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## EFFECT OF AMYGDALIN ON ANEUPLOIDY INCIDENCE IN RABBIT

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### ABSTRACT

The aim of this study was to examine possible effect of amygdalin substance applied to rabbit by different ways on the occurrence of aneuploidy. Totally 30 adult females of the New Zealand White line were used in the experiment. Animals were divided into four experimental groups and one control group, each consisted of five individuals. In groups P1 and P2, isolated amygdalin substance was administered intramuscularly (P1: 0.6 mg.kg<sup>-1</sup>; P2: 3 mg.kg<sup>-1</sup> of body weight), whilst in P3 and P4 the animals were fed by a mixture of commercial diet and apricot seeds (P3: 60 mg.kg<sup>-1</sup>; P4: 300 mg.kg<sup>-1</sup> of body weight). Experimental treatment lasted one month of continuous amygdalin application (one experimental dose applied per day in each group). Aneuploidy assay was performed on peripheral blood lymphocytes arrested at the metaphase stage by colcemid solution (0.6 µg.ml<sup>-1</sup>). The average occurrence of diploidy versus aneuploidy (presented by hypodiploid nuclei) was detected as follows: P1- 76 % vs 21.6 %; P2 - 72.8 % vs 27.2 %; P3 - 82.4 % vs 17.6 %; P4 - 70.4 % vs 29.6 %; Control - 70.4 % vs 26.8 %. Low incidence of polyploid cells was found in P1 (2.4 %) and control (2.8 %) groups. In conclusion, no significant effect of amygdalin on aneuploidy occurrence in rabbit blood cells was found.

**Key words:** amygdalin; aneuploidy; lymphocyte; chromosome; rabbit

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### INTRODUCTION

Amygdalin, the substance hidden under various nicknames, including: vitamin B17, nitriloside, mandelonitrile, etc. (Fukuda *et al.*, 2003) is widely distributed in plants, especially in the *rosaceus* plant seed, such as: apricot and peach (Santos Pimenta *et al.*, 2014). It can hydrolyze and generate prunasin and mandelonitrile under the glucosidase action, such as amygdalase and prunase, and ultimately decomposed into benzaldehyde and hydrocyanic acid. Amygdalin itself is non-toxic, but its production of hydrocyanic acid decomposed by some enzymes is poisonous substance (Suchard *et al.*, 1998). There are number of studies describing the effect of amygdalin and derived substances on the recipient body. For example: anti-tussive and anti-asthmatic effects by the amygdalin decomposition to hydrocyanic acid which could inhibit the respiratory center to a certain level (Chang *et al.*,

2005; Do *et al.*, 2006); effects on the digestive system by inhibition of the pepsin activity (Song and Xu, 2014); analgesic effect by inhibiting prostaglandins E2 and nitric oxide synthesis (Yang *et al.*, 2007; Paoletti *et al.*, 2013); promoting apoptosis of human renal fibroblast by enhancing the activity of type I collagenase (Guo *et al.*, 2013); improving the immune function of organism by the significant increase of polyhydroxyalkanoates inducing human peripheral blood T lymphocyte proliferation (Baroni *et al.*, 2005); the anti-tumor effect of amygdalin presented by the hydrocyanic acid, which is an anti-tumor compound formed from the amygdalin decomposition (Kwon *et al.*, 2003). In other studies, amygdalin significantly inhibited sperm hyaluronidase activity and spermatozoa motility of bull sperm *in vitro* (Tanyildizy and Bozkurt, 2004). Recent data indicated that amygdalin reduced proliferation potential, decreased mitochondrial activity of cervical cancer cells, accumulated cells in the G1-phase and led to their death

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(Jarocho and Majka, 2011).

Various ways of amygdalin application play a different role in recipient organism, what was confirmed by Moertel *et al.* (1981), who demonstrated in human, that intravenous infusion of amygdalin produced neither cyanidemia nor signs of toxicity, but oral administration resulted in significant blood cyanide levels.

Cytogenetic analysis plays an important role in livestock breeding. The chromosomal abnormalities, caused by various factors, are generally divided into structural and/or numerical changes. Commonly observed aneuploidies are hypo (loss) and hyper (gain) haploidy, as a result of meiotic and mitotic non-disjunction events (Goepfert *et al.*, 2000). Normal organisms are characterised by the presence of aneuploid cells at very low frequency. In rabbits, the rate of aneuploidy varies depending on the tissue of origin, from 5 % in *in vitro* fertilized oocytes (Asakawa *et al.*, 1988), 16-18 % in blood cells (Zartman and Fehheimer, 1967; Parkanyi, 1981), 35-51 % in bone marrow (Parkanyi, 1981; Curlej *et al.*, 2007), up to 56-83 % in embryos (Austin, 1967; Shi *et al.*, 2004; Curlej *et al.*, 2010). Diseases, assisted reproductive technologies, or genetic manipulations might cause an increase in the incidence of aneuploidy (Hegedus *et al.*, 2008).

The present study was focused to reveal the effect of various amygdalin concentrations, administrated by different ways, to evoke the aneuploidy on the rabbit model. To achieve the paper goals chromosomes were recovered from lymphocyte's nuclei isolated from the peripheral blood.

## MATERIAL AND METHODS

### Animals

A total of 30 adult rabbit females (20 individuals assigned to the experimental groups P1-P4 and 10 rabbits selected to control group) of the New Zealand White line were used in the experiment. The animals were bred at the farm of the Research Institute for Animal Production, NAFC Nitra. Prior to start of the experiment, animals were selected according to similar age and good health status which was continuously monitored from the start up to the end of the experimental treatment. Environmental conditions were maintained using a control system for the light (16:8 light/dark photoperiod), air ventilation and the temperature (18-25 °C). Animals were fed *ad libitum* with treated/non-treated commercial rabbit diet. The experiment was lasted one month of continuous amygdalin application (one experimental dose applied per day in each group) as described in the text below.

### Experimental groups

Group P1 (n = 5): intramuscular application of isolated amygdalin substance at the final concentration of 0.6 mg.kg<sup>-1</sup> of body weight.

Group P2 (n = 5): intramuscular application of isolated amygdalin substance at the final concentration of 3 mg.kg<sup>-1</sup> of body weight.

Group P3 (n = 5): feeding by a mixture of commercial diet and apricot seeds at the final concentration of 60 mg.kg<sup>-1</sup> of body weight.

Group P4 (n = 5): feeding by a mixture of commercial diet and apricot seeds at the final concentration of 300 mg.kg<sup>-1</sup> of body weight.

### Chromosome preparation

Venous blood samples were collected aseptically from the intermediate auricular vein (*vena auricularis caudalis*) of the experimental (on the last day of experimental treatment) and control animals using sterile needles and heparinized plastic syringes. Three drops of blood were added to 4 ml of the complete blood cultivation medium PB Max Karyotyping (Gibco BRL). The blood cultures were incubated at 37 °C for 72 h. Colcemide at the final concentration of 0.6 µg.ml<sup>-1</sup> (Gibco BRL) was added 60 min prior to cell harvest. After the hypotonic treatment with 0.075 M potassium chloride solution (Gibco BRL) for 15 min and fixation in modified Carnoy's solution (3 : 1, methanol : acetic acid), the resuspended cells were placed onto frozen glass microslides, air-dried and stained for 6 min with 2 % Giemsa solution (Gibco BRL). Stained microslides were observed under the Leica light microscope. The chromosomal analysis was carried out using 25 c-metaphase spreads per rabbit taken from chromosome microphotographs (Parkanyi *et al.*, 2004).

The  $\chi^2$  test was used for statistical evaluation of the results.

## RESULTS AND DISCUSSION

The study was focused on examining a possible effect of amygdalin substance applied to rabbit at different concentrations and by different ways on the occurrence of aneuploidy. Proportion of the cells with diploid chromosome sets in the cell nuclei was ranged from 70.4 % in the P4 group to 82.4 % in the P1 group of rabbits (Table 1). The same occurrence of diploid cells (70.4 %) was found in the control group. Aneuploidy was represented exclusively by hypodiploid cells for experimental groups as well as for control group. The lowest percentage of aneuploidy was found in P3 rabbits (17.6 %), whilst the P4 group exhibited the highest

**Table 1: Evaluation of chromosomal number from c-metaphase spreads**

Rabbit groups	Diploidy	Hypodiploidy	Polyploidy
Experimental			
P1	76.0 % (n = 95)	21.6 % (n = 27)	2.4 % (n = 3 )
P2	72.8 % (n = 91)	27.2 % (n = 34)	-
P3	82.4 % (n = 103)	17.6 % (n = 22)	-
P4	70.4 % (n = 88)	29.6 % (n = 37)	-
Control			
C	70.4 % (n = 176)	26.8 % (n = 67)	2.8 % (n = 7)

\*n – the number of evaluated metaphase plates

presence of aneuploid nuclei (29.6 %) in comparison to control animals (26.8 %). According to the results of  $\chi^2$ -test, no significant differences were found. Polyploid cells were found at low percentage in P1 (2.4 %) and control (2.8 %) groups.

Previous studies, performed specifically to reveal the rate of chromosome number variability, show that almost all tissues exhibit aneuploid cells (Iourov *et al.*, 2008). The incidence of chromosomal aneuploidies may depend on many factors, such as the source of cells, animal species, animal age, cell culture conditions and genetic manipulations (Czepulkowski, 2001). Aneuploidy in the present study was represented by hypodiploid cells ( $2n < 44$ ). Average value of hypodiploid cells regardless to experimental groups was represented by 24 % (21.6, 27.2, 17.6 and 29.6, respectively). Control group exhibited 26.8 %. These relatively small differences among the groups may be explained by a random selection of evaluated nuclei. Higher proportion of hypodiploid cells (at average 41.47 % and 54.8 %, resp.) for non-treated rabbits have been recorded in the study of bone marrow cells, published by Curlej *et al.* (2007). Parkanyi *et al.* (2004) detected 38 % and 26 % presence of hypodiploid cells (average value 32 %) in the blood lymphocytes of control rabbits. Occurrence of cells with normal chromosome sets (diploid cells) in the present study is represented by average value of 75.4 % for experimental groups (measured values 76 %, 72.8 %, 82.4 %, and 70.4 %, resp.) in comparison to 70.4 % for control group.

Numbers of studies have been focused to reveal potential action of amygdalin substance to cell culture, especially those from cancer tissues. But still there is a lack of scientific records derived from the experiments with the model organisms about effect of amygdalin-derived substances on the structure or number of chromosomes in the cell nuclei. Studies by other authors suggest that the amygdalin in “safety” concentrations

and admission way affect the viability of human cervical cancer HeLa cells (Chen *et al.*, 2013). Such information brings a therapeutic option to use of this substance. Nevertheless, the anti-tumor mechanism of amygdalin is not completely clear (Song and Xu, 2014). Clinical trials and large retrospective studies showed some adverse reactions after large dose application, such as gastrointestinal tract reaction and headache (Barwina *et al.*, 2013; Yang *et al.*, 2013; Karabulutlu, 2014). The ways of amygdalin application to rabbits in our study were chosen basing on the knowledge, that the toxicity of oral administration route is far greater than the intravenous route. The mean lethal dose (LD50) of amygdalin in rats was reported to be 880 mg.kg<sup>-1</sup> body weight by oral administration (Adewusi and Oke, 1985; Park *et al.*, 2013). For the rabbits, mice and dogs the maximum tolerance dose for oral amygdalin intake has been published as 75 mg.kg<sup>-1</sup> (Zhang *et al.*, 1986). We have decided for „safe“ 60 mg.kg<sup>-1</sup> and risky 300 mg.kg<sup>-1</sup> doses. The LD50 of intravenous injection in mice is 25 g.kg<sup>-1</sup>, while intra-peritoneal injection is 8 g.kg<sup>-1</sup>. The maximum tolerance dose of intravenous and intramuscular injection of amygdalin in mice, rabbits and dogs is 3 g.kg<sup>-1</sup> (Rauws *et al.*, 1982). On the basis of this records, intra-muscular doses 0.6 and 3 mg.kg<sup>-1</sup> used in our experiment may be considered as safe. The human’s maximum tolerant dose of intravenous injection is approximately 70 mg.kg<sup>-1</sup> (Rauws *et al.*, 1982). In human, systemic toxicity occurs after oral administration of 4 g amygdalin per day, lasted for half a month or intravenous injection of a month. If the dose is reduced to daily oral doses of 0.6 ~ 1g, it can avoid toxicity (Bromley *et al.*, 2005; O’Brien *et al.*, 2005).

In conclusion, according to our results, amygdalin applied at chosen concentrations orally or intramuscularly, showed no significant adverse effect represented by extra-creation of aneuploid cells in rabbits.

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## REFERENCES

- ADEWUSI, S. R. – OKE, O. L. 1985. On the metabolism of amygdalin. 1. The LD50 and biochemical changes in rats. *Canadian Journal of Physiology and Pharmacology*, vol. 63 (9), 1985, p. 1080–1083.
- ASAKAWA, T. – ISHIKAWA, M. – SHIMIZU, T. – DUKELOW, W. R. 1988. The chromosomal normality of *in vitro* fertilized rabbit oocytes. *Biology of Reproduction*, vol. 38 (2), 1988, p. 292–295.
- AUSTIN, C. R. 1967. Biological mechanisms in aging. *Nature*, vol. 213, 1967, p. 1018–1019.
- BARONI, A. – PAOLETTI, I. – GRECO, R. – SATRIANO, R. A. – RUOCCO, E. – TUFANO, M. A. – PEREZ J. J. 2005. Immunomodulatory effects of a set of amygdalin analogues on human keratinocyte cells. *Experimental Dermatology*, vol. 14 (11), 2005, p. 854–859.
- BARWINA, M. – WIERGOWSKI, M. – SEIN-ANAND, J. 2013. Accidental poisoning with peach seeds used as anticancer therapy—report of two cases. *Przegląd Lekarski*, vol. 70 (8), 2013, p. 687–689.
- BROMLEY, J. – HUGHES, B. G. – LEONG, D. C. – BUCKLEY, N. A. 2005. Life-threatening interaction between complementary medicines: Cyanide toxicity following ingestion of amygdalin and vitamin C. *The Annals of Pharmacotherapy*, vol. 39 (9), 2005, p. 1566–1569.
- CURLEJ, J. – BULLA, J. – CHRENEK, P. 2010. Occurrence of chromosomal aneuploidy in rabbit oocytes and embryos at different developmental stages. *Zygote*, vol. 18, 2010, p. 203–207.
- CURLEJ, J. – PARKANYI, V. – BULLA, J. – JURCIK, R. – CHRENEK, P. 2007. The effect of hFVIII transgene on the chromosomal aneuploidy rate in rabbits. *Folia Biologica (Kraków)*, vol. 55 (3-4), 2007, p. 161–164.
- CZEPULKOWSKI, B. 2001. Staining and banding of chromosome slides. In: CZEPULKOWSKI, B. (ed.): *Analysing chromosomes*. Oxford UK : BIOS Scientific Publishers, 2001, p. 49–67.
- DO, J. S. – HWANG, J. K. – SEO, H. J. – WOO, W. H. – NAM, S. Y. 2006. Antiasthmatic activity and selective inhibition of type 2 helper T cell response by aqueous extract of semen armeniacae amarum. *Immunopharmacology and Immunotoxicology*, vol. 28 (2), 2006, p. 213–225.
- FUKUDA, T. – ITO, H. – MUKAINAKA, T. – TOKUDA, H. – NISHINO, H. – YOSHIDA, T. 2003. Anti-tumor promoting effect of glycosides from *Prunus persica* seeds. *Biological and Pharmaceutical Bulletin*, vol. 26 (2), 2003, p. 271–273.
- GOEPFERT, T. M. – MCCARTHY, M. – KITTRELL, F. S. – STEPHENS, C. – ULLRICH, R. L. – BRINKLEY, B. R. – MEDINA, D. 2000. Progesterone facilitates chromosome instability (aneuploidy) in p53 null normal mammary epithelial cells. *The FASEB Journal*, vol. 14 (14), 2000, p. 2221–2229.
- GUO, J. – WU, W. – SHENG M. – YANG, S. – TAN, J. 2013. Amygdalin inhibits renal fibrosis in chronic kidney disease. *Molecular Medicine Reports*, vol. 7 (5), 2013, p. 1453–1457.
- HEGEDUS, C. M. – CONWAY, D. I. – GORNBEIN, J. A. – SURREY, M. W. – DANZER, H. C. 2008. The effect of day 3 embryo biopsy on thawed embryo transfer outcomes. *Fertility and Sterility*, vol. 90, 2008, S296.
- CHANG, L. W. – ZHU, H. P. – LI, W. B. – LIU, H. C. – ZHANG, Q. S. – CHEN, H. B. 2005. Protective effects of amygdalin on hyperoxia-exposed type II alveolar epithelial cells isolated from premature rat lungs *in vitro*. *Zhonghua Er Ke Za Zhi, Chinese Journal of Pediatrics*, vol. 43 (2), 2005, p. 118–123.
- CHEN, Y. – MA, J. – WANG, F. – HU, J. – CUI, A. – WEI, C. – YANG, Q. – LI, F. 2013. Amygdalin induces apoptosis in human cervical cancer cell line HeLa cells. *Immunopharmacology and Immunotoxicology*, vol. 35, 2013, p. 43–51.
- IOUROV, I. Y. – VORSANOVA, S. G. – YUROV, Y. B. 2008. Chromosomal mosaicism goes global. *Molecular Cytogenetics*, vol. 1, 2008, p. 26.
- JAROCHA, D. – MAJKA, M. 2011. Influence of amygdalin on biology of cervical carcinoma cells. In: *Abstracts of the 2<sup>nd</sup> Congress of Biochemistry and Cell Biology*. Krakow, 2011, p. 280.
- KARABULUTLU, E. Y. 2014. Coping with stress of family caregivers of cancer patients in Turkey. *Asia-Pacific Journal of Oncology Nursing*, vol. 1 (1), 2014, p. 55–60.
- KWON, H. Y. – HONG, S. P. – HAHN, D. H. – KIM, J. H. 2003. Apoptosis induction of *Persicae Semen* extract in human promyelocytic leukemia (HL-60) cells. *Archives of Pharmacal Research*, vol. 26 (2), 2003, p. 157–161.
- MOERTEL, C. G. – AMES, M. M. – KOVACH, J. S. – MOYER, T. P. – RUBIN, J. R. – TINKER, J. H. 1981. A pharmacologic and toxicological study of amygdalin. *JAMA*, vol. 245 (6), 1981, p. 591–594.
- O'BRIEN, B. – QUIGG, C. – LEONG, T. 2005. Severe cyanide toxicity from “vitamin supplements”. *European Journal of Emergency Medicine*, vol. 12 (5), 2005, p. 257–258.

