; Martinez-Tome et al., 2001).

The aim of the study was to test the commercial feed mixtures supplemented by propolis extract and to verify its influence on oxidative stability of the most valuable parts of Hubbard JV chicken carcass during the storage process in freezer.

***Correspondence:** Peter Haščík, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Products Evaluation and Processing, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, e-mail: peter.hascik@uniag.sk

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2. Material and methods

The experiment was under taken in poultry test station Zámostie Company (Slovak republic). The testing chickens were Hubbard JV hybrid combination. Totally, 300 one-day-old chickens were included in the experiment and there were created three groups: control group – C (without propolis extract application) and two experimental groups (E1 and E2) with different doses of propolis extract. Each group consisted of 100 chickens. Fattening lasted 42 days. Chickens were bred using technology on deep litter (wood shavings).

Feed mixture was administered by the tube feeders. Feed mixtures, applied in this experiment, were prepared and mixed according to requirements of Gazette of Ministry of Agriculture and Rural Development (2004) in Biofeed Company with the seat in Kolárovo (Slovak Republic). Feed mixtures were analysed in term of basic nutrients and energy value at the Department of Animal Nutrition (Faculty of Agrobiology and Food Resources, SUA in Nitra). Composition of feed mixtures is in the table 1.

Feed was manually administered in regular intervals each day. Chickens were fed by *ad libitum* system. They were fed to 21st day by the same starter feed mixture HYD-01 (powder form) and from 22nd to 42nd day of fattening, they were fed by the same feed mixture HYD-02 (powder form) in all evaluated groups. Feed mixtures HYD-01 and HYD-02 were produced without antibiotics and coccidiostats. Nutritional value of feed mixtures was the same in all groups during the experiment, but experimental groups had feed mixtures HYD-01 and HYD-02 supplemented by propolis extract at a dose of 600 mg kg^{-1} (E1) and 800 mg kg^{-1} (E2). Propolis extract was prepared from milled propolis originated in Slovak Republic. Milled propolis was mixed with 80 % ethanol (Krell, 1996). Propolis solution was extracted in water bath at 80 °C under the reflux for 1 hour. After the extraction, mixture was cooled and centrifuged. Obtained supernatant was evaporated on rotary vacuum evaporator at the temperature of water bath 40–50 °C and then it was weighted. The evaporation residue at

Table 1 Composition of the diets

Ingredients in %	Starter (1 to 21 days of age)	Grower (22 to 42 days of age)
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48 % N)	21.30	18.70
Fish meal (71 % N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysin	0.05	0.07
Methionin	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5 %*	0.50	0.50
Analysed composition in g kg^{-1}		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
Linoleic acid	13.51	14.19
MEN in MJ kg^{-1} by calculation	12.02	12.03

* active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 50 000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg

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amount of 60 and 80 g was separately solved in 1000 cm^3 of ethanol (80% concentration) and applied to 100 kg of feed mixture for the experimental group of Hubbard JV chickens. Water was administered *ad libitum* by self-powered system using nipple drinkers with drip tray.

At the end of fattening (42nd day), 60 pieces of chickens (30 male chickens and 30 female chickens) from each group in experiment were selected for slaughter analysis. To determine the degradation changes of fat (determination of thiobarbituric number - TBA), samples of breast and thigh muscles without bones were packed to polyethylene bags and stored at -18 °C for 6 months. TBA values, expressed as malondialdehyde amount, were determined in the 1st day and 1st, 2nd, 3rd, 4th, 5th and 6th months of storage process. TBA number was found using method according to Marcinčák et al. (2004). Absorbance of samples was measured on UV-VIS spectrophotometer T 80 (PG Instruments Limited, Great Britain) at wave length of 532 nm and result was calculated on malondialdehyde (MDA) amount in 1 kg of sample.

Results (mean and standard error) were processed in statistical programme Statgraphics 5.0. *F*-test followed by *t*-test was used for determination of significant differences between the groups of experiment.

3. Results and discussion

Results of oxidative stability in breast and thigh muscles of Hubbard JV chickens during the storage at -18 °C for 6 months are in the table 2.