- PAOLETTI, I. – DE GREGORIO, V. – BARONI, A. – TUFANO, M. A. – DONNARUMMA, G. – PEREZ, J. J. 2013. Amygdalin analogues inhibit IFN-gamma signalling and reduce the inflammatory response in human epidermal keratinocytes. *Inflammation*, vol. 36, 2013, p. 1316–1326.
- PARK, J. H. – SEO, B. I. – CHO, S. Y. – PARK, K. R. – CHOI, S. H. – HAN, C. K. – SONG, C. H. – PARK, S. J. – KU, S. K. 2013. Single oral dose toxicity study of prebrewed armeniacaee semen in rats. *Toxicological Research*, vol. 29, 2013, p. 91–98.
- PARKANYI, V. 1981. Utilization of some methods of cytogenetic analysis in the detection of chromosome anomalies in the species *Oryctolagus cuniculus*. *Agriculture*, vol. 27, 1981, p. 148–154.
- PARKANYI, V. – CHRENEK, P. – RAFAY, J. – SUVEGOVA, K. – JURCIK, R. – MAKAREVICH, A. V. – PIVKO, J. – HETENYI, L. – PALEYANDA, R. K. 2004. Aneuploidy in the transgenic rabbit. *Folia Biologica (Praha)*, vol. 50, 2004, p. 194–199.
- RAUWS, A. G. – OLLING, M. – TIMMERMAN, A. 1982. The pharmacokinetics of prunasin, a metabolite of amygdalin. *Journal of Toxicology. Clinical Toxicology*, vol. 19 (8), 1982, p. 851–856.
- SANTOS PIMENTA, L. P. – SCHILTHUIZEN, M. – VERPOORTE, R. – CHOI, Y. H. 2014. Quantitative analysis of amygdalin and prunasin in *Prunus serotina* Ehrh. using (1)H-NMR spectroscopy. *Phytochemical Analysis*, vol. 25 (2), 2014, p. 122–126.
- SHI, W. – DIRIM, F. – WOLF, E. – ZAKHARTCHENKO, V. – HAAF, T. 2004. Methylation reprogramming and chromosomal neuploidy in *in vivo* fertilized and cloned rabbit preimplantation embryos. *Biology of Reproduction*, vol. 71 (1), 2004, p. 340–347.
- SONG, A. Z. – XU, X. 2014. Advanced research on anti-tumor effects of amygdalin. *Journal of Cancer Research and Therapeutics*, vol. 10, 2014, p. 3–7.
- SUCHARD, J. R. – WALLACE, K. L. – GERKIN, R. D. 1998. Acute cyanide toxicity caused by apricot kernel ingestion. *Annals of Emergency Medicine*, vol. 32 (6), 1998, p. 742–744.
- TANYILDIZI, S. – BOZKURT, T. 2004. *In vitro* effects of linamarin, amygdalin and gossypol acetic acid on hyaluronidase activity, sperm motility and morphological abnormality in bull sperm. *Turkish Journal of Veterinary and Animal Sciences*, vol. 28 (5), 2004, p. 819–824.
- YANG, D. – QIU, M. – ZOU L.Q. – ZHANG, W. – JIANG, Y. – ZHANG, D. Y. – YAN, X. 2013. The role of palliative chemotherapy for terminally ill patients with advanced NSCLC. *Thoracic Cancer*, vol. 4, 2013, p. 153–160.
- YANG, H. Y. – CHANG, H. K. – LEE, J. W. – KIM, Y. S. – KIM, H. – LEE, M. H. – SHIN, M.,S. – HAM, D. H. – PARK, H. K. – LEE, H. – KIM, C. J. 2007. Amygdalin suppresses lipopolysaccharide-induced expressions of cyclooxygenase-2 and inducible nitric oxide synthase in mouse BV2 microglial cells. *Neurological Research*, vol. 29, 2007, S59–S64.
- ZARTMAN, D. L. – FECHHEIMER, N. S. 1967. Somatic aneuploidy and polyploidy in inbred and linecross cattle. *Journal of Animal Science*, vol. 26, 1967, p. 252–258.
- ZHANG, G. M. – JIN, B. Q. 1986. Pharmacokinetics of amygdalin in rabbits. *Zhongguo Yao Li Xue Bao*, vol. 7, 1986, p. 460–462.

## GENETIC CHARACTERIZATION OF NITRA RABBITS AND ZOBOR RABBITS

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### ABSTRACT

In an attempt to characterize the two rabbit breeds concerning the shift on the genetic level we studied microsatellite and genetic markers associated with coat color. These two local rabbit breeds, Nitra rabbit (Ni) and Zobor rabbit (Zo) with different phenotypes have a common origin. Based on the highest polymorphic six microsatellites we chose more than 300 rabbits microsatellites. In two analysed experimental groups Ni (n = 8) and Zo (n = 8) the number of detected alleles for six chosen microsatellites was ranged from two to four alleles (4 alleles per INRACCDDV0106, 3 alleles per INRACCDDV0183, 4 alleles per INRACCDDV0259, 4 alleles per Sol08, 2 alleles per SOL28 and 3 alleles per Sol33). Genetic markers were chosen based on the unique characteristics of the two analyzed breeds: mutations c.585delG in the MLPH gene (dilution of coat color), c.5<sub>6</sub>insA in the ASIP gene (characters of wild type rabbit) and c.1118C → A in the tyrosinase gene (albinism). The mutation c.585delG in Ni rabbits is represented only by homozygous genotype del/del. Ni is a rabbit breed with a blue-grey coloring, therefore, in principle, other genotype for c.585delG is not expected in this breed. For Zo breed, only G/G homozygous genotype should be represented, but even in that small group of Zo rabbits (n = 8) one heterozygote del/G has occurred, with the recessive allele hidden under the black phenotype. Concerning ASIP gene, from 8 Ni rabbits 7 animals were homozygous ins/ins, which were blue-coated, and only one was Wt/ins, which was Ni wild-blue, whilst Wt/Wt homozygote was not represented. In Ni and Zo breeds the mutation c.1118C → A in the tyrosinase gene was represented only by CC homozygotes, whereas albinos Hyla hybrid was represented only by AA homozygotes. Here we propose steps to make more efficient the process of cross-breeding of the analyzed Ni and Zo rabbit breeds in order to stabilize and then standardize these two unique rabbit breeds, which could be preserved in the gene bank for possible future use.

**Key words:** coat color; cross-breeding; genetic marker; microsatellite; rabbit

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### INTRODUCTION

Rabbit breeds with different names may sometimes have recent common origin, while in other cases their uniqueness has been eroded by cross-breeding. Breeds of rabbits as well as all animals are characterized by the standard of breed, by a set of precisely defined characteristics or parameters established on the basis of phenotypic assessment. The aim of this study is to shift this characterization on the genetic level through a microsatellite and genetic markers associated with coat coloring in two local rabbit breeds with common origin

but so uniquely different phenotype, Nitra rabbit (Ni) and Zobor rabbit (Zo).

Microsatellite markers are useful tool recommended by ISAG / FAO, used for the analysis of genetic diversity in many programs of the livestock preservation (FAO, 2011). Research program ResGen-CT95-60 in 1995-2000 was similarly focused on rabbits in which the European Union was working to inventory, evaluate, conserve and utilize the genetic resources of European rabbits (Bolet *et al.*, 2000), but Slovakia did not participate in this program. For this study, polymorphic rabbit microsatellites

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Sol08, SOL28, Sol33 were chosen from articles written by Rico *et al.* (1994) and SurrIDGE *et al.* (1997) and microsatellites INRACCDDV0106, INRACCDDV0183, INRACCDDV0259 were published by Chantry-Darmon *et al.* (2006).

Genetic markers were chosen base on the unique characteristics of the two analyzed breeds. As a genetic marker for similar study of differentiation analysis, in particular in the case of the definition of genetic resources, traits such as reproduction must not be used such as reproduction characteristics, for which is the effort to achieve in all aspects of their a cost-effectived and balanced level, no go to extremes (Bolet *et al.*, 2000).

In this case it was not possible to evaluate carcasses characteristics, because two similar meat breeds of rabbit were examined. Suitable markers associated with coat color had to be found. Today several genetic mutations associated with coloring are known, from which those were chosen for analysis, which define some of the unique characteristics of these two specific analysed rabbit breeds. The purpose for breeding of these two breeds, Ni and Zo, was to create a white meat rabbit. First, Ni was bred from the cross-breeding of the French silver rabbit with Russian rabbit breed and subsequently with Californian rabbit. Zo breed is the result of cross-breeding of Ni with the New Zealand White rabbit. The uniqueness of Ni and Zo is in specific distribution of pigment areas (color pattern) and their different color. Ni breed is unique by Himalayan color pattern in blue color (nose, ears, feet and tail are blue with clear division between these areas and the white body color), originally in conjunction with characters of wild type rabbit. Ni has clear bright blood red eyes. The Himalayan drawing is typical for Russian rabbit and Californian rabbit, but in black color, while Ni has this color pattern in blue, as previously mentioned. Zo breed has a unique Zobor color pattern (head white, tail black and hindlegs cuffs black with clear division from the white body color). Zo has clear bright blood red eyes, too. Typical Zobor color pattern is a result of combination of Dutch color pattern and partial albinism.

The purpose of this study was the characterization of the unique features of Ni and Zo at the genetic level, which corresponds with genetic markers associated with coat color: mutations c.585delG in the MLPH gene (melanophiline gene) (dilution of coat color, Lehner *et al.*, 2013), c.5\_ginsA in ASIP gene (characters of wild type rabbit, Fontanesi *et al.*, 2010) and c.1118C → A in the tyrosinase gene (albinism, Aigner *et al.*, 2000). Epistatic deletion c.585delG in the MLPH gene inhibits the full expression of pigment formation with manifestation of weaker coloring, called dilution

of coat color. Dominantly inherited mutation c.5\_ginsA in the ASIP gene A<sup>Wt</sup> is associated with the characters of wild type rabbit (white inside ears, white underside of the tail, as well as the whole ventral part of the body). Recessive inherited, but also epistatic to all mutation associated with color changes, mutation c.1118C → A in the tyrosinase gene, which in a homozygous recessive line up repressed all pigment production, including the eyes, so-called complete albinism.

We propose steps to make more efficient the process of cross-breeding of analyzed breeds Ni and Zo rabbit to be able to stabilize and then breed-standardize the genetic material of these two unique rabbit breeds, which could be preserved in the gene bank for possible future use.

Ni and Zo are the result of combination of genes of four high quality foreign breeds (Silver French, Russian, California and New Zealand White). In the breed Ni and Zo not only the combination of high quality genetic material is encoded, but at the same time adaptability of this gene combination to our environmental conditions, its efficient use of local natural food sources of forage and its resistance to the pathogenic pressure in our animal production. But choosing the breeds to be evaluated, we did not take into account the risk of extinction of the breed, but their historical status and their potential zootechnical interest (Bolet *et al.*, 2000).

## MATERIAL AND METHODS

### Animals

The total set of analyzed animals was represented by total of 77 animals from 31 rabbit breeds (Table 4). In this study, three analyzed systems of three rabbit breeds Ni (n = 8), Zo (n = 8) and albinos of hybrid Hyla (n = 3) are considered the key results. From small populations of Ni and Zo rabbits the selection has been made so that the animals conform to the characteristics of the breed standard.

### Isolation of DNA

Genomic DNA was extracted from blood using DNA Purification kit Maxwell16<sup>®</sup> (Promega).

### Multiplex PCR

The sequence of six polymorphic microsatellite loci was chosen from published articles (Sol08, SOL28 and Sol33 by Rico *et al.*, 1994 and SurrIDGE *et al.*, 1997 and INRACCDDV0106, INRACCDDV0183 and INRACCDDV0259 by Chantry-Darmon *et al.*, (2006), Table 1). Sequences of microsatellites were obtained from GenBank (NCBI). Primers for multiplex PCR were chosen using software QDD2 (MeglécZ *et al.*, 2010).

**Table 1: Primer sequences for analysed microsatellite**

Microsatellite marker	GenBank. Acc. No	Fluorescence labeling and sequence of forward and reverse primers in 5'-3'	No. of publ. alleles	Reference
INRACCDDV0106	AJ874448	6-FAM: TTGGGAGTGAACCAGCAGAT AATCCGACCCATCTTTCGAT	6	Chantry-Darmon <i>et al.</i> , 2006
INRACCDDV0183	AJ874521	6-FAM: TCACCTTCTACTGCCTGCAC CGCTCTCACTAACTCTGCCTG	7	Chantry-Darmon <i>et al.</i> , 2006
INRACCDDV0259	AJ874589	VIC: CTTTGCATGGGCAATTGAATGCT ACTGAGACAGGGAGGAG	7	Chantry-Darmon <i>et al.</i> , 2006
Sol08	X79217	NED: ATTGGGCCCTTTGCTCACTCGC AGCCATATCTGAGAGAA	1-10	Rico <i>et al.</i> , 1994; Surridge <i>et al.</i> , 1997
Sol28	X79216	PET: AACCACTGACGGAAGTGGAATT ATTATTCAGGGCAGA	1-7	Rico <i>et al.</i> , 1994; Surridge <i>et al.</i> , 1997
Sol33	X94683	6-FAM: CCCATGTGTGCTACTGATTTG GATGTCAAATCTGCCCTCCC	1-15	Rico <i>et al.</i> , 1994; Surridge <i>et al.</i> , 1997

**Multiplex PCR conditions and fragment analysis**

Multiplex PCR reactions were performed at a total volume of 16 µl using QIAGEN Multiplex PCR Kit (Qiagen), 10-50 ng genomic DNA as template and 0.2 µmol.l<sup>-1</sup> of each primer. The PCR profile included an initial denaturation step at 95 °C for 15 min, 28 cycles: 94 °C (60 s), 60 °C (90 s), 72 °C (60 s) and a final extension step of 30 min at 60 °C in C1000 thermal cycler (Bio-Rad). The PCR products were analysed on ABI PRISM 310 and the fragment analysis was performed using the Gene Mapper software 4.0.

**PCR**

PCR primers sequence for ASIP was taken from Fontanesi *et al.* (2010) and for MPLH from Lehner *et al.* (2013) (Table 2).

**STAS-PCR**

STAS-PCR for tyrosinase c.1118C-A (T373K) polymorphism. Briefly, two allele-specific internal primers (INT1, INT2) recognized with their 3' nucleotide the allelic variation (Table 2). In order to increase the allele specific annealing an additional mismatch position 3 from the 3' end of each primer was introduced (lower case). The external primers are located at different distances from point mutation, so that different fragment lengths were generated for each allele. In case of nt118C and nt118A homozygotes only one fragment of 79 bp or 99 bp respectively was amplified, whereas in heterozygotes C/A both fragments were detected. Independently of the genotype an additional product (131 bp) of the common external primers (EXT1, EXT2) were amplified.

**Table 2: Primer sequences for analysed chosen genetic markers for rabbit coat color**

Genetic marker	GenBank Acc. No.	Sequence of primers forward and reverse in 5'-3'	PCR product	Temp. of annealing	Restriction endonuclease
ASIP c.5_6insA	FN547134 Fontanesi <i>et al.</i> , (2010)	CAGGAAGGCACATCCTCTTT GGGTGGCCTGGAGCAGGCGAGTGGAATT	95 bp	57 °C	<i>EcoR</i> I
MPLH c.585delG	KC791692 Lehner <i>et al.</i> , (2013)	GTGAGCAGGCATGTGCAG GGGCCTGAGCAGAGACAC	111 bp	60 °C	<i>HpyF3I</i> ( <i>Dde</i> I)
TYR c.1118C→A	AF210660 Aigner <i>et al.</i> , (2000)	EXT1 TGCTAATCCACTTACTGGGATAGC INT 1 GCTTTGCATATGTATATGAATGGcAC INT 2 GATCCCTGTACCTGGGACAcCT EXT 2 CATGGTGAAGGAGAAAAATGGGATC	131 99 79	60 °C	-

### The PCR conditions

The PCR reactions were performed at a total volume of 25 µl using 1X Colorless GoTaq Flexi buffer, 2.0 mmol.l<sup>-1</sup> MgCl<sub>2</sub>, 0.2 mmol.l<sup>-1</sup> of each dNTP, 1 U GoTaq G2 Hot Start DNA polymerase (Promega, USA), 50-100 ng genomic DNA as template and 0.4 µmol.l<sup>-1</sup> of each primer. The PCR profile included an initial denaturation step at 94 °C for 2 min, 35 cycles of 94 °C (20 s), 57 °C/60 °C (30 s), 72 °C (30 s) and a final extension step of 10 min at 72 °C in C1000 thermal cycler (Bio-rad).

### RFLP

The appropriate restriction endonuclease (ThermoScientific, Germany): EcoRI for ASIP and *HpyF3I* (Dde I) for MLPH polymorphism was used to digest PCR products (Table 2).

### Microchip electrophoresis of DNA fragments

PCR products were analysed by the automated microchip electrophoresis system MCE<sup>®</sup>-202 MultiNA (Shimadzu, Japan) with a DNA-500 kit according to the manufacturer's protocol. A SYBR Gold fluorescent dye for DNA staining (Invitrogen, USA) and a 25 bp DNA Ladder (Invitrogen, USA) were used to determine the size of the restriction fragments.

## RESULTS AND DISCUSSION

The overall population size of Ni and Zo rabbit breeds is very low. Stability of these two breeds is constantly eroded by cross-breeding efforts. Frequent is the occurrence of weak coloring intensity and the lack of compact coloring markings with the appearance of pigment spots on various parts of the body. The selection of the necessary amount of individuals to conform to the breed standard characteristics among them was difficult. However, it 8 specimen of Ni rabbits and 8 specimen of Zo rabbits were selected for the experiment. In order to find genetic variability and to define two breeds on the genetic level, from more than 300 rabbit microsatellites we chose the six highest polymorphic microsatellites. Number of published rabbit microsatellite alleles ranges from 2 to 15 (SurrIDGE *et al.*, 1997). For chosen microsatellites 6 alleles per INRACCDDV0106 (Chantry-Darmon *et al.*, 2006), 7 alleles per INRACCDDV0183 (Chantry-Darmon *et al.*, 2006), 7 alleles per INRACCDDV0259 (Chantry-Darmon *et al.*, 2006), 10 alleles per Sol08 (SurrIDGE *et al.*, 1997), 7 alleles per Sol28 (Rico *et al.*, 1994) and 15 alleles per Sol33 (SurrIDGE *et al.*, 1997) are described.

In our two analysed rabbit experimental groups Ni (n = 8) and Zo (n = 8) the number of detected alleles for six chosen microsatellites ranges from 2 to 4

alleles (4 alleles per INRACCDDV0106, 3 alleles per INRACCDDV0183, 4 alleles per INRACCDDV0259, 4 alleles per Sol08, 2 alleles per SOL28 and 3 alleles per Sol33) (Table 3).

Here we avoided traditional statistical processing, determinations of heterozygosity and genetic distances which would lead to erroneous conclusions rather than to achieve reasonable results, both precisely because of the small numbers of analyzed groups of rabbits, and also because two populations that are genetically distant need not phenotypically differ (Edding and Laval, 1999), and *vice versa*.

Microsatellite is a mean particularly to distinguish individuals under paternity testing. For many species (H cattle, buffalo, sheep, goats, horses, donkeys, camels, pigs and chickens) the sets of microsatellites to identify on the genetic level (FAO 2011) are recommended. For rabbits such microsatellite sets are not recommended yet, although the number of the rabbit microsatellites is described. Since in rabbits very intensive cross-breeding has been done, the origins of the rabbit breeds significantly overlap and this complicates the ability to find appropriate specific microsatellites. Nevertheless, we tried to look for characteristics of these two breeds through microsatellites too. No reallocation alleles in the study of rabbit breeds Ni and Zo were observed as is shown in Table 3. The results of genotyped rabbits for the three genetic mutations associated with change of the coat color c.585delG in MLPH gene, c.5\_6insA in ASIP gene and c.1118C→A in tyrosinase gene are listed in the Table 5.

Table 4 shows the results of analysis of mutation c.585delG MLPH in the MLPH gene for 31 rabbit breeds with different coat coloring for a total of 77 animals. This mutation was described by Lehner *et al.* (2013) and Fontanesi *et al.* (2014). Dilution of coat color effect of this mutation was confirmed in breeds Ni, Czech Spot blue, Havana blue, Moravian blue, Gray-blue Rex, Dwarf grey-blue Rex, French Lop blue and Vienna blue (Table 5).

We also thought that coloring in New Zealand red is caused by interaction of the mutation c.585delG in the MLPH gene, too. It is possible however, that in this case the dilution or mixing of pigments is caused by another interactions. One known mutation is now associated with the dilution coat color c.111-5C→A gene MLPH (Lehner *et al.*, 2013) but it was not the subject of the study.

Mutation c.585delG in Ni rabbits is represented only by homozygous genotype del/del. Ni is rabbit breed with blue-grey coloring, therefore, in principle, another genotype for c.585delG is not possible in this breed. On the contrary, it should be for Zo breed, which should be represented only by homozygous genotype G/G for c.585delG, therefore, any likelihood of excision

**Table 3: Number of detected alleles for chosen microsatellites in Ni and Zo rabbits**

Microsatellite name	Rabbit of Nitra (n = 8)		Zobor rabbit (n = 8)	
	Allele number	Allele size (bp)	Allele number	Allele size (bp)
INRACCDDV0106	4	184_188_190_193	4	184_188_190_193
INRACCDDV 0183	3	152_159_161	2	152_161
INRACCDDV 0259	4	105_116_118_120	4	105_116_118_120
Sol08	4	99_101_104_106	4	99_101_104_106
Sol28	2	100_102	3	96_100_102
Sol33	3	76_79_84	3	76_79_84

**Table 4: Results of rabbit genotyping for the c.585 in the MLPH gene**

		c.585delG in melanophiline gene		
		del/del	del/G	G/G
Rabbit of Nitra (8)	del/del	8	-	-
Zobor rabbit (8)	del/G, G/G	-	1	7
Californian (8)	G/G	-	-	8
Dwarf Russian (Dwarf Himalayan) (1)	del/G	-	1	-
Czech Spot (5)	G/G	-	-	5
Czech Spot blue (1)	del/del	1	-	-
Czech Spot red (1)	G/G	-	-	1
German Giant Spot (1)	del/G	-	1	-
Spot of Mecklenburg (1)	G/G	-	-	1
Belgian Giant black (2)	G/G	-	-	1
Dutch black (4)	G/G	-	-	4
Havana blue (1)	del/del	1	-	-
Moravian blue (2)	del/del	3	-	-
Grey-blue Rex (2)	del/del	2	-	-
Dwarf grey-blue Rex (1)	del/del	1	-	-
French Lop blue (1)	del/del	1	-	-
French Lop wild type (1)	G/G	-	-	1
Blue of Vienna (2)	del/del	2	-	-
Vienna rabbit (wild coloring) (4)	G/G	-	-	4
European rabbit (3)	G/G	-	-	3
Dwarf european rabbit (1)	del/G	-	1	-
Big Light Silver (3)	G/G	-	-	3
Big Chinchilla (3)	G/G	-	-	3
Rabbit of Rhön (2)	G/G	-	-	2
Dwarf Black (2)	G/G	-	-	2
Dwarf Casto Rex (2)	del/G	-	2	-
Thuringer (1)	del/G	-	1	-
Dwarf Lynx (1)	del/del	1	-	-
New Zealand Red (1)	G/G	-	-	1
Fox-Rabbit white (1)	del/G	-	1	-
Albinos-hybrid HYLA (3)	G/G	-	-	3

**Table 5: Results of rabbit genotyping for three analyzed genetic mutations**

		c.585delG in melanophilin gene		
		del/del	del/G	G/G
Rabbit of Nitra	8	8	-	-
Zobor rabbit	8	-	1	7
Albinos	3	-	-	3
		c.5_6insA in ASIP gene		
		ins/ins	ins/Wt	Wt/Wt
Rabbit of Nitra	8	7	1	-
Zobor rabbit	8	5	3	-
Albinos	3	-	1	3
		c.1118C→A in TYR gene		
		A/A	A/C	C/C
Rabbit of Nitra	8	-	-	8
Zobor rabbit	8	-	-	8
Albinos	3	3	-	-

offspring with dilution coat color would be eliminated. But even in that small group of Zo rabbits ( $n = 8$ ), one heterozygote del/G is occurred with the recessive allele hidden under the black phenotype. Here is an example, that even if the individual is recognized as a breeding animal, it does not mean that it is „cleans“ (homozygous) for a given reference characters, as many farmers/breeders believe. In case of two heterozygotes del/G for c.585delG in mating plans, their use brings 25 % likelihood of progeny with inadmissible coat color due to dilution. In this way, recognized heterozygotes for c.585delG need not be negatively selected as they will be properly combined with a truly „pure“ homozygote G/G, and all the offspring will have a 100 % full black pigment.

Concerning the ASIP gene, within our small experimental group of 8 pieces Ni 7 rabbits were homozygous ins/ins, these were Ni blue, only one was Wt/ins, and this was Ni wild-blue, whilst homozygote Wt/Wt was not represented. In this case it is necessary to point out one fact when assessing. Ni rabbit breed is bred in two color surges, blue and wild-blue. Blue Ni was bred from wild-blue, Ni blue has intense darker blue-grey color and is without characteristics of wild type rabbit. Currently there is a gradual reduction of wild-blue Ni rabbits, which is the original Ni rabbit with a unique combination of the Himalayan pattern in blue color with wild characters. Modern cross-breeding steps should be reviewed in order to prevent the elimination of unique color lines like that of the wild-blue Ni breed. In relation to the ASIP gene, another major problem was found

in Zo breed too. In our small experimental group ( $n = 8$ ) three Zo rabbits had clear signs of wildness (heterozygotes ins/Wt). Characters of wild type rabbit are inadmissible in Zo rabbit breed, because white hairs move to the pigment black areas. When the allele Wt is present, black characters with white hairs create the effect of less intense coloring. C.5\_6insA mutation in the ASIP gene is dominantly inherited and effect of allele Wt is clearly identifiable. According to this it is necessary to put more emphasis on checking wildness in the evaluation of breeding animals of Zo rabbit breed. A clear indicator of the presence of the Wt allele and therefore wildness is the white underside of tail.

Mutation c.1118C→A in the tyrosinase gene, a key enzyme for melanin production, is associated with the expression of complete albinism. In the breeds Ni and Zo mutation c.1118C→A in the tyrosinase gene is represented only by homozygotes CC, whereas albinos Hyla hybrid is represented only by AA homozygote. The results, therefore, confirm the complete suppression of pigment in albino AA assembly, and only homozygous genotypes CC for c.1118C→A in the tyrosinase gene in breeds Ni and Zo. In conjunction with suppression the production of pigment is not specifically defined, concepts/phenomena such as acromelanizmus, partial albinism and leucizmus are misunderstood and often confused, especially in these two breeds. These are therefore excellent experimental material for study and subsequent understanding of these concepts, but this is subject to further studies of the Ni and Zo breeds. Basing on the results of analysis of Ni and Zo rabbit

**Table 6: Recommended genetic assembly for three analyzed genetic mutations in two rabbit breeds**

	Rabbit of Nitra Wild-blue	Rabbit of Nitra Blue	Zobor rabbit
c.585delG in MLPH gene	del/del	del/del	G/G
c.5_6insA in ASIP gene	Wt/Wt	ins/ins	ins/ins
c.1118C→A in TYR gene	C/C	C/C	C/C

breeds of three systems we propose following genetic assembly shown in the Table 6. For both color surges (blue and wild-blue) of Ni rabbits for c.585delG it should be homozygous assembly del/del, which determines the dilution coat color. For c.585delG of Zo rabbit breed it should be homozygous assembly G/G, ensuring full pigment production on the site-specific staining characters. For c.5\_6insA of wild-blue Ni, which has characters of wild-type rabbits as a part of its breeding standard, it should be homozygous assembly Wt/Wt, whilst the ins/ins homozygous assembly is necessary for Blue Ni and Zo, which must be without characters of wild-type rabbit. This system is very effectively monitored thanks to the underside of the tail as effective indicator of wildness (only genotype ins/ins has coloring underside of tail in coloring rabbit breeds, but presence of only one Wt allele is associated with characters of wild-type rabbits). Regarding the last analyzed genetic system TYR\_1118, both breeds are important to maintain in the clean homozygous assembly C/C, without the presence of allele A, which needs to be eliminated if clean lines are disrupted by cross-breeding to avoid useless of the negative selection of born albinos. Simply on the basis of the presence of albinos in the litter, without doing genetic analysis, it is possible to confirm whether the parental pair, both male and female, has heterozygous assembly A/C for c.1118C→A in the tyrosinase gene.

## CONCLUSION

The implementation of genetic analyses to rabbit breeding is important in order to use all available information and knowledge of similar genetic studies for more efficient breeding techniques to avoid unnecessary false steps, such as the inclusion Marder breed in cross-breeding programs Ni in mistaken belief that the phenomenon acromelanism improves the coloring characteristics of the partial albinism.

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## REFERENCES

- AIGNER, B. – BESENFELDER, U. – MÜLLER, M. – BREM, G. 2000. Tyrosinase gene variants in different rabbit strains. *Mammalian Genome*, vol. 11, 2000, p. 700–702.
- BOLET, G. – BRUN, J. M. – MONNEROT, M. – ABENI, F. – ARNAL, C. – ARNOLD, J. – BELL, D. – BERGOGLIO, G. – BESENFELDER, U. – BOSZE, Z. – BOUCHER, S. – CHANTELOUP, N. – DUCOUROUBLE, M. C. – DURAND-TARDIF, M. – ESTEVES, P. J. – FERRAND, N. – GAUTIER, A. – HAAS, C. – HEWITT, G. – JEHL, N. – JOLY, T. – KOEHL, P. F. – LAUBE, T. – LECHEVESTRIER, S. – LOPEZ, M. – MASOERO, G. – MENIGOZ, J. J. – PICCININ, R. – QUENEY, G. – SALEIL, G. – SURRIDGE, A. – van der LOO, W. – VICENTE, J. S. – VIUDES de CASTRO, M. P. – VIRAG, J. S. – ZIMMERMANN, J. M. 2000. Evaluation and utilisation of European rabbit (*Oryctolagus cuniculus*) genetic resources. First results and inferences. In: Proceedings of 7<sup>th</sup> World Rabbit Congress, 4–7 July 2000, Valencia, Spain, Volume A, p. 281–315. (<http://world-rabbit-science.com/WRSA-Proceedings/Congress-2000-Valencia/Valencia-2000a.htm>)
- EDDING, J. H. – LAVAL, G. 1999. Cit. by BOLET, G. *et al.* 2000. Evaluation and utilisation of European rabbit (*Oryctolagus cuniculus*) genetic resources. First results and inferences. In: Proceedings of 7<sup>th</sup> World Rabbit Congress, 4–7 July 2000, Valencia, Spain, Volume A, p. 281–315. (<http://world-rabbit-science.com/WRSA-Proceedings/Congress-2000-Valencia/Valencia-2000a.htm>)

- CHANTRY-DARMON, C. – URIEN, C. – DE ROCHAMBEAU, H. – ALLAIN, D. – PENA, B. – HAYES, H. – GROHS, C. – CRIBIU, E. P. – DERETZ-PICOULET, S. – LARZUL, C. – SAVE, J. C. – NEAU, A. – CHARDON, P. – ROGEL-GAILLARD, C. 2006. A first-generation microsatellite-based integrated genetic and cytogenetic map for the European rabbit (*Oryctolagus cuniculus*) and localization of angora and albino. *Animal Genetics*, vol. 37, 2006, p. 335–341.
- FAO. 2011. Molecular genetic characterization of animal genetic resource. *FAO Animal Production and Health Guidelines*. 2011, No. 9. Rome.
- FONTANESI, L. – FORESTIER, L. – ALLAIN, D. – SCOTTI, E. – BERETTI, F. – DERETZ-PICOULET, S. – PECCHIOLI, E. – VERNESI, C. – ROBINSON, T. J. – MALANEY, J. L. – RUSSO, V. – OULMOUDEN, A. 2010. Characterization of the rabbit agouti signaling protein (ASIP) gene: Transcripts and phylogenetic analyses and identification of the causative mutation of the nonagouti black coat colour. *Genomics*, vol. 95, 2010, p. 166–75.
- FONTANESI, L. – SCOTTI, E. – ALLAIN, D. – DALL’OLIO, S. 2014. A frameshift mutation in the melanophilin gene causes the dilute coat colour in rabbit (*Oryctolagus cuniculus*) breeds. *Animal Genetics*, vol. 45 (2), 2014, p. 248–55.
- KALINOWSKI, S. T. – TAPER, M. L. – MARSHALL, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, vol. 16, 2007, p. 1099–1006.
- LEHNER, S. – GÄHLE, M. – DIERKS, C. – STELTER, R. – GERBER, J. – BREHM, R. – DISTL, O. 2013. Two-exon skipping within MLPB is associated with coat color dilution in Rabbits. *PLOS ONE*, vol. 8 (12), 2013, p. 1–11.
- MEGLÉCZ, E. – COSTEDOAT, C. – DUBUT, V. – GILLES, A. – MALAUSA, T. – PECH, N. – MARTIN J. F. 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics*, vol. 26 (3) 2010, p. 403–404.
- RICO, C. – RICO, I. – WEBB, N. – SMITH, S. – BELL, D. – HEWITT, G. 1994. Four polymorphic microsatellite loci for the European wild rabbit, *Oryctolagus cuniculus*. *Animal Genetics*, vol. 25, 1994, p. 367.
- SURRIDGE, A. K. – BELL, D. J. – RICO, C. – HEWITT, G. M. 1997. Polymorphic microsatellite loci in the European rabbit (*Oryctolagus cuniculus*) are also amplified in other lagomorph species. *Animal Genetics*, vol. 28, 1997, p. 302–305.