Results are expressed as TBA number and evaluated by malondialdehyde (MDA) amount. In the 1st day of storage, MDA values were low in the breast muscle (from

Table 2 Effect of storage in freeze (-18 °C) on the concentration of malondialdehyde in mg kg^{-1} in breast and thigh muscle (mean ± SE)

Time of storage	Group			S
	control (no propolis added)	E1 (propolis extract 600 mg kg^{-1})	E2 (propolis extract 800 mg kg^{-1})	
Breast muscle				
Day – 1	0.212a±0.025	0.186b±0.020	0.175b±0.011	C : E1* C : E2**
Month – 1	0.247a±0.007	0.206b±0.037	0.202b±0.018	C : E1* C : E2***
Month – 2	0.261a±0.024	0.216b±0.013	0.217b±0.021	C : E1*** C : E2**
Month – 3	0.279a±0.037	0.241b±0.013	0.230b±0.024	C : E1* C : E2**
Month – 4	0.289a±0.013	0.246b±0.007	0.238b±0.005	C : E1*** C : E2***
Month – 5	0.299a±0.014	0.249b±0.007	0.247b±0.011	C : E1*** C : E2***
Month – 6	0.313a±0.008	0.264b±0.020	0.259b±0.026	C : E1*** C : E2***
Thigh muscle				
Day – 1	0.255a±0,026	0.196b±0,022	0.194b±0,012	C : E1*** C : E2***
Month – 1	0.271a±0.013	0.223b±0.018	0.214b±0.019	C : E1*** C : E2***
Month – 2	0.287a±0.027	0.241b±0.021	0.242b±0.013	C : E1** C : E2**
Month – 3	0.299a±0.020	0.243b±0.021	0.243b±0.019	C : E1*** C : E2***
Month – 4	0.314a±0.014	0.244b±0.016	0.252b±0.020	C : E1*** C : E2***
Month – 5	0.326a±0.021	0.264b±0.013	0.258b±0.029	C : E1*** C : E2***
Month – 6	0.339a±0.033	0.279b±0.026	0.268b±0.020	C : E1** C : E2***

a, b – means with different superscripts differ significantly, determined by t test, S – siginificance (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS – not significant), SE – standard error

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0.175 mg kg^{-1} in E2 to 0.212 mg kg^{-1} in C) as well as in the thigh muscle (from 0.194 mg kg^{-1} in E2 to 0.255 mg kg^{-1} in C). These results are in accordance with statements of Marcinčák et al. (2010).

In further storage of breast muscle, MDA values were increased gradually: after the 1st month, we found 0.247 mg kg^{-1} in control group, 0.206 mg kg^{-1} in E1 and 0.202 mg kg^{-1} in E2. And after the 1st month of thigh muscle storage, we found followed MDA values: 0.271 mg kg^{-1} in control group, 0.223 mg kg^{-1} in E1 and 0.214 mg kg^{-1} in E2. MDA values were higher in further months of breast and thigh muscles storage by freezing. In the 5th month, we found the highest MDA value in control group (0.299 mg kg^{-1} – breast muscle, 0.326 mg kg^{-1} – thigh muscle), the lower MDA value in E1 (0.249 mg kg^{-1} – breast muscle, 0.264 mg kg^{-1} – thigh muscle) and the lowest MDA value in E2 (0.247 mg kg^{-1} – breast muscle, 0.258 mg kg^{-1} – thigh muscle). Experimental group E2 was the group with the highest amount of propolis extract in Hubbard JV chicken nutrition.

After the 6 months of storage, tendency of increasing MDA values was confirmed and the highest average concentration of MDA in breast muscle was found in control group (0.313 mg kg^{-1}), the lower MDA value in E1 (0.264 mg kg^{-1}) and the lowest MDA value in E2 (0.259 mg kg^{-1}). Significant differences ($P \leq 0.05$) between the control and experimental groups were found in oxidative stability of breast muscle from the 1st day. The breast muscle of E2 group (after the application of 800 mg kg^{-1} propolis extract in Hubbard JV chickens nutrition during the whole period of fattening) can be considered as the most stable breast muscle.