## EFFECTS OF DIETARY SUPPLEMENTATION WITH ISOFLAVONES ON EXTERIOR DEVELOPMENT AND TIBIA BONE QUALITY OF LAYING HENS

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### ABSTRACT

The experiment was conducted to evaluate the effect of dietary supplementation with isoflavones on exterior development and tibia bone quality of laying hens. Eighty laying hens, 20 weeks old, were divided into 5 groups, 20 in each and fed with different amount of 300, 600, 1200 and 1800 mg.kg<sup>-1</sup> supplemented isoflavones (SI) in the feed. Dietary isoflavones supplemented in different concentrations to the ISA Brown laying hens diet caused increase of the body weight ( $P < 0.05$ ). The heaviest body weight was noticed in the group fed with supplementation of 1800 mg.kg<sup>-1</sup> SI ( $2142.5 \pm 190.5$  g). In addition, shank length as an important measurement of skeletal development was significantly longest in the group fed with supplementation of 1800 mg.kg<sup>-1</sup> SI ( $29.50 \pm 1.73$  cm). There were no differences in other exterior parameters among the experimental groups ( $P > 0.05$ ). High SI treatment (1800 mg.kg<sup>-1</sup> feed) had a beneficial effect ( $P < 0.05$ ) on bone quality of aged laying hens (weight, volume, ash/tibia and calcium), but do not affect on the tibial phosphorus ( $P > 0.05$ ). Results of the present study indicates that isoflavones are effective supplements to improve the body weight and calcium bone content during the late laying period, even though their supplementation was high.

**Key words:** isoflavones; genistein; daidzein; bone quality; laying hens

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### INTRODUCTION

Commercial laying hens have a problem with the loss of bone strength during their egg production cycle. When the hens reached sexual maturity, the osteoporosis initiated serious welfare problems (Webster, 2004; Whitehead and Fleming, 2000). In their ordinary diet the soybean and its products are commonly used as a protein source for layers. Soybean is a great source of isoflavones, such as genistein and daidzein, which are believed to be biologically active in several animals, including humans and poultry. Supplementation of daidzein improves the laying performance during post peak laying in Shaoxing duck (Zhao *et al.*, 2005) and the amount of cracked eggs decreases and eggshell thickness and egg production increases (Ni *et al.*,

2007). Sahin N. *et al.* (2006) reported that soybean isoflavones have a significant effect on the egg quality and bone mineralization in quails.

The experiment will thus provide knowledge on the effect of dietary supplementation with isoflavones on the tibia bone quality in aging laying hens and may provide as a means to increase the welfare of laying hens.

### MATERIAL AND METHODS

#### Animal Treatments and Doses

The experiment was performed on ISA Brown laying hens in a commercial poultry farm. Eighty laying hens, 20 weeks old, were divided into 5 groups,

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20 in each (4 cages for a group), set to a 16L:8D cycle and fed with different amount of supplemented isoflavones (SI) in the feed. The control group was blank and fed with no SI in the feed and the other 4 experimental groups was fed with SI in the feed

**Table 1: Composition of the 40 % isoflavone product**

Isoflavone	%
1. Genistin	7.30
2. Genistein	1.26
3. Daidzin	22.12
4. Daidzein	1.74
5. Glycitin	8.01
6. Glycitein	0.45
Total	40.88

in amount of 300, 600, 1200 and 1800 mg.kg<sup>-1</sup>. Water was offered for *ad libitum* consumption throughout the experiment, which was conducted for 40 weeks. The experimental feed was supplemented with a concentrated product, 40 % isoflavones, produced by the North China Pharmaceutical Corporation. The isoflavone composition of the product is presented in Table 1.

The composition and nutritive value of the basal diet is presented in Table 2.

The laying hens received additional isoflavones from 20 to 60 weeks of age.

#### Exterior Parameters

The body weight of the laying hens was measured with a special digital balance at the beginning and at the end of the experiment. The body length, the breast circumference and the length of the legs were measured using the clot tape.

**Table 2: Composition and nutritive value of the basal feed (BF)**

Ingredient, g.kg <sup>-1</sup>	Basal feed (BF)	Basal feed (BF)	Basal feed (BF)
	Phase I of egg production	Phase II of egg production	Phase III of egg production
Yellow corn	40.01	40.24	43.13
Soybean meal, 44 % protein	18.96	15.60	14.43
Sunflower meal, 33 % protein	15.00	15.00	15.30
Wheat middilings	9.40	12.39	10.70
Soybean oil	5.10	4.94	4.32
Methionine, 99 %	0.12	0.07	0.04
Calcium carbonate	9.12	9.61	9.94
Monocalcium phosphate	1.10	0.90	0.76
NaHCO <sub>3</sub>	0.15	0.20	0.30
Potassium carbonate	-	0.04	0.09
Zeolites	0.30	0.30	0.30
Salt	0.24	0.22	0.19
Vitamin and mineral mixture	0.50	0.50	0.50
Total	100.00	100.00	100.00
Chemical composition, calculated			
Dry matter, g.kg <sup>-1</sup>	904.30	904.00	903.10
Metabolic energy, kcal.kg <sup>-1</sup>	2800.00	2750.00	2750.00
Crude proteins, g.kg <sup>-1</sup>	179.90	169.40	165.00
Crude fat, g.kg <sup>-1</sup>	71.50	70.90	65.20
Crude fiber, g.kg <sup>-1</sup>	69.80	72.00	70.00
Total ash, g.kg <sup>-1</sup>	125.30	130.00	130.00
Calcium, g.kg <sup>-1</sup>	37.50	39.00	40.00
Phosphorus (available), g.kg <sup>-1</sup>	3.80	3.40	3.00
Lysine, g.kg <sup>-1</sup>	8.50	7.70	7.40
DL Methionine, g.kg <sup>-1</sup>	4.60	3.90	3.60
Methionine + cystine, g.kg <sup>-1</sup>	7.30	6.50	6.10

### Bone Sample Collection

At the end of the experiment 5 laying hens from each experimental group were sacrificed. The laying hens were anaesthetized by inhalation with diethyl ether and killed. At necropsy, the right tibia was excised from the body and defleshed without boiling. The tibia was then sealed in a plastic bag to minimize moisture loss and stored at -20 °C until analysis. The bone volume was taken by the weight change in water method. Tibias were measured on the air and in the water. The weight change equalled the weight of water replaced by the bone as the specific gravity of water is 1.0 g.cm<sup>-3</sup>. The weight was measured with a digital balance of 0.01 g accuracy. Bone length was measured with a calliper with 0.01 cm accuracy. The tibia was defatted, dried at 105 °C for 24 h and placed in a dessicator. The bone ash weight was obtained after ashing at 600 °C for 24 h. Bone ash concentration was calculated as bone ash weight per unit of volume. The content of calcium and phosphorus were determined by potassium permanganate titration and vanadate-molybdate colorimetric methods, respectively.

### Statistical analysis

Statistical analysis was performed by Statgraph 3 software package. One-way analysis of variance (ANOVA)

was used for the differences between groups. When the F values were significant, the Duncan's Multiple Range Test was performed.

## RESULTS AND DISCUSSION

The obtained results of the body weight and the exterior parameters are presented in Table 3.

There are some information (Payne *et al.*, 2001; Jiang *et al.*, 2007) which indicate that phytohormones added to a large amount to the feed of chicken cause changes in the body weight and appearance that have influence on some other exterior characteristics and the whole body conformation. In our experiment dietary isoflavones supplemented in different concentrations to the ISA Brown laying hens' diet caused increase in the body weight ( $P < 0.05$ ). The heaviest body weight was noticed in the group fed with supplementation of 1800 mg.kg<sup>-1</sup> SI ( $2142.5 \pm 190.5$  g).

In addition, shank length is an important measurement of skeletal development (Yilmaz Dickmen and Ipek, 2006). It is related with the productive traits of the laying hens (Petek *et al.*, 2000). The longest shank length was noticed in the group fed with supplementation

**Table 3: Body weight and exterior parameters of the ISA brown laying hens**

	Basal feed (BF)	Basal feed (BF) + 300 mg.kg <sup>-1</sup> SI	Basal feed (BF) + 600 mg.kg <sup>-1</sup> SI	Basal feed (BF) + 1200 mg.kg <sup>-1</sup> SI	Basal feed (BF) + 1800 mg.kg <sup>-1</sup> SI
Body weight, g	1987.5 ± 125.8 <sup>b</sup>	1967.5 ± 100.1 <sup>bc</sup>	2045.0 ± 92.9 <sup>b</sup>	1955.0 ± 92.6 <sup>bc</sup>	2142.5 ± 190.5 <sup>a</sup>
Body length, cm	28.88 ± 1.55 <sup>ab</sup>	27.13 ± 0.85 <sup>b</sup>	28.50 ± 1.29 <sup>ab</sup>	27.50 ± 1.29 <sup>ab</sup>	29.50 ± 1.73 <sup>a</sup>
Breast circumference, cm	21.50 ± 1.73	20.63 ± 1.25	20.75 ± 0.65	20.75 ± 1.26	22.50 ± 1.29
Length of femur, cm	11.25 ± 0.96	11.00 ± 0.05	11.00 ± 0.82	10.75 ± 0.50	11.00 ± 0.71
Length of tibia, cm	20.50 ± 1.29	20.38 ± 0.95	22.50 ± 0.58	21.50 ± 0.58	21.38 ± 1.80
Length of metatarsus, cm	29.50 ± 1.29	29.00 ± 1.41	29.75 ± 0.50	29.50 ± 0.58	29.75 ± 1.50

SI – supplemented isoflavones; a,b,c – values in the same row with no common superscript differ significantly ( $P < 0.05$ ).

of 1800 mg.kg<sup>-1</sup> SI ( $29.50 \pm 1.73$  cm). This result is in agreement with the results of our previous study conducted with ISA Brown pullets (Gjorgovska *et al.*, 2014).

There were no differences in other exterior parameters among the experimental groups ( $P > 0.05$ ).

There are few studies investigating the effects of the soy phytoestrogens on bone strength in animals, but this is one of the newest studies to investigate the

effect of genistein and daidzein on the bone quality of laying hens during late egg production.

These results suggest that the high supplemented isoflavones treatment (1800 mg.kg<sup>-1</sup> feed) has a beneficial effect ( $P < 0.05$ ) on bone quality of aged laying hens (weight, volume, ash/tibia and calcium), but do not affect on the tibial phosphorus ( $P > 0.05$ ). Sahin *et al.* (2007) reported that supplementation with soy isoflavones significantly improve bone mineral density in Japanese

**Table 4: Bone quality and mineralization of the tibia (calculated on dry matter)**

	Basal feed (BF)	Basal feed (BF) + 300 mg.kg <sup>-1</sup> SI	Basal feed (BF) + 600 mg.kg <sup>-1</sup> SI	Basal feed (BF) + 1200 mg.kg <sup>-1</sup> SI	Basal feed (BF) + 1800 mg.kg <sup>-1</sup> SI
1. Weight of the tibia, g	7.08 ± 0.56 <sup>ab</sup>	6.71 ± 1.24 <sup>b</sup>	7.45 ± 0.77 <sup>ab</sup>	7.06 ± 0.77 <sup>ab</sup>	8.38 ± 1.01 <sup>a</sup>
2. Volume of the tibia, cm <sup>3</sup>	9.75 ± 0.50 <sup>b</sup>	8.38 ± 0.75 <sup>a</sup>	9.63 ± 0.75 <sup>b</sup>	9.83 ± 0.29 <sup>b</sup>	10.50 ± 0.58 <sup>b</sup>
3. Ash/ tibia, g	4.10 ± 0.37 <sup>ab</sup>	4.12 ± 0.62 <sup>ab</sup>	4.18 ± 0.53 <sup>ab</sup>	3.89 ± 0.56 <sup>b</sup>	4.88 ± 0.74 <sup>a</sup>
4. Calcium, g	2.68 ± 0.17 <sup>b</sup>	2.53 ± 0.47 <sup>b</sup>	2.97 ± 0.27 <sup>ab</sup>	2.61 ± 0.21 <sup>b</sup>	3.31 ± 0.50 <sup>a</sup>
5. Phosphorus, g	1.03 ± 0.32	1.16 ± 0.20	1.21 ± 0.42	1.22 ± 0.17	1.39 ± 0.15

SI – supplemented isoflavones; Ash concentration I (g.cm<sup>-3</sup>) (Ash concentration = ash weight/bone volume); a, b – values in the same row with no common superscript differ significantly (P < 0.05).

quail. Recent studies also suggested that higher isoflavone intake is associated with increased mineral content (Saiafzadeh and Jahanian, 2013).

## CONCLUSIONS

The results of the present study show that supplementation with 600 and 1800 mg isoflavones/kg diet increased the body weight significantly (P < 0.05). The longest shank length was noticed in the group fed with supplementation of 1800 mg.kg<sup>-1</sup> SI (P < 0.05). These findings also suggest that isoflavones have a stimulatory effect on calcium content in bone in groups supplemented with 1800 mg isoflavones .kg<sup>-1</sup> diet (P < 0.05). These results indicate that isoflavones are effective supplements to improve the body weight, body length and calcium bone content during the late laying period, even though their supplementation is high.

## REFERENCES

- GJORGOVSKA, N. – FILEV, K. – PAVLOVA, V. – LEVKOV, V. 2014. Effects of the soy isoflavones on the growth and the exterior development of the ISA Brown pullets. *Emirates Journal of Food and Agriculture*, vol. 26 (9), 2014, p. 822–827.
- JIANG, Z. Y. – JIANG, S. Q. – LIN, Y. C. – XI, P. B. – YU, D. Q. – WU, T. X. 2007. Effects of soybean isoflavone on growth performance, meat quality, and antioxidation in male broilers. *Poultry Science*, vol. 86 (7), 2007, p. 1356–1362.
- NI, Y. – ZHU, Q. – ZHOU, Z. – GROSSMANN, R. – CHEN, J. – ZHAO, R. 2007. Effect of Dietary Daidzein on Egg Production, Shell Quality, and Gene Expression of ER- $\alpha$ , GH-R, and IGF-IR in Shell Glands of Laying Hens. *Journal of Agricultural and Food Chemistry*, vol. 55 (17), 2007, p. 6997–7001.
- PAYNE, R. L. – BIDNER, T. D. – SOUTHERN, L. L. – MCMILLIN, K. W. 2001. Dietary Effects of Soy Isoflavones on Growth and Carcass Traits of Commercial Broilers. *Poultry Science*, vol. 80 (8), 2001, p. 1201–1207.
- PETEK, M. – BALCI, F. – BASPINAR, H. – OGAN, M. – DIKMEN, S. 2000. The effects of shank length on some production traits of a commercial layer flock. *Uludag University Journal of Faculty of Veterinary Medicine*, vol. 19 (3), 2000, p. 129–134.
- SAHIN, N. – ONDERCI, M. – BALCI, T. A. – CIKIM, G. – SAHIN, K. – KUCUK, O. 2007. The effects of soy isoflavones on egg quality and bone mineralisation during the late period of quail. *British Poultry Science*, vol. 48 (3), 2007, p. 363–369.
- SAIAFZADEH, S. – JAHANIAN, R. 2013. Effect of soy isoflavones on ovarian follicle dynamics and bone mineral content in leghorn laying hens. In: 19<sup>th</sup> European Symposium on Poultry Nutrition: Proc. Potsdam, Germany, August 26-29, 2013, p. 1-5.
- WEBSTER, A. 2004. Welfare implications of avian osteoporosis. *Poultry Science*, vol. 83 (2), 2004, p. 184–192.
- WHITEHEAD, C. – FLEMING, R. 2000. Osteoporosis in cage layers. *Poultry Science*, vol. 79 (7), 2000, p. 1033-1041.
- YILMAZ-DIKMEN, B. – IPEK, A. 2006. The Effects of shank length on egg production and Egg quality traits of Japanese quails (*Coturnix coturnix japonica*). In: EPC 2006 – XII European Poultry Conference: Proc. Verona, Italy, September 10-14. 2006.
- ZHAO, R. Q. – ZHOU, Y. C. – NI, Y. D. – LU, L. Z. – TAO, Z. R. – CHEN, W. H. – CHEN, J. 2005. Effect of daidzein on egg-laying performance in Shaoxing duck breeders during different stages of the egg production cycle. *British Poultry Science*, vol. 46 (2), 2005, p. 175–181.

## SURFACE TEMPERATURE OF WARM-WATER PADS FOR HEATING PIGLETS IN FARROWING PENS

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### ABSTRACT

The objective of this study was to evaluate the supplementary piglet heating on the basis of surface temperature of warm-water pads (WWP) in strawless farrowing house. Two WWPs (0.48 m<sup>2</sup>) were installed in each farrowing pen for piglet heating. Surface temperature of all double-size pads was measured by infrared thermometer (GIM 3590) in 120 farrowing pens during winter season. Average surface temperature of the pads was assessed in 8 groups in section rows (in the order of 1<sup>st</sup> till the 8<sup>th</sup> pen) including frequency distribution of temperatures in the range 34–43 °C (with scale of 1 °C), in the range 37–43 °C and 39–41 °C, and in the 3 specific temperature limits (< 37.8 °C, 37.8–38.9 °C, and > 38.9 °C). The data were analyzed using the Descriptive Statistics procedure and a General Linear Model ANOVA by the statistical package STATISTIX 10.0. Significance of differences between WWP in pens was determined by LSD All-Pairwise Comparisons Test (at  $\alpha \leq 0.01$ ). Outdoor average air temperature was -0.6 °C and average temperature in the farrowing sections was 18.7 °C during the measuring period. Warm-water pads in the group of second pens had the highest average surface temperature 40.59 ± 0.96 °C. The pads in the eighth group of pens had the lowest temperature 37.91 ± 1.83 °C ( $P < 0.001$ ), because these were situated at the external enclosure wall of stable. In these pens, internal wall was not thermally insulated sidelong the heating pads, as it was in the first group of pens. As far as the average surface temperature is concerned, almost 94.17 % of double-size pads had advantageous temperature (37–43 °C) and 67.50 % had the optimum temperature (39–41 °C). It is possible to get improvement of temperature conditions by additional insulation of the external enclosure wall in the area of pens as well as by optimal heat regulation of the pads base on the position of lying piglets.

**Key words:** warm-water pads; temperature; farrowing pen; piglets

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### INTRODUCTION

The newborn pig is extremely susceptible to cold, damp conditions. It has little fat and hair, a thin skin and a small mass in relation to its body surface area (Roese and Taylor, 2006). Pigs have a normal temperature range of 38–40 °C. Piglets do not have the ability of thermoregulation at birth, because they need heat immediately to survive and grow (Blecha, 2001). Immediately after birth, a piglet's temperature can drop by 1 °C or more within the first half-hour, depending on environmental conditions. Under favourable conditions, this decrease in temperature is regained in about 24–48 hours (Roese and Taylor, 2006).

This is particularly stressful for a newborn piglet which after birth experiences a sudden 15–20 °C decrease in ambient temperature (Herpin *et al.*, 2002). Piglet after its birth leaves a warm draft-free environment of 38.9 °C which is the sow's womb temperature and so for the newborn piglet even the temperature of 35 °C is cold during the first hours its life. At temperatures below 37.8 °C the piglet uses the sow milk also for warming its body (AHSI, 2010). For new born pigs in its resting (creep) area the temperature should be range from 32 °C (Lean, 1994; Curtis, 1995) to 35 °C (Xin and Zhang, 2000), which gradually decreases till 22–24 °C weaning (Herpin *et al.*, 2002; Ilsters *et al.*, 2009). When the temperature drops below the piglets'

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thermo-neutral zone (34 – 36 °C), piglets try to increase their heat production by means of energetically demanding muscular shivering thermogenesis (Berthou *et al.*, 1994), and they try to reduce their heat loss by social and individual thermoregulation (Vasdal *et al.*, 2009).

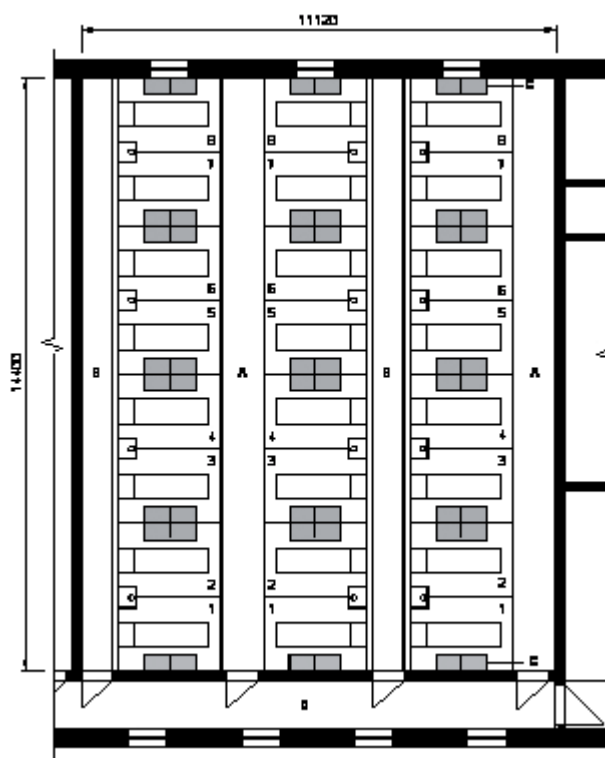
Recommended optimum range of the air temperature in farrowing house for lactating sows is 16 – 22 °C only (Odehna *et al.*, 2008; Pig Welfare Standards, 2012). The temperature of 22 °C is upper critical thermal limit for the lactating sow (Quiniou and Noblet, 1999). Because room temperature in the farrowing unit is usually kept within the sows' thermal comfort zone (around 20 °C), it is necessary to provide a suitable temperature (30 – 34 °C) in the creep area by external heat sources and some sort of insulated flooring to avoid hypothermia in piglets. Several studies have found that newborn piglets prefer to huddle near the sow and littermates despite unfavourable thermal conditions in the sow area, instead of staying in the heated creep area during the first days after birth (Andersen *et al.*, 2007; Vasdal *et al.*, 2009). Hypothermia might often predispose piglets to starvation and crushing by the sow (Edwards, 2002), especially in the first three days of life when crushing risk is at its greatest (Berg *et al.*, 2006).

Newborn piglets have remarkably higher requirements for warmth. Because the neonatal piglet requires quite a different environment from the sow, heated creep area (away from the sow) is essential in the farrowing pen (Roese and Taylor, 2006). It is necessary to use local heating of their area, the best by heated pads. To keep minimum ambient temperature of 20°C till piglets' age of 1 month it is necessary to use local heating of their area.

Electric or warm-water pads for bottom local heating are used. Warmth transmission to piglets occurs by their body contact with the surface of heated pad. The surface temperature of pad (mat) is usually preset to certain levels (commonly 30 – 40 °C) according to the age of piglets, room temperature, and management style (Zhang and Xin, 2000a). Ilsters *et al.* (2009) suggested that the temperature of the resting place for piglets comprising of concrete floor panels should range from 35 °C to 40 °C. Zhang and Xin (2000b) proposed an acceptable mat surface temperature of 34 – 43°C for creep heating and for surface heating of piglets. de Baey-Ernsten *et al.* (1995) suggested a tolerable surface temperature range of 37 – 43 °C. If the goal is to increase piglets' use of the creep area during the first critical days after farrowing, it seems important to increase the attractiveness of the area itself by using temperature gradients (Lynch, 1983; Barber and Bourne, 1987). Vasdal *et al.* (2010) showed that piglets had a significant preference for 42 °C compared to 34 °C ( $P < 0.05$ ) when

different types of infrared temperatures in the creep area at 24 hours of age were tested.

Recommended surface temperature of WWP at the birth of piglets is about 38 – 41 °C (MIK International, 2014). The laying position of the litter determines the WWP temperature's control variable. Since the temperature needs of the litter cannot be established according to age, the lying position of the litter must be observed and the temperature adjusted as needed. According to DLG Fokus Test warm-water pads are tested from the point of view of the surface share with advantageous temperatures in the range of 37 – 43 °C and with optimum temperatures ranging from 39 °C to 41 °C (MIK International, 2009). From the viewpoint of comfort zone for piglets (AHSI, 2010), correct regulation of temperature, either optimum or advantageous surface temperatures of heated pads are important factors. The control of the surface temperature of heating areas is very important to keep the optimal temperature for the sucking piglets.



**Fig. 1: Layout of the 1<sup>st</sup> farrowing section (from the total number of 5) with 3 x 8 farrowing pens with crate and warm-water pads for floor heating of piglets**

1 to 8 - farrowing pens (pen groups according to their order), A - manipulating alley, B - feeding alley, C - warm-water pads, D - marginal lengthwise passage in stable

## MATERIAL AND METHODS

The objective of this study was to evaluate the supplementary piglet heating on the basis of surface temperature of warm-water pads (WWP) in strawless farrowing house. In the farrowing house there were 5 sections with 3 rows of pens, 2 feeding and 2 manipulating alleys (Figure 1). In each row there was 8 farrowing pens with crate and a plastic slotted floor. The pens were situated across the alley in sections. Vacuum ventilation was regulated in all the sections.

The system of warm-water pads was used for supplementary heating of piglets. Two WWP with dimensions 400 x 600 mm were installed in each farrowing pen at the left or right side. They were mounted to the floor pen next to each other so as to create a long strip of 1 200 mm with a total area of 0.48 m<sup>2</sup> (double-size pad). Piglets had tipping covers at their disposal with plastic sheets. Warm-water pads in first farrowing pens were situated at the enclosure wall adjacent to marginal lengthwise passage of the farrowing unit. WWPs in the last group of (eighth) pens were situated in section rows at the external enclosure wall of the stable.

Surface temperature of double-size warm-water pad was measured in all the 120 farrowing pens by infrared thermometer GIM 3590 with an internal data logger (Greisinger Electronic GmbH, Regenstauf, Germany; accuracy  $\pm 0.75$  °C or  $\pm 0.75$  %, whichever was greater) in winter season. The measurements were taken vertically from a distance of 500 mm above the WWP surface as a whole by continuous scanning line in 4 selected longitudinal lines for a period of time about 11 seconds. Response time of this device was 150 ms, which correspond to a data set with about 70 values. Measured values of data sets (Max, Min, Avg) were continuous stored in device memory as the measurement protocols. Maximum, minimum and average values were related to the duration of measurement.

For data evaluation of 120 double-size pads farrowing pens were divided according to their order in the lateral section rows into eight longitudinal groups with 15 pens in each group. The average

surface temperature of double-size pads was assessed in first to the eighth group of pens in the farrowing house including frequency distribution of temperature. Frequency distribution of surface temperature of WWPs was evaluated in the range 34 – 43 °C with scale of 1 °C (8 temperature zones with difference of 9 °C), than in the range from 37 °C to 43 °C and from 39 °C to 41 °C (as advantageous and optimum temperatures; MIK International, 2009) and in the three specific temperature limits (< 37.8 °C, 37.8 – 38.9 °C, and > 38.9 °C).

Temperature and the relative humidity of air were registered in the farrowing sections and in the external environment during the measuring period by electronic data loggers HDL (HIVUS Ltd., Žilina, Slovakia).

The data were analyzed using the Descriptive Statistics procedure and a General Linear Model ANOVA by the statistical package STATISTIX 10.0 was used (Analytical Software, Tallahassee, Florida, USA). Significance of differences between the average surface temperatures of warm-water pads in pens was determined by LSD All-Pairwise Comparisons Test (at  $\alpha \leq 0.001$ ).

## RESULTS AND DISCUSSION

Average temperature of air in the farrowing house was  $18.7 \pm 0.62$  °C during the measuring period and average air temperature in the external environment was  $-0.6 \pm 3.72$  °C (Table 1). Indoor air temperature ranged from 17.4 °C to 20.6 °C and the outdoor air temperature ranged from -7.9 °C to 4.8 °C. Average relative humidity of air in the farrowing house was  $54.6 \pm 3.72$  % and in external environment it was  $73.8 \pm 9.88$  %. The indoor relative humidity of air ranged from 39.8 % to 64.6 % and the outdoor air relative humidity ranged from 49.1 % to 91.9 %. All the measured internal temperatures of air were suitable for sows (Odehnaľova *et al.*, 2008; Pig Welfare Standards, 2012) but not for sucking piglets. Registered room temperatures were lower than 22 °C which is the bottom temperature limit for piglets in the time of their weaning (Herpin *et al.*, 2002) and supplementary heating by WWPs was essential to maintain the optimal temperature for the sucking piglets (Zhang and Xin, 2000;

**Table 1: Temperature and relative humidity of air during the measuring period**

Parameter	Air temperature, °C		Relative humidity of air, %	
	Farrowing house	External environment	Farrowing house	External environment
Average	18.7	-0.6	54.6	73.8
SD	0.62	3.72	4.67	9.88
Minimum	17.4	-7.9	39.8	49.1
Maximum	20.6	4.8	64.6	91.9

**Table 2: Surface temperatures of double-size warm-water pads in farrowing pens according to their order in section rows (pen groups)**

Parameter	Order of pens in a row in sections (pen groups in farrowing house)							
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>
Average*	40.20 <sup>a</sup>	40.59 <sup>a</sup>	40.27 <sup>a</sup>	40.25 <sup>a</sup>	40.26 <sup>a</sup>	39.97 <sup>a</sup>	39.30 <sup>ab</sup>	37.91 <sup>b</sup>
SD	1.29	0.96	1.08	1.19	0.55	0.72	1.27	1.83
Minimum	36.8	38.5	38.8	38.7	38.9	38.8	35.9	35.3
Maximum	41.9	42.1	42.0	42.7	41.0	40.8	40.7	40.5

\*Significance of differences at  $P < 0.001$ , <sup>a-b</sup>Data with an equal superscript are not significantly different from one another (LSD test at  $\alpha = 0.001$ ),  $n = 15$  data sets (i.e. data set includes about 70 measured values)

Roese and Taylor, 2006; Vasdal *et al.*, 2010).

Warm-water pads in the group of second pens in the farrowing house had the highest average surface temperature of  $40.59 \pm 0.96$  °C (Table 2). The lowest temperature of  $37.91 \pm 1.83$  °C ( $P < 0.001$ ) was recorded for the pads in the group of eighth pens (last group of pens in section rows), which were situated at external enclosure wall. The internal wall in the area of these pens was not thermally insulated (by plastic plate) as most of the first group of pens (80 %). Warm-water pads in the group of the first group of pens, which were situated closest to the regulation unit, had average surface temperature of  $40.20 \pm 1.29$  °C, i.e. by 0.39 °C lower than WWPs in the group of second pens in farrowing unit. However, this difference was non-significant statistically. Most likely it could be affected by the fact that the pads in the group of first pens were situated at the enclosure wall adjacent to marginal lengthwise passage in farrowing house. In this passage the temperature of air was lower than in the individual sections. Each of farrowing sections had four doors which were not always closed when the attendant staff was entering or exiting. The side pen walls in all the outlying first pens in section rows (in the area with heating pads) were made from the ceramic tiles, but three farrowing pens in the middle section in this area did not have insulated wall in the form of plastic plate (like all the pens in the eighth in section rows). The surface temperature of ceramic tiles was lower than the surface

temperature of the plastic plate. All mentioned factors could also affect the surface temperature of WWPs in the group of first farrowing pens.

Frequency distribution of 120 average surface temperatures of double-side pads (Table 3) indicated that the largest share of pad temperatures were in the range of 40 – 41 °C (40.8 %) and in the range of 39 – 40 °C (26.7 %). Ten pads (8.3 %) had surface temperatures lower than 38 °C and 16 pads (13.4 %) had surface temperatures higher than 41 °C. All measured surface temperatures were higher than 35 °C and lower than 43 °C, which corresponds with acceptable mat surface temperature range (34 – 43 °C) for creep heating as proposed by Zhang and Xin (2000b). Surface temperatures in the range of 37 – 43 °C, i.e. advantageous temperature (MIK International, 2009) were recorded for 113 double-size pads (94.17 %). This corresponds with a tolerable surface temperature range for surface heating of piglets as suggested by de Baey-Ernsten *et al.* (1995). Surface temperatures of 81 double-size pads (67.50 %) were in the range of 39 – 41 °C, which was also deemed as optimum temperatures by us (MIK International, 2009).

Frequency distribution of surface temperatures according to specific temperature limits (Table 4) suggested that 10 % of double-size warm-water pads had average surface temperature below 37.8 °C and 13 % of pads had the surface temperature between 38.7 °C and 38.9 °C. This means that the pads with temperature

**Table 3: Frequency distribution of average surface temperatures of double-size warm-water pads in the farrowing house**

Parameter	Range of surface temperatures in °C							
	35-36*	36-37	37-38	38-39	39-40	40-41	41-42	42-43
Number	4	3	3	13	32	49	11	5
%	3.3	2.5	2.5	10.8	26.7	40.8	9.2	4.2

\*The lower limit of range includes the value and the upper limit excludes the value.

**Table 4: Specific frequency distribution of average surface temperatures of double-size warm-water pads**

Parameter	Specific range of surface temperatures in °C		
	< 37.8 °C <sup>1)</sup>	37.8 °C <sup>2)</sup> – 38.9 °C <sup>3)</sup>	> 38.9 °C
Number of values	10	13	97
Percentage share	8.33	10.83	80.84

<sup>1)</sup> the temperatures at which milk can be used for the heating of piglet body and not to growth, <sup>2)</sup> the temperature providing comfort zone for piglets, <sup>3)</sup> the temperature of the environment in the sow's womb

below 37.8 °C do not provide the comfort zone for piglets and a certain amount of milk received can be used for the heating of their body and not to growth (AHSI, 2010). Surface temperature of 38.7 °C or more may provide comfort zone for piglets and the value of 38.9 °C is the temperature of environment in the sow's womb. The largest share (97 %) of all 120 double-size pads had the surface temperature above 38.9 °C, which is favourable in terms of providing temperature comfortable for piglets during the first critical days after farrowing.

## CONCLUSION

Among 8 groups of 15 double-size warm-water pads (total 120 pads with dimensions 400 x 1200 mm) the lowest average surface temperature (37.9 °C) was recorded in pads in the group of eighth (last) farrowing pens in section rows (by their order), which were situated at external enclosure wall without thermal insulation of side pen wall from ceramic tiles in the area with local floor heating. All the measured surface temperatures were higher than 35 °C and lower than 43 °C. As far as the average surface temperature is concerned, almost 94.2 % of double-size warm-water pads had advantageous (acceptable) temperature in the range of 37 – 43 °C and 67.5 % of double-size pads had the optimum temperature in the range of 39 – 41 °C.

It is possible to get improvement of temperature conditions by additional insulation of the external enclosure wall in the area of pens as well as by optimal heat regulation of water heated pads based on the position of lying piglets.