After 6 months of storage in freezer, results of thigh muscle were very similar as in the breast muscle. We found the higher MDA values in control group (0.339 mg kg^{-1}) in compare with experimental groups (0.279 mg kg^{-1} – group E1 and 0.268 mg kg^{-1} – group E2). Significant differences in oxidative stability of thigh muscle between the control and experimental groups were found from the 1st day of storage. Thigh muscle of E2 group (with the highest dose of propolis extract) was considered as the most stabile.

Results of oxidative stability in chicken meat, obtained in this experiment confirm the statements of other researchers (Kennedy et al., 2005; Imik et al., 2010), that oxidative stability of chicken meat decreases by cooling or freezing. Higgins et al. (1998), Jensen et al. (1998), Fellenberg and Speisky (2006) warned that oxidative yellowing of fat is one of the main reasons causing deterioration of human food and this factor is responsible for unpleasant odour, loss of taste, consistency, appearance, nutritional value, pigment, polyunsaturated fatty acids, fat-soluble vitamins, decreases the meat quality and eventually decrease its storability and safety. Different alternative supplements tested in poultry nutrition (including propolis and propolis extract) contain various antioxidants and prevent oxidation of lipids (Govaris et al., 2004; Kennedy et al., 2005; Šperňáková et al., 2007; Marcinčák et al., 2010; Skřivan et al., 2010) and increase the stability of meat during its storage by cooling or freezing. This statement was confirmed by results in this experiment, too.

Sheehy et al. (1993) and Florou-Paneri et al. (2005) stated that higher antioxidant concentration in raw and cooked chicken meat resulted in decreasing of lipid oxidation and TBA values in storing and freezing. It was confirmed in our experiment, in evaluation of breast and thigh muscles of Hubbard JV chickens with application of propolis extract in their nutrition. Results of our experiment did not confirm the results of Šperňáková et al. (2007) and Luna et al. (2010), who applied extract of thymol and carvacrol (powdered rosemary) and found the higher stability of thigh muscle compared with breast muscle. In our experiment, we found opposite effect. It related with higher fat content in thigh muscle as in breast muscle.

4. Conclusions

In the experiment, we tested the influence of propolis extract applied to feed mixtures of Hubbard JV broiler chickens on oxidative stability of the breast and thigh muscles stored at -18 °C for 6 months. During the storage, the highest MDA values were found in control group and the best results (it means the lowest MDA value) were found in E2 group with application of propolis extract at a dose of 800 mg kg^{-1}. Obtained results indicate that propolis extract, tested as a supplement in Hubbard JV chicken nutrition, had a positive effect ($P \leq 0.05$) on storability and oxidative stability of breast and thigh muscles.

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6. References

BANKOVA, V. et al. (2002) Chemical composition of European propolis: expected and unexpected results. In *Zeitschrift für Naturforschung*, vol. 57c, no. 5–6, pp. 530–533.

BARBUT, S. (2002) Poultry meat processing and product technology. In *Industry Guide : Poultry Products Processing*. Boca Raton: CRC Press, pp. 1–29.

BYSTRICKÝ, P. and DIČÁKOVÁ, Z. (1998) Animal fats in food. In *Slovenský veterinársky časopis*, Suppl. 1, vol. 1, no. 23, pp. 1–45 (in Slovak).

DIMOV, V. et al. (1991) Immunomodulatory action of propolis. Influence on anti-infectious protection and macrophage function. In *Apidologie*, vol. 22, no. 2, pp. 155–162.
DOI: http://dx.doi.org/10.1051/apido:19910208.

FLOROU-PANERI, P. et al. (2005) Oregano herb versus oregano essential oil as feed supplements to increase the oxidative stability of turkey meat. In *International Journal of Poultry Science*, vol. 4, no. 11, pp. 866–871.
DOI: http://dx.doi.org/10.3923/ijps.2005.866.871.

Faculty of Agriobiology and Food Resources, 2014
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2014.17.02.47–51

FELLENBERG, M.A. and SPEISKY, H. (2006) Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. In *World's Poultry Science Journal*, vol. 62, no. 1, pp. 53–70. DOI: http://dx.doi.org/10.1079/WPS200584.