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## REFERENCES

- AHSI. Hog Hearth heat mat. AHSI (Alternative Heating Systems Inc.), Available at: <http://www.ahsi.ca:81/hog-hearth-advantages.page.htm>. [accessed 19.05.2015].
- ANDERSEN, I. L. – TAJET, G. M. – HAUKVIK, I. A. – KONGSRUD, S. – BØE, K. E. 2007. Relationship between postnatal piglet mortality, environmental factors and management around farrowing in herds with loose-housed, lactating sows. *Acta Agriculturae Scandinavica, Section A - Animal Science*, vol. 57, 2007, p. 38–45.
- BARBER, J. – BOURNE, R. A. 1987. The effect of farrowing house temperature on piglet behaviour and creep use. *Animal Science*, vol. 44, 1987, p. 478.
- BERG, S. – ANDERSEN, I. L. – TAJET, G. M. – HAUKVIK, I. A. – KONGSRUD, S. – BØE, K. E. 2006. Piglet use of the creep area and piglet mortality - effects of closing the piglets inside the creep area during sow feeding time in pens for individually loose-housed sows. *Animal Science*, vol. 82, 2006, p. 277–281.
- BERTHON, D. – HERPIN, P. – LE DIVIDICH, J. L. 1994. Shivering thermogenesis in the neonatal pig. *Journal of Thermal Biology*, vol. 19, 1994, p. 413–418.
- BLECHA, F. 2001. Immunology. In: *Biology of the Domestic Pig*. (ed.) Pond, W. G and H. J. Mersmann. Cornell University Press, Ithaca, NY., 2001, p. 688–712.
- CURTIS, S. E. 1995. The physical environment and mortality. In: *VARLEY, M. A. (ed.). The Neonatal Pig. Development and Survival*. Oxon; CAB International, Wallingford, 1995, p. 269–285.
- de BAEY-ERNSTEN, H. – von der HAAR, F. – BICHMANN, M. – CLAUSEN, N. 1995. Heating



- systems for piglets in a practical comparison. Institute for agricultural Process Engineering (Institut für Landwirtschaftliche Verfahrenstechnik), University of Kiel, Germany, 1995.
- EDWARDS, S. A. 2002. Perinatal mortality in the pig: environmental or physiological solutions? *Livestock Production Science*, vol. 78, 2002, p. 3–12.
- HERPIN, P. – DAMON, M. – LE DIVIDICH, J. 2002: Development of thermoregulation and neonatal survival in pigs. *Livestock Production Science*, vol. 78, 2002, p. 25–45.
- ILSTERS, A. – ZIEMELIS, I. – KRISTUTIS, I., 2009. Possibilities of heat pump usage for heating piglet resting places. Engineering for Rural Development Jelgava, 2009, p. 202–206, [http://www.tf.llu.lv/conference/proceedings2009/Papers/34\\_Andrievs\\_Ilsters.pdf](http://www.tf.llu.lv/conference/proceedings2009/Papers/34_Andrievs_Ilsters.pdf).
- LEAN, I. J. 1994. Pigs. In: Universities Federation for Animal Welfare (UFAW). Herts; UFAW, 1994, p. 9.
- LYNCH, P. B. 1983. Heat seeking behaviour of newborn pigs as affected by house temperature and level of lighting. *Animal Science*, vol. 36, 1983, p. 531 (abstract).
- MIK International. 2009. Water heated heating panels. Technical report no. 7134 8988. TÜV SÜD Product Service, Frankfurt am Main, 2009, 18 p. Available at: [http://download.mik-online.de/agrar/en/news/thermow\\_TUEV\\_english.pdf](http://download.mik-online.de/agrar/en/news/thermow_TUEV_english.pdf). [accessed 19.05.2015].
- MIK International. 2014. Operating Instruction Thermo W 400x600. Ransbach-Baumbach, 2014, 20 p. Available at: [http://root.mik-ag.de/download/agrar/en/Bedienungsanleitung\\_Thermo\\_W\\_400x600\\_engl\\_02\\_09\\_2014.pdf](http://root.mik-ag.de/download/agrar/en/Bedienungsanleitung_Thermo_W_400x600_engl_02_09_2014.pdf). [accessed 19.05.2015].
- ODEHNALOVA, S. – VINKLER, A. – NOVAK, P. – DRABEK, J. 2008. The dynamics of changes in selected parameters in relation to different air temperature in the farrowing house for sows. *Czech Journal of Animal Science*, vol. 53 (5), 2008, p. 195–203.
- PIG WELFARE STANDARDS. 2012. Victorian Standards and Guidelines for the Welfare of Pigs. Revision 1. Melbourne, Victoria, Department of Primary Industries, Biosecurity Victoria, 2012. 40 p., ISBN 9781742647920 (print), 9781742647937 (online), Available at: <http://agriculture.vic.gov.au/agriculture/animal-health-and-welfare> [accessed 9.12.2015].
- QUINIOU, N. – NOBLET, J. 1999. Influence of high ambient temperatures on performance of multiparous lactating sows. *Journal of Animal Science*, vol. 77, 1999, p. 2124–2134.
- ROESE, G. – TAYLOR, G. 2006. Basic pig husbandry – the litter. PRIMEFACT 71, NSW Department of Primary Industries, 2006, 8 p.
- VASDAL, G. – MØGEDAL, I. – BØE, K. E. – KIRKDEN, R. – ANDERSEN, I. L. 2010. Piglet preference for infrared temperature and flooring. *Applied Animal Behaviour Science*, vol. 122, 2010, p. 92–97.
- VASDAL, G. – WHEELER, E. F. – BØE, K. E. 2009. Effect of infrared temperature on thermoregulatory behaviour in suckling piglets. *Animal*, vol. 3, 2009, p. 1449–1454.
- XIN, H. – ZHANG, Q. 2000. Effects of drying off birth fluid on the newborn piglet. ASAE Paper No. 004112. St. Joseph, Michigan, American Society of Agricultural Engineers, 2000.
- ZHANG, Q. – XIN, H. 2000a. Modeling heat mat operation for piglet creep heating. *Transactions of the ASAE*, vol. 43 (5), 2000, p. 1261–126.
- ZHANG, Q. – XIN, H. 2000b. Static and dynamic temperature distribution of heat mats for swine farrowing creep heating. *Applied Engineering in Agriculture*, vol. 16 (5), 2000, p. 563–569.

Review

## HEAVY METALS – ENVIRONMENTAL CONTAMINANTS AND THEIR OCCURRENCE IN DIFFERENT TYPES OF MILK

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### ABSTRACT

The aim of this review is to summarize the findings about heavy metals and their effects on animal organism and to describe a source of contamination and inputs of heavy metals into food chain. Furthermore, the comparison of the occurrence of selected heavy metals in different types of milk on the basis of previous studies has been made. Based on available information and according to level of contamination, we want to draw an attention to suitability of using milk for further processing in selected areas of the world.

**Key words:** heavy metals; toxic elements; contaminants; milk

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### INTRODUCTION

Industrial progress, industrialization, urbanization and agricultural production have become permanent resources of extraneous chemicals for living organisms. In recent years, food safety is regarded as a carrier theme for food industry. The increase in agricultural production efficiency causes using of large quantities of chemical products not even in animal feed production, but also on farms with milk production. Due to non-compliance with right technologies, these substances are becoming a part of agricultural products, including milk from cows, ewes or goats. These contaminants are not a natural part of milk, but they are secreted into the milk from animal body. Success in food production significantly depends on the abundance of quality materials and good manufacturing practice. Therefore, an attention should be paid to understanding the problems and ways of possible penetration of contaminants into the food chain.

### Heavy metals and trace elements

Heavy metals are widely dispersed in the environment. The toxicities induced by excessive levels of some of these elements, such as chromium (Cr), cadmium (Cd), lead (Pb) and mercury (Hg) are well known (Llobet *et al.*, 2003). Heavy metals like cadmium, lead, mercury and arsenic are the major toxic metals posing a threat to human health. Their concentrations in animal organisms and their milk concentrations may increase very fast, although their excretion through milk is very low (Miller, 1971; Houpert *et al.*, 1997). Their ecosystem accumulation (water-soil-plant-animal) makes them very toxic and leads to undesirable consequences for live organisms (Bogut *et al.*, 2000; Piskorova *et al.*, 2003). Free-living animals are important indicators of the environmental pollution with heavy metals (Kottferová and Koréneková, 1998). Sheep and cattle reared freely on pasture are also indicators of the environmental pollution (Gallo *et al.*, 1996). Increased concentrations of heavy metals in body

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of domestic animals result in low fitness of animals, reproduction problems, immunity decline and occurrence of cancerous and teratogenic diseases (Bires *et al.*, 1995).

In the local studies, cadmium, lead, copper, zinc, iron, chromium and manganese are found at toxic levels in soils and vegetables (Ghafoor and Rasool, 1999; Quadir *et al.*, 2000). These studies guide to hypothesize that fodders grown on such soils will also accumulate these heavy metals, and animals reared on contaminated fodder will contain heavy metal residues in edible tissues, such as milk. The general public eating such contaminated edible products may accumulate toxic levels of heavy metals (Licata *et al.*, 2004).

Pollution of the environment with metals, such as lead, is a world-wide problem. Lead alkyl additives into petrol are combusted and emitted into the atmosphere and can be responsible for high concentration of lead in some vegetation, roadside, soil, air, water and plants (Burguera and Randon, 1987).

Lead has been added to petrol (gasoline) as an anti-knocking agent since the 1920s in order to improve fuel performance and reduce wear on vehicle engines. Since this time, leaded petrol has been reported to cause more lead exposures than any other source worldwide (Landrigan, 2002). During the 1970s, health impacts associated with lead emissions from vehicles became a widely discussed issue. Many studies have reported that environmental lead emissions have resulted in significant health effects to the central nervous system, haem-synthesis, reproductive system, as well as psychological and neurobehavioral functions, and may even increase the risk of cancer (Bellinger, 2005; Fewtrell *et al.*, 2004; Tong *et al.*, 2000). It has been observed throughout the world that the lead content of various environmental components has been decreased after the replacement of leaded petrol (Bridbord and Hanson, 2009; Landrigan, 2002).

The use of leaded petrol resulted in the emission of large quantities of lead that are still present in the ambient environment, which may continue to cause concerns for health (Mielke *et al.*, 2011). Although the United States were responsible for 80 % of all leaded petrol sold globally prior to 1970 (Kristensen, 2015), Australia was a substantial consumer of lead petrol products. The use of leaded petrol in Australia over a 70 years period, from 1932 to 2002 (Cook and Gale, 2005) was a major contributor to atmospheric lead levels (Kristensen, 2015). With the introduction of unleaded petrol in Australia in 1985 and the subsequent phase out of leaded petrol by 2002, ambient lead levels have fallen in metropolitan

urban centers to levels less than 10 % of Australia's current guideline for lead in ambient air ( $0.5 \mu\text{g}\cdot\text{m}^{-3}$ ) (Abeeb *et al.*, 2003).

Manufacturing processes, incineration of refuse and combustion of coal, are also the other sources which contribute to lead occurrence in the atmosphere; hence it is not surprising that lead levels are highest in area of intense industrialization (Burguera *et al.*, 1988; Shakour *et al.*, 2006). Lead is toxic to the blood and the nervous, urinary, gastric and genital systems. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis and teratogenesis in experimental animals (Pitot and Dragan, 1996; Baht and Moy, 1997). Accumulation of lead in the organism produces damaging effects in the hematopoetical, hematic, renal and gastrointestinal systems (Correia *et al.*, 2000). On the other hand, cadmium is also easily volatilized at the operating temperatures of common industrial processes, much of the cadmium in the atmosphere results from incineration of ferrous scrap and metallurgy processes (Thomas *et al.*, 1972). Cadmium is considered to be one of the most toxic metals. In addition, it is implicated in high blood pressure (Perry *et al.*, 1979), prostate cancer, mutations and fetal (embryonic) death (Pitot and Dragan, 1996). Chromium, nickel and cobalt are also toxic metals which are released to the environment. They originated from dumping industrial wastes in the rivers, as well as the application of phosphatic fertilizers (Venugopal and Luckey, 1978).

Toxicity of metal is closely related to age, sex, route of exposure, level of intake, solubility metal oxidation state, duration of exposure, frequency of intake, absorption rate and mechanisms/efficiency of extraction (Venugopal, 1978; Mertz, 1986).

Milk and milk products are the most diversified of the natural food stuffs in terms of composition and contain more than twenty different trace elements. Most of them are essential and very important, such as copper, zinc, manganese and iron (Schroeder, 1973; Somer, 1974). These metals are cofactors in many enzymes and play an important role in many physiological functions of man and animals. Lack of these metals causes disturbance and pathological conditions (Koh, 1986; Schuhmacher, 1991). The amount of metals in non-contaminated milk is admittedly minute, but their content may be significantly altered through manufacturing and packaging process. Also, metals that can contaminate different cattle feed and environment, such as lead, cadmium, chromium, nickel and cobalt, could be excreted into milk at various levels and cause serious problems (Abou-Arab, 1994, 1997).

**Table 1: Classification of elements according to toxicity (Toman *et al.*, 2003)**

Low toxicity			Very toxic, relatively accessible			Toxic, low solubility		
Na	C	F	Be	As	Au	Ti	Ga	Hf
K	P	Li	Co	Se	Hg	La	Zr	Os
Mg	Fe	Rb	Ni	Te	Cu	W	Rh	Nb
Ca	S	Sr	Pd	Pb	Zn	Ir	Ta	Ru
H	Cl	Al	Ag	Sb	Sn	Re	Ba	
O	Br	Si	Cd	Bi	Pt			

### The effect of toxic elements

Cadmium and lead are heavy metals which have caused most concern in terms of adverse effects on human health. This is because they are readily transferred through the food chains and are not known to serve any essential biological function (Liu, 2003). Children have been shown to be more sensitive to cadmium and lead than adults and the effects are cumulative. As a result, the regular absorption of small amounts of certain elements, such as lead, may cause serious effects on the health of growing children, including retardation of metal development and deficiencies in concentration, adverse effects on kidney function, blood chemistry and the cardiovascular system, as well as hearing degradation (Ataro *et al.*, 2008; Salma *et al.*, 2000).

Cadmium is ubiquitous environmental contaminant arising primarily from electroplating, plastics manufacturing, mining, paint pigments, alloy preparation, and batteries. Food is the most important source of cadmium in the non-smoking, non-occupationally exposed population (Järup, 2003). Cadmium causes tissue damage in humans and animals and many toxicological studies have found the functional and structural changes in the kidneys, liver, lungs, bones, ovaries and fetal effects (Kukner *et al.*, 2007; Massányi *et al.*, 2007).

Cadmium can induce both carcinogenic and non-carcinogenic effects on various organs including the lung, liver, kidney, bone and vascular system (Waalkes, 2003). At the cellular level, cadmium induces oxidative stress, cell proliferation and apoptosis (Tremellen, 2008; Turner and Lysiak, 2008). Cadmium is a known endocrine disruptor and reproductive toxicant (Henson and Chedrese, 2004; Cheng *et al.*, 2011) which affects male fertility through altered function of hypothalamic-pituitary-testicular axis (Lafente, 2013) and/or through direct gonadotoxic and spermiotoxic effects (Thompson and Bannigan, 2008). The disruption of functional structures within the blood-testis (Wong *et al.*, 2004; Siu *et al.*, 2009) and blood-epididymis barrier (Toman *et al.*, 2002; Dubé and Cyr, 2013) results in impaired spermatogenesis and sperm maturation processes associated with infertility (Cheng and Mruk, 2012). Cadmium has been suggested to have some of its toxic effects by disturbing metabolism of essential metals, such as selenium. Zinc and selenium are believed to be the antagonists of cadmium toxic effects (Toman *et al.*, 2009).

Selenium is known due to its antioxidant role in living systems and, therefore, it is considered to be an essential element for humans and animals. However, routine selenium supplementation is not recommended,

**Table 2: Mean  $\pm$  SE values (mg.L<sup>-1</sup>) of Cd, Cr, Ni and Pb in the milk of cattle and goats collected from two areas of Pakistan during the period of November, 2006- April, 2007 (Ijaz *et al.*, 2009)**

Metal	Cattle milk	Goat milk
Cd	0.076 $\pm$ 0.014*	0.084 $\pm$ 0.003
Cr	1.066 $\pm$ 0.074 <sup>NS</sup>	1.152 $\pm$ 0.045
Ni	22.395 $\pm$ 0.988*	19.522 $\pm$ 0.011
Pb	18.870 $\pm$ 2.912*	42.687 $\pm$ 0.051

<sup>NS</sup>Non-significantly ( $p > 0.05$ ) different from the respective value.

\*Significantly ( $p < 0.05$ ) different from the respective value.

if the Se intake is equal to 80 µg per day or greater (Burk, 2002). The best way of adequate selenium intake is via food and increase of its content in plants by soil or foliar applications is under investigation during the last decade (Ducsay and Ložek, 2006; Zhao and McGrath, 2009). The treatment with Se during Cd exposure has been demonstrated to have beneficial effects on Cd-induced toxicity (Kimáková *et al.*, 2006; El-Sharaky *et al.*, 2007). However, the co-effect of the trace elements on the toxicity caused by Cd is not yet well studied. Xiao *et al.* (2002) assessed the protective effect of Se and Zn on Cd-induced oxidative stress only in the kidney of the rat, besides, Cd was administrated using the intraperitoneal route.

Nickel is an essential trace element. Its industrial application has a broad spectrum and primarily it is used in alloys (Das *et al.*, 2008). Nickel is also an essential trace metal that is vital for growth enhancement in very low doses for birds and mammals (Lukáč *et al.*, 2009). High quantity of nickel is known to be injurious for animal and human health. Its effects on various aspects of reproduction have been previously described. Animal studies demonstrate that nickel has negative effects on the structure and function of the testis, seminal vesicles and prostate gland; there is similar report on adverse effect on spermatozoa (Pandey *et al.*, 2000; Forgacs *et al.*, 2001). Lukáč *et al.* (2014) reported about negative effect of nickel on spermatogenesis. The decrease in the relative volume of germinal epithelium indicates on alterations of the spermatozoa production.

Accelerated industrial and agricultural development considerably affects environmental emissions of selected toxic elements: cadmium, lead, mercury and arsenic (Lopez *et al.*, 2002). Their concentrations in animal organisms and their milk

concentrations may increase very fast, although their excretion through milk is very low (Houper *et al.*, 1997). Their ecosystem accumulation (water-soil-plant-animal) makes them very toxic and leads to undesirable consequences for live organisms (Bogut *et al.*, 2000; Piskorova *et al.*, 2003). Free-living animals are important indicators of the environmental pollution with heavy metals (Kottferová and Koréneková, 1998). Sheep and cattle reared freely on pasture are also indicators of the environmental pollution (Gallo *et al.*, 1996). It is also known that metal (Cd, Pb and Hg) excretion is significantly lower in the offspring (Oskarsson *et al.*, 1995).

### The content of heavy metals in different types of milk

Concentrations of heavy metals in milk were mainly described in cows (Cerkvenik *et al.*, 2000; Pilsbacher and Grubhofer, 2002; Sikirič *et al.*, 2003). Ijaz *et al.* (2009) compared the content of heavy metals (Cd, Cr, Ni, Pb) in cattle and goat milk in Pakistan. They found difference in concentrations of these metals, which are listed in Table 1. The goat milk was found strongly ( $p < 0.05$ ) contaminated with Cd than cattle milk, 0.084 vs. 0.076 mg.L<sup>-1</sup>, respectively.

Licata *et al.* (2004) and Tripathi *et al.* (1999) reported the levels of Cd in the milk of cattle 0.0228 and 0.00007 mg.L<sup>-1</sup> in area of Calabria-Italy and India. Baldini *et al.* (1990) (Rome-Italy), Cerutti (1999) (Milano-Italy) and Martino *et al.* (2001) (Spain) found Cd concentration in cattle milk in the range between 0.0002 and 0.03 mg.L<sup>-1</sup>, which is quite lower than the measured values in the study of Ijaz *et al.* (2009). Ijaz *et al.* (2009) states in his study, that the higher values of Cd residues in the milk of goats (0.084 mg.L<sup>-1</sup>), than in the cattle milk (0.076 mg.L<sup>-1</sup>) are also in accordance with the values determined by Coni *et al.* (1996) in the milk of goat

**Table 3: Metal content (mg.kg<sup>-1</sup>) in buffalo's and cow's raw milk samples from area of Giza-Egypt (Enb *et al.*, 2009)**

Metals	Buffalo's milk		Cow's milk	
	Mean ± SD	Range	Mean ± SD	Range
Iron	0.980 ± 0.442	0.786-1.242	0.682 ± 0.406	0.607-0.794
Copper	0.212 ± 0.102	0.188-0.542	0.142 ± 0.116	0.108-0.194
Manganese	0.076 ± 0.044	0.051-0.916	0.056 ± 0.038	0.048-0.084
Zinc	4.366 ± 0.814	3.966-6.814	3.146 ± 1.081	3.001-3.940
Lead	0.084 ± 0.042	0.044-1.088	0.066 ± 0.056	0.040-0.960
Cadmium	0.118 ± 0.086	0.094-0.142	0.086 ± 0.062	0.070-0.112
Chromium	0.042 ± 0.022	0.036-0.058	0.034 ± 0.014	0.028-0.066
Nickel	0.006 ± 0.010	0.003-0.009	0.004 ± 0.002	0.002-0.009
Cobalt	0.008 ± 0.010	0.003-0.014	0.004 ± 0.001	0.003-0.006
Tin	0.006 ± 0.010	0.004-0.010	0.003 ± 0.006	0.002-0.005

(0.15 mg.L<sup>-1</sup>) and by Licata *et al.* (2004) in cattle milk (0.0228 mg.L<sup>-1</sup>). However, 0.05 mg.L<sup>-1</sup> of Cd in the milk of goat has been reported by Caggiano *et al.* (2005) in the region of southern Italy.

Ijaz *et al.* (2009) indicated in his work, that the higher milk concentration of heavy metals can be attributed to the use of sewerage water for agricultural purposes. It has also been observed that the animals have direct access to this sewerage water for drinking. The uptake of heavy metals in the soil, vegetables, fodder and other herbage produced in the investigated areas of Faisalabad city the main sewerage drains may have a definite role in the contamination of the milk composition.

Enb *et al.* (2009) stated, that higher levels of heavy metals in buffalo's and cow's milk (Table 3) in his study may be attributed to the high contamination of animal feed and water by such pollutants. Abou-Arab (1994; 1997) indicated that these pollutants could be excreted into the milk at various levels and also could reach milk through handling procedures. In this respect, several studies have been carried out to assess metal contents in milk from different areas. Tripathi and Raghunath (1999) reported that metals (Zn, Cu, Pb and Cd) were detected in cow's and buffalo's milk samples at following levels: Zn 3.177-3.697 mg.L<sup>-1</sup>, Cu 0.043-0.195 mg.L<sup>-1</sup>, Pb 1.70-3.35 µg.L<sup>-1</sup> and Cd 0.07-0.10 µg.L<sup>-1</sup>. However, Onianwa *et al.* (1999) determined levels of Zn 0.39-2.75, Cu 0.07-0.67, Fe 1.68-15.1, Cr 0.005-0.030, Pb 0.03-0.18, Cd 0.004-0.009, Co 0.03-0.12 and Ni 0.04-0.09 mg.L<sup>-1</sup> in cow's milk in Nigeria. Regarding to the investigation of Lante *et al.* (2004), they reported that Zn (4.631 mg.L<sup>-1</sup>), Cu (0.0518 mg.L<sup>-1</sup>), Fe (0.290 mg.L<sup>-1</sup>), Mn (0.0291 mg.L<sup>-1</sup>), Cr (0.004 mg.L<sup>-1</sup>), Pb (5.23 µg.L<sup>-1</sup>) and Cd (0.40 µg.L<sup>-1</sup>) were detected at various levels in Italy. On the other hand, Caggiano *et al.* (2005) reported that metals in cow's milk were detected in southern Italy at the following

levels: Mn: 0.13, Cr: 0.20, Pb: 0.20 and Cd: 0.06 mg.kg<sup>-1</sup>. The average lead concentration 7.9 ng.L<sup>-1</sup> in raw milk samples collected from milk tanker in Iran was recorded by Tajkarimi *et al.* (2008) and they ranged from 1 to 46 ng.L<sup>-1</sup>.

In our study we found the levels of copper, mercury, nickel and lead in sheep and cow milk from selected area of Slovakia below the limit of quantification (LOQ) (Cu: <0.50; Hg: <0.002; Ni: <0.10; Pb: <0.010 mg.kg<sup>-1</sup>) in spring and autumn season. We analyzed contents of some trace elements in sheep milk: Fe 0.67 mg.kg<sup>-1</sup> in spring season; 0.69 mg.kg<sup>-1</sup> in autumn season, Mg 153 mg.kg<sup>-1</sup> in spring; 176 mg.kg<sup>-1</sup> in autumn and Zn 4.4 mg.kg<sup>-1</sup> in spring season; 3.8 mg.kg<sup>-1</sup> in autumn season. In cow milk we found content of Fe below the LOQ (<0.50 mg.kg<sup>-1</sup>) in both seasons, Mg 80 mg.kg<sup>-1</sup> in spring season; 103 mg.kg<sup>-1</sup> in autumn season and Zn 3.4 mg.kg<sup>-1</sup> in spring season; 4.1 mg.kg<sup>-1</sup> in autumn season (unpublished data).

According to Anutovič *et al.* (2005), concentrations of selected toxic elements in ewe's milk from the area of Slavonian region in Croatia can be different depending on lactation stage. In colostrum (2<sup>nd</sup> lactation day), Cd and Pb (0.011 and 0.035 mg.kg<sup>-1</sup>, respectively) concentrations were significantly higher (P<0.01), whereas As (0.011 mg.kg<sup>-1</sup>) concentrations were lower in comparison with milk on the 10<sup>th</sup> (Cd: 0.004; Pb: 0.022; As: 0.025 mg.kg<sup>-1</sup>), 30<sup>th</sup> (Cd: 0.005; Pb: 0.024; As: 0.028 mg.kg<sup>-1</sup>) and 60<sup>th</sup> (Cd: 0.006; Pb: 0.026; As: 0.025 mg.kg<sup>-1</sup>) lactation day.

Rodríguez *et al.* (1999) determined concentrations of Pb and Cd in samples of human, cow (raw and pasteurized) and goat milk and powdered infant formula. The following mean Cd concentrations were recorded: in human milk - 2.70 µg.L<sup>-1</sup>, in raw cow's milk - 4.88 µg.L<sup>-1</sup>, in pasteurized cow's milk - 4.30 µg.L<sup>-1</sup>, in goat's milk - 7.81 µg.L<sup>-1</sup> and in powdered infant formula - 3.81 µg.L<sup>-1</sup>.

**Table 4: Toxic heavy metals and trace elements in milk from lactating cows reared in different environments of India (Patra *et al.*, 2008)**

	Toxic and trace elements in milk					
	Lead	Cadmium	Copper	Cobalt	Zinc	Iron
Unpolluted area (n = 52)	0.25±0.03	0.033±0.02	0.101±0.006	0.18±0.009	3.95±0.40	5.10±1.06
Closed lead cum zinc smelter (n = 14)	0.26±0.047	0.052±0.005	0.14±0.007	0.23±0.037	12.50±0.73	8.68±1.60
Phosphate fertilizer and mining areas (n = 21)	0.28±0.039	0.037±0.005	0.08±0.006	0.25±0.010	6.34±0.63	4.11±1.15
Coal mining areas (n = 46)	0.35±0.024	0.057±0.003	0.08±0.004	0.21±0.008	4.79±0.31	2.19±0.33
Steel manufacturing plant (n = 46)	0.50±0.04	0.256±0.02	0.09±0.006	0.26±0.020	3.68±0.25	2.78±0.42
Aluminium processing plant (n = 25)	0.65±0.020	0.87±0.003	0.05±0.006	0.12±0.014	3.04±0.25	2.46±0.13
Lead zinc smelter (n = 21)	0.85±0.114	0.78±0.014	0.08±0.009	0.17±0.013	6.18±0.55	3.62±0.95

Respective milk samples were collected from 201 lactating cows and allowed to graze around industrial units; P < 0.05.

**Table 5: Concentrations of trace elements in raw cow's milk samples from the southern and northern Croatia (Bilandžič *et al.*, 2011)**

Element	Region	Number of samples	Mean ( $\mu\text{g.L}^{-1}$ )	Range (min.-max.) ( $\mu\text{g.L}^{-1}$ )	SD
As	Southern	90	18.5	1.0283.0	38.9
	Northern	67	43.5	1.0-1019.0	131.6
Cd	Southern	90	1.76*	1.0-11.0	1.94
	Northern	67	3.40*	1.0-20.0	3.89
Cu	Southern	90	931.9	2.0-17.077	2448.0
	Northern	67	848.4	1.0-377.0	1190.1
Hg	Southern	90	1.59*	1.0-0	1.45
	Northern	67	7.10*	1.0-90.0	16.6
Pb	Southern	90	58.7	1.0-370.0	82.9
	Northern	67	36.2	1.0-476.0	72.3

\*Significant difference between two regions:  $p < 0.001$ .

The concentrations of Pb were following: in human milk -  $8.34 \mu\text{g.L}^{-1}$ , in raw cow's milk -  $14.82 \mu\text{g.L}^{-1}$ , in pasteurized cow's milk -  $10.82 \mu\text{g.L}^{-1}$ , in goat's milk -  $11.86 \mu\text{g.L}^{-1}$ , and in powdered infant formula -  $8.30 \mu\text{g.L}^{-1}$ . Data from this study were within the normal ranges for each kind of milk. The Cd and Pb concentrations in goat's milk were significantly higher than the concentrations observed in the other kinds of milk, whereas human milk and powdered infant formula presented the lowest Cd and Pb concentrations. A considerable decrease in the concentration of Cd within the stage of lactation was observed. The concentrations of Cd and Pb in human, cow's and goat's milk also varied according to the time of year. The concentrations of Pb and Cd in different kinds of milk did not represent any risk to human health (infants or adults).

Tunegová *et al.* (2016) compared the content of selected toxic and trace elements in sheep and cow milk during two seasons (spring and autumn) in Slovakia. They reported that the content of Cd in cow milk was less than  $0.0040 \text{ mg.kg}^{-1}$  in spring season and less than  $0.0040 \text{ mg.kg}^{-1}$  in autumn season. The content of arsenic was below  $0.030 \text{ mg.kg}^{-1}$  in both seasons. In sheep milk they recorded content of Cd below  $0.0040 \text{ mg.kg}^{-1}$  and As below  $0.030 \text{ mg.kg}^{-1}$  in both seasons. The content of Se was below  $0.030 \text{ mg.kg}^{-1}$  in sheep and cow milks. In cow milk, value of calcium was  $915 \text{ mg.kg}^{-1}$  in spring season and  $1210 \text{ mg.kg}^{-1}$  in autumn season. Content of calcium in sheep milk was  $1770 \text{ mg.kg}^{-1}$  in spring and  $2170 \text{ mg.kg}^{-1}$  in autumn.

Weglarzy (2010) reported that cows are susceptible to contamination of the environment with the lead, which deposits in meat and liver, especially at the beginning of the growing season. He found that

there is more lead in the cow's cheese than in milk with the difference being the highest in April and with decreasing during the growing season. In May, the amount of cadmium in milk was lower. Cadmium and lead were depositing mostly in liver, and their amount in this organ was higher in autumn than in spring. Furthermore, the content of cadmium in meat was much higher than the acceptable level.

## CONCLUSION

The aim of this review was to document and compare the results of previous studies about prevalence of heavy metals in different types of milk and from different parts of the world. We pointed out effects of heavy metals on animal organism and their accumulation in human and animal organism. Majority of the literature sources shows that industry and agriculture are the most common sources of contaminants that are entering the food chain. Therefore, it is important to monitor the areas where milk is produced in order to avoid negative effects of these substances on animal and human organism and to improve the quality of milk, which is the raw material for further food processing.

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## REFERENCES

- ABEED, F. – MITCHELL, R. – HOPE, L. 2003. Future Air Quality Monitoring for Lead in Metropolitan Adelaide- a Report the National Environment Protection Council, 2003, South Australia.
- ABOU-ARAB, A. A. K. – KHOLIF, A. M. – ABOU EL-NOR, S. A. H. 1994. Effect of spraying diazinon to control the external parasites on the productive performance of dairy animals. Yield and composition of buffalo's and Friesian cow's milks. *Egyptian Journal of Dairy Science*, vol. 22, 1994, p.145–154.
- ANUTOVIČ, Z. – BOGUT, I. – SENCIC, D. – KATIC, M. – MIJIC, P. 2005. Concentrations of selected toxic elements (cadmium, lead, mercury and arsenic) in ewe milk in dependence on lactation stage. *Czech Journal of Animal Science*, vol. 50, 2005, p. 369–357.
- ATARO, A. – MCCRINDLE, R. I. – BOTHA, B. M. – MCCRINDLE, C. M. E. – NDIBEWU, P. P. 2008. Quantification of trace elements in raw cow's milk by inductively coupled plasma mass spectrometry (ICP-MS). *Food Chemistry*, vol. 111, 2008, p. 243–248.
- BAHT, R. V. – MOY, G. G. 1997. Monitoring and assessment of dietary exposure to chemical contaminants. WHO, Geneva. *Technical Report*, vol. 50, 1997, p. 132–149.
- BALDINI, M. – CONI, E. – STACCHINI, A. – STACCHINI, P. 1990. Presence and assessment of xenobiotic substances in milk and dairy products. *Annali dell'Istituto Superiore di Sanità*, vol. 26, 1990, p. 167–176.
- BELINGER, D. C. 2005. Teratogen update: lead and pregnancy. *Birth Defects Research a Clinical and Molecular Teratology*, vol. 73, 2005, p. 409–420.
- BILANDŽIČ, N. – DOKIČ, M. – SEDAK, M. – SOLOMUN, B. – VARENINA, I. – KNEŽEVIČ, Z. – BENIČ, M. 2011. Trace element levels in raw milk from northern and southern regions of Croatia. *Food Chemistry*, vol. 127, 2011, p. 63–66.
- BIRES, J. – DIANOVSKY, J. – BARTKO, P. – JUHASOVA, Z. 1995. Effects on enzymes and the genetic apparatus of sheep after administration of samples from industrial emissions. *Bio Metals*, vol. 8, 1995, p. 53–58.
- BOGUT, I. – HAS-SCHÖN, E. – JANSON, R. – ANTUNOVIĆ, Z. – BODAKOŠ, D. 2000. Concentrations of Hg, Pb, Cd and As in meat of fish-pond carp (*Cyprinus carpio*). *Agriculture*, vol. 6, 2000, p. 123–125.
- BRIDBOARD, K. – HANSON D. 2009. A personal perspective on the initial federal health-based regulation to remove lead from gasoline. *Environmental Health Perspect*, vol. 117, 2009, p. 1195–1201.
- BURGUERA, J. L. – RONDON, C. 1987. Lead content in hair, as related to petrol-lead exposure in Merida city, Venezuela, in S.E. Lindberg and T.C. Hutchinson (Eds.), *Heavy metals in the Environmental*, Int. Conf. 6<sup>th</sup>, New Orleans, LA, September 1987. CEP Consultanta Ltd, Edinburgh, vol. 2, 1987, p. 274–276.
- BURGUERA, J. L. – BURGUERA, M. – RONON, C. 1988. Lead in roadside soils of Merida City, Venezuela. *Science of the Total Environment*, vol. 77, 1988, p. 45–49.
- BURK, R. F. 2002. Selenium, an antioxidant nutrient. *Nutrition in Clinical Care*, vol. 2, 2002, p. 75–79.
- CAGGIANO, R. – SERENA, S. – MARIAGRAZIA, D. E. – MARIA, M. – ANIELLO, A. – MARIA, R. – SALVATORE, P. 2005. Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of southern Italy. *Environmental Research*, vol. 99, 2005, p. 48–57.
- CAGGIANO, R. – SABIA, S. – D'EMILIO, M. – ANASTASIO, A. – RAGOSTA, M. 2005. Metal levels in fodder, milk, dairy products and tissues sampled in bovine farms of Southern Italy. *Environmental Research*, vol. 99, 2005, p. 48–57.
- CERKVENIK, V. – DOGANOC, D. Z. – JAN, J. 2000. Evidence of some trace elements, organochlorine pesticides and PCBs in Slovenian cow's milk. *Food Technology and Biotechnology*, vol. 38, 2000, p. 155–160.
- CERUTTI, G. 1999. Residui additive contaminanti degli alimenti. 1<sup>st</sup> ed. Milano Tecniche Nuove.
- CONI, E. – BOCCA, A. – COPPOLELLI, P. – CAROLI, S. – CAVALLUCCI, C. – MARINUCCI, M. T. 1996. Minor and trace element content in sheep and goat milk and dairy products. *Food Chemistry*, vol. 57, 1996, p. 253–260.
- COOK, D. E. – GALE, S. J. 2005. The curious case of the date of introduction of leaded fuel to Australia: implications for the history of Southern Hemisphere atmospheric lead pollution. *Atmospheric Environment*, vol. 39, 2005, p. 2553–2557.
- CORREIA, P. R. M. – OLIVEIRA, E. – OLIVEIRA, P. V. 2000. Simultaneous determination of Cd and Pb in foodstuffs by electro thermal atomic absorption spectrometry. *Analytica Chimica Acta*, vol. 405, 2000, p. 205–211.
- DAS, K. K. – DAS, S. N. – DHUNDASI, S. A. 2008. Nickel, its adverse health effects and oxidative stress. *Indian Journal of Medical Research*, vol. 128, 2008, p. 412–425.
- DUBÉ, E. – CYR, D. G. 2013. The blood-epididymis barrier and human male fertility. *Advances in Experimental Medicine and Biology*, vol. 763, 2013, p. 218–236.