GARLÍK, J. et al. (2011) Impact of propolis applications on chemical composition of chicken meat. In *Potravinárstvo*, vol. 5, no. 2, pp. 349–354.

GOVARIS, A. et al. (2004) Dietary versus post-mortem use of oregano oil and/or α-tocopherol in turkeys to inhibit development of lipid oxidation in meat during refrigerated storage. In *International Journal of Food Science and Nutrition*, vol. 55, no. 2, pp. 115–123. DOI: http://dx.doi.org/10.1080/09637480410001666487.

HAŠČÍK, P. et al. (2005a) Relationship between probiotic preparation application and abdominal fat amount in fattening chickens. In *Agriculture*, vol. 51, no. 11, pp. 574–579 (in Slovak).

HAŠČÍK, P. et al. (2005b) Effect of probiotic preparation in feed mixtures on meat chemical composition in broiler chickens Ross 308. In *Acta fytotechnica et zootechnica*, vol. 8, no. 1, pp. 20–24 (in Slovak).

HAŠČÍK, P. et al. (2007) Effect of probiotic on production of fta in body of chickens. In *Abstracts of the International conference of the VII. Slovak conference of animal physiology*, Nitra: Slovak University of Agriculture.

HIGGINS, F. M. et al. (1998) Effect of dietary α-tocopherol acetate supplementation on β-tocopherol distribution in raw turkey muscles and its effect on the storage stability of cooked turkey meat. In *Meat Science*, vol. 50, no. 3, pp. 373–383.

IMIK, H. et al. (2010) Effect of dietary supplementation of some antioxidants on growth performance, carcass composition and breast meat characteristics in quails reared under heat stress. In *Czech Journal Animal Science*, vol. 55, no. 5, pp. 209–220.

JENSEN, C., LAURIDSEN, C. and BERTELSEN, G. (1998) Dietary vitamin E: quality and storage stability of pork and poultry. In *Trends in Food Science and Technology*, vol. 9, no. 2, pp. 62–72. DOI: http://dx.doi.org/10.1016/S0924-2244(98)00004-1.

KENNEDY, O.B. et al. (2005) Vitamin E supplementation, cereal feed type and consumer sensory perceptions of poultry meat quality. In *British Journal of Nutrition*, vol. 93, no. 3, pp. 333–338. DOI: http://dx.doi.org/10.1079/BJN20041336.

KRELL, R. (1996) Value-added products from bee keeping. Milan: FAO, 395 p.

KUMAZAWA, S., HAMASKA, T. and NAKAYAMA, T. (2004) Antioxidant activity of propolis of various geographic origins. In *Food Chemistry*, vol. 84, no. 3, pp. 329–339.

DOI: http://dx.doi.org/10.1016/S0308-8146(03)00216-4.

LUNA, A. et al. (2010) Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. In *Poultry Science*, vol. 89, no. 2, pp. 366–370.

DOI: http://dx.doi.org/10.3382/ps.2009-00130.

MARCINČÁK, S. et al. (2004) Determination of lipid oxidation level in broiler meat by liquid chroatography. In *Journal of AOAC International*, vol. 87, no. 5, pp.1148–1152.

MARCINČÁK, S. et al. (2010) Oxidative stability of chilled chicken meat after feeding of selected plants. In *Potravinárstvo*, vol. 4, no. 3, pp. 46–49. DOI: http://dx.doi.org/10.5219/38.

MARTINEZ-TOME, M. et al. (2001) Antioxidant properties of Mediterranean spices compared with common food additives. In *Journal of Food Protection*, vol. 64, no. 9, pp. 1412–1419.

MOREKI, J.C., DIKEME, R. and POROGA, B. (2010) The role of village poultry in food security and HIV/AIDS mitigation in Chobe District of Botswana. In *Livest. Res. Rural Dev.*, vol. 22, no. 5.

MOURA, S.A., NEGRI, G., SALATINO, A. (2009) Aqueous extract of brazilian green propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. In *eCAM*.

DOI: http://dx.doi.org/10.1093/ecam/nep112.

NAGEI, T. et al. (2003) Preparation and antioxidant properties of water extract of propolis. In *Food Chemistry*, vol. 80, pp. 29–33. DOI: http://dx.doi.org/10.1016/S0308-8146(02)00231-5.

NAMGOONG, S.Y. et al. (1994) Effects of naturally occurring flavonoids on mitogen-induced lymphocyte proliferation and mixed lymphocyte culture. In *Life Science*, vol. 54, no. 5, pp. 313–320. DOI: http://dx.doi.org/10.1016/0024-3205(94)00787-X.

PERRY, B.D. et al. (2002) Investing in animal health research to alleviate poverty. Nairobi: International Livestock Research Institute, 148 p.

PRYTZYK, E. et al. (2003) Flavonoids and trypanocidal activity of bulgarian propolis. In *Journal of Ethnopharmacoly*, vol. 88, no. 2–3, pp. 189–193.

DOI: http://dx.doi.org/10.1016/S0378-8741(03)00210-1.

SEVEN, T.P. et al. (2008) The effect of Turkish propolis on growth and carcass characteristics in broilers under heat stress. In *Animal Feed Science and Technology*, vol. 146, no. 1–2, pp. 137–148. DOI: http://dx.doi.org/10.1016/j.anifeedsci.2007.11.003

SHALMANY, S. and SHIVAZAD, M. (2006) The effect of diet propolis supplementation on Ross broiler chicks performance. In *International Journal of Poultry Science*, vol. 5, no. 1, pp. 84–88. DOI: http://dx.doi.org/10.3923/ijps.2006.84.88.

SHEEHY, P.J.A., MORRISSEY, P.A. and FLYNN, A. (1993) Increased storage stability of chicken muscle by dietary alpha-tocopherol supplementation. In *Irish Journal of Agricultural Food Research*, vol. 32, pp. 67–73.

SKŘIVAN, M. et al. (2010) Effects of different levels of dietary supplemental caprylic acid and vitamin E on performance, breast muscle vitamin E and A, and oxidative stability in broilers. In *Czech Journal of Animal Science*, vol. 55, no. 4, pp. 167–173.

ŠPERŇÁKOVÁ, D. et al. (2007) Effects of dietary use of rosemary powder and a-tocopherol on performance of chicken, inhibition of lipid oxidation during storage at chilling conditions and increasing of meat quality. In *Bulletin of the Veterinary Institute in Pulawy*, vol. 51, pp. 585–589.

TRUSHEVA, B., POPOVA, M., BANKOVA, V., SIMOVA, S., MARCUCCI, M.C., MIORIN, P.L., DAROCHA, P.F., TSVETKOVA, I. (2006) Bioactive constituents of brazilian red propolis. In *eCAM.*, vol. 3, no. 2, pp. 249–254. DOI: http://dx.doi.org/10.1093/ecam/nel006.

VENNAT, B., ARVOUETGRAND, A., GROSS, D., POURRAT, A. (1995) Qualitative and quantitative analysis of flavonoids and identification of phenolic acids from a propolis extract. In *Journal de pharmacie de Belgique*, vol. 50, no. 5, pp. 438–444.

REGULATION of Ministry of Agriculture of Slovak republic from 23th of August 2004 No. 2145/2004-100, which change and supplement regulation of Ministry of Agriculture of Slovak republic from 7th of October 1997 No. 1497/4/1997-100 on offcial sample collection nad laboratory testing and feed evaluation in clang of regulation of Ministry of Agriculture of Slovak republic from 1lth of February 2003, No. 149/2/2003-100, vol. 36, 2004, part 22.

WANG, B.J., LIEN, Y.H., YU, Z.R. (2004) Supercritical fluid extractive fractionation–study of the antioxidant activities of propolis. In *Food Chemistry,* vol. 86, no. 2, pp. 237–243, DOI: http://dx.doi.org/10.1016/j.foodchem.2003.09.031.

Faculty of Agriobiology and Food Resources, 2014
http://www.fapz.uniag.sk/
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