- EL-SHARAKY, A. S. – NEWAIRY, A. A. – BADRELDEEN, M. – EWEDA, S. M. SHEWEITA, S. A. 2007. Protective role of selenium against renal toxicity induced by cadmium in rats. *Toxicology*, vol. 235, 2007, p. 185–193.
- ENB, A. – DONIA ABOU, M. A. – ABD-RABOU, N. S. – ABOU-ARAB, A. A. K. – EL SENAITY, M. H. 2009. Chemical composition of raw milk and heavy metals behavior during processing of milk products. *Global Veterinaria*, vol. 3, 2009, p. 268–275.
- FEWTRELL, L. J. – PRUSS-USTUN, A. – LANDRIGAN, P. – AYUSO-MATEOS, J. L. 2004. Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. *Environmental Research*, vol. 94, 2004, p. 120–123.
- FORGACS, Z. – NEMETHY, Z. – REVESZ, CS. – LAZAR, P. 2001. Specific amino acids moderate the effects on Ni<sup>2+</sup> on the testosterone production of mouse Leydig cells *in vitro*. *Journal of Toxicology and Environmental Health*, vol. 62, 2001, p. 349–358.
- GALLO, M. – MLYNÁR, R. – RAJČÁKOVÁ, L. 1996. Porovnanie obsahu ťažkých kovov v tkanivách dojnic zo Spišských Vlách a Lubenika. In: *Sympóziu o ekológii vo vybraných aglomeráciách Jelšavy a Lubenika a Stredného Spiša*, Hrádok, 1996, p. 29–31.
- HENSON, M. C. – CHEDRESE, P. K. 2004. Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. *Experimental Biology and Medicine*, vol. 229, 2004, p. 283–392.
- HOUPT, P. – MEHENNAOUI, S. – FEDERSPIEL, B. – KOLF-CLAUW, M. – JOSEPH-ENRIQUEZ, B. – MILHAUD, G. 1997. Transfer of cadmium from feed to ewe food products: variations in transfer induced by lead and zinc. *Journal of Environmental Sciences*, vol. 5, 1997, p. 127–138.
- CHENG, C. Y. – WOND, E. W. P. – LIE, P. P. Y. 2011. Environmental toxicants and male reproductive function. *Spermatogenesis*, vol. 1, 2011, p. 2–13.
- CHENG, C. Y. – MRUK, D. D. 2012. The blood-testis barrier and its implications for male contraception. *Pharmacological Reviews*, vol. 64, 2012, p. 16–64.
- IJAZ, J. – IBADULLAH, J. – FAQIR, M. – ZIA-UR, R. – BILAL, A. – ZARGHAM, K. – JAVED, I. S. 2009. Heavy metal residues in the milk of cattle and goats during winter season. *Bulletin of Environmental Contamination and Toxicology*, vol. 82, 2009, p. 616–620.
- JÄRUP, L. 2003. Hazards of heavy metal contamination. *British Medical Bulletin*, vol. 68, 2003, p. 167–182.
- KIMÁKOVÁ, T. – KORÉNEKOVÁ, B. – BERNASOVSKÁ, K. 2006. Comparison of effects of cadmium and selenium on renal histological changes in Japanese Quails. *Second Central and Eastern Europe Conference on Health and the Environment: Health Effects Abstracts*. Bratislava, 2006, p. 36.
- KOH, M. 1986. Trace elements in sheep grazing near a lead-zinc smelting complex at Port Pirie South Australia. *Bulletin of Environmental Contamination and Toxicology*, vol. 37, 1986, p. 87–95.
- KOTTFFEROVÁ, J. – KORÉNEKOVÁ, B. 1998. Distribution of Cd and Pb in the Tissues and Organs of Free-Living Animals in the Territory of Slovakia. *Bulletin of Environmental Contamination and Toxicology*, vol. 60, 1998, p. 171–176.
- KUKNER, A. – COLAKOGLU, N. – KARA, H. – ONER, H. – OZOGUL, C. – OZAN, E. 2007. Ultrastructural changes in the kidney of rats with acute exposure to cadmium and effects of exogenous metallothionein. *Journal of Biology and Trace Elements*, vol. 119, 2007, p. 137–146.
- KRISTENSEN, L. J. 2015. Quantification of atmospheric lead emissions from 70 years of leaded petrol consumption in Australia. *Atmospheric Environment*, vol. 111, 2015, p. 195–201.
- LAFUENTE, A. 2013. The hypothalamic-pituitary-gonadal axis is target of cadmium toxicity. And update of recent studies and potential therapeutic approaches. *Food and Chemical Toxicology*, vol. 59, 2013, p. 395–404.
- LANDRIGAN, P. L. 2002. The worldwide problem of lead in petrol. *Bulletin of World Health Organization*, vol. 80, 2002, p. 768.
- LANTE, A. – LOMOLINO, G. – CAGNIN, M. – SPETTOLI, P. 2004. Content and characterization of minerals in milk and in crescenza and squacquerone Italian fresh cheese by ICP-OES. *Food Control*, vol. 17, 2004, p. 229–233.
- LICATA, P. – TROMBETTA, D. – CRISTANI, M. – GIOFRE, F. – MARTINO, D. – CALO, M. – NACCARI, F. 2004. Levels of “toxic” and “essential” metals in samples of bovine milk from various dairy farms in Calabria, Italy. *Environment International*, vol. 30, 2004, p. 1–6.
- LIU, Z. P. 2003. Lead poisoning combined with cadmium in sheep and horses in the vicinity of non-ferrous metal smelters. *Science of the Total Environment*, vol. 309, 2003, p. 117–126.
- LLOBET, J. M. – FALCO, G. – CASAS, C. – TEIXIDO, A. – DOMINGO, J. L. 2003. Concentrations of Arsenic, Cadmium, Mercury and Lead in common foods and estimated daily Intake by children, adolescents, adult and seniors of Catalonia, Spain. *Journal of Agricultural and Food Chemistry*, 2003, vol. 51, p. 838–842.

- LOPEZ, A. M. – BENEDITO, J. L. – MIRANDA, M. – CASTILLO, C. – HERNANDEZ, J. – SHORE, R. F. 2002. Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution. *Archives of Environmental Contamination and Toxicology*, vol. 42, 2002, p. 165–172.
- LUKÁČ, N. – MASSÁNYI, P. – KROČKOVÁ, J. – NAĐ, P. – SLAMEČKA, J. – ONDRUŠKA, E. – FORMICKI, G. – TRANDŽÍK, J. 2009. Relationship between trace element concentrations and spermatozoa quality in rabbit semen. *Slovak Journal of Animal Science*, vol. 42, Suppl. 1, 2009, p. 46–50.
- LUKÁČ, N. – MASSÁNYI, P. – KROČKOVÁ, J. – TOMAN, R. – DANKO, J. – STAWARZ, R. – FORMICKI, G. 2014. Effect of Nickel on Male Reproduction. *Universal Journal of Agricultural Research*, vol. 7, 2014, p. 250–252.
- MARTINO, F. A. R. – SANCHEZ, M. L. F. – SANZ-MEDEL, A. 2001. The potential of double focusing-ICP-MS for studying elemental distribution patterns in whole milk, skimmed milk and milk whey of different milks. *Analytica Chimica Acta*, vol. 442, 2001, p. 91–100.
- MERTZ, W. E. D. 1986. Trace elements in human and animal nutrition, vol. I and II, 5<sup>th</sup> ed. Academic Press, New York.
- MASSÁNYI, P. – LUKÁČ, N. – UHRÍN, V. – TOMAN, R. – PIVKO, J. – RAFAY, J. – FORGÁCS, ZS. – SOMOSY, Z. 2007. Female reproductive toxicology of cadmium. *Acta Biologica Hungarica*, vol. 58, 2007, p. 287–299.
- MIELKE, H. W. – LAIDLAW, M. A. S. – GONZALES, C. R. 2011. Estimation of leaded (Pb) gasoline's continuing material and health impacts on 90 US urbanized areas. *Environment International*, vol. 37, 2011, p. 248–257.
- NRIAGU, J. O. 1990. The rise and fall of leaded gasoline. *Science of the Total Environment*, vol. 92, 1990, p. 13–28.
- ONIANWA, P. C. – ADETOLA, I. G. – IWEGBUE C. M. A. – OJO, M. F. – TELLA, O. O. 1999. Trace heavy metals composition of some Nigerian beverages and food drinks. *Food Chemistry*, vol. 66, 1999, p. 275–279.
- OSKARSSON, A. – HALLEN, I. P. – SUNDBERG, J. 1995. Exposure to toxic elements via breast milk. *Analyst*, vol. 120, 1995, p. 765–770.
- PANDEY, R. – SRIVASTAVA, S. P. 2000. Spermatotoxic effects on nickel in mice. *Bulletin of Environmental Contamination and Toxicology*, vol. 64, 2000, p. 161–167.
- PATRA, R. C. – SWARUP, D. – KUMAR, P. – NANDI, D. – NARESH, R. – ALI, S. L. 2008. Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units. *Science of the Total Environment*, vol. 404, 2008, p. 36–43.
- PERRY, H. M. – ERLANGER, M. – PERRY, F. 1979. Increase in the systolic pressure of rats chronically fed cadmium. *Environmental Health Perspectives*, vol. 28, 1979, p. 261.
- PILSBACHER, L. – GRUBHOFER, F. 2002. Mercury, lead and cadmium in Austrian raw milk – a comparison to earlier analyses and to analyses from foreign countries. *Wiener Tierärztliche Monatsschrift*, vol. 89, 2002, p. 249–253.
- PISKOROVÁ, L. – VASILKOVÁ, Z. – KRUPICER, I. 2003. Heavy metal residues in tissues of wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*) in the Central Zemplin region of the Slovak Republic. *Czech Journal of Animal Sciences*, vol. 48, 2003, p. 134–138.
- PITOT, C. H. – DRAGAN, P. Y. 1996. Chemical carcinogenesis. In: Casarett and Doull's Toxicology, 5<sup>th</sup> Edn. International Edition, Mc Graw Hill, New York, p. 201–260.
- QADIR, M. – GHAFOR, A. – MURTAZA, G. 2000. Cadmium concentration in vegetables grown on urban soils irrigated with untreated municipal sewage. *Environment, Development Sustainability*, vol. 2, 2000, p. 11–19.
- RASOOL, I. 1999. Zinc, copper, iron and manganese in soils at different canal and water course sections in rice-wheat cropping zone. *International Journal of Agricultural and Biological Engineering*, vol. 1, 1999, p. 218–221.
- RODRIGUÉZ, E. M. – DELGADO URETA, E. – DÍAZ ROMERO, C. 1999. Concentrations of cadmium and lead in different types of milk. *Zeitschrift für Lebensmitteluntersuchung und-Forschung*, vol. 208, 1999, p. 162–168.
- SALMA, I. – MAENHAUT, W. – DUBTSOV, S. – PAPP, E. Z. – ZARAY, G. 2000. Impact of phase out of leaded gasoline on the air quality in Budapest. *Microchemical Journal*, vol. 67, 2000, p. 127–133.
- SHAKOUR, A. A. – EL-TAIEB, N. M. – HASSAN, S. K. 2006. Seasonal variation of some heavy metals in total suspended particulate mater in Great Cairo atmosphere. *The 2<sup>nd</sup> International Conference of Environmental Science and Technology*, Egypt, 2006, p. 4–6.
- SCHROEDER, H. A. 1973. The trace elements and nutrition. London: Faber and Faber.
- SCHUHMACHER, M. 1991. Dietary intake of lead and cadmium from foods in Tarragona Province, Spain. *Bulletin of Environmental Contamination and Toxicology*, vol. 46, 1991, p. 320–328.
- SIKIRIĆ, M. – BRAJENOVIĆ, M. – PAVLOVIĆ, I. – HAVRANEK, J. L. – PAVLJANIĆ, N. 2003.

- Determination of metals in cow's milk by flame atomic absorption spectrophotometry. *Czech Journal of Animal Sciences*, vol. 48, 2003, p. 481–486.
- SIU, E. R. – MRUK, D. D. – PORTO, C. S. – CHENG, C. Y. 2009. Cadmium-induced testicular injury. *Toxicology and Applied Pharmacology*, vol. 238, 2009, p. 240–249.
- SOMERS, E. 1974. Toxic potential of trace metals in foods. *Journal Food Science*, vol. 39, 1974, p. 215–217.
- TAJKARIMI, M. – AHMADI FAGIH, M. – POURSOULTANI, H. – SASTRY, V. N. – SALAH NEJAD, A. – MOTALLEBI, A. A. – MAHDAVI, H. 2008. Lead residue levels in raw milk from different regions of Iran. *Food Chemistry*, vol.19, 2008, p. 495-498.
- THOMAS, B. – ROUGHAN, J. A. – WATTERS, E. D. 1972. Lead and cadmium content of some vegetable foodstuffs. *Journal of Science and Food Agriculture*, vol. 23, 1972, p. 1493–1498.
- THOMPSON, J. – BANNIGAN, J. 2008. Cadmium: toxic effects on the reproductive system and the embryo. *Reproductive Toxicology*, vol. 25, 2008, p. 304–315.
- TOMAN, R. – MASSÁNYI, P. – UHRÍN, V. 2002. Changes in the testis and epididymis of rabbits after an intraperitoneal and peroral administration of cadmium. *Trace Elements and Electrolytes*, vol. 19, 2002, p. 114–117.
- TOMAN, R. – MASSÁNYI, P. – GOLIAN, J. 2003. *Toxikológia potravín*. Nitra: SPU. 116 p. ISBN 80-80-69-166-5.
- TOMAN, R. – GOLIAN, J. – ŠIŠKA, B. – MASSÁNYI, P. – LUKÁČ, N. – ADAMKOVIČOVÁ, M. 2009. Cadmium and selenium in animal tissues and their interactions after an experimental administration to rats. *Slovak Journal of Animal Science*, vol. 42, 2009, p. 115–118.
- TONG, S. – VON SCHIRNDING, Y. E. – PRAPAMONTOL, T. 2000. Environmental lead exposure: a public health problem of global dimensions. *Bulletin of World Health Organization*, vo. 78, 2000, p. 1068–1077.
- TREMELLEN, K. 2008. Oxidative stress and male infertility- a clinical perspective. *Human reproduction Update*, vol. 14, 2005, p. 243–258.
- TRIPATHI, R. M. – RAGHUNATH, R. – SASTRY, V. N. – KRISHNAMOORTHY, T. M. 1999. Daily intake of heavy metals by infants through milk and milk products. *The Science of the Total Environment*, vol. 227, 1999, p. 229–235.
- TURNER, T. T. – LYSIAK, J. J. 2008. Oxidative stress: a common factor in testicular dysfunction. *Journal of Andrology*, vol. 29, 2008, p. 488–498.
- VENUGOPAL, B. – LUCKEY, T. D. 1978. Metal toxicity to mammals, vol. II. Chemical Toxicity of metals and metalloids. Plenum Press, New York.
- WAALKES, M. P. 2003. Cadmium carcinogenesis. *Mutation Research*, vol. 533, 2003, p. 107–120.
- WEGLARZY, K. 2010. Effect of lead, cadmium, copper and zinc content in soil on their deposition in pasture sward as well as in products and tissues of the cows grazed on pasture. *Journal of Food Agriculture and Environment*, vol. 8, 2010, p. 1209–1217.
- WONG, C. H. – MRUK, D. D. – LUI, W. Y. – CHENG, C. Y. 2004. Regulation of blood-testis barrier Dynamics: and *in vivo* study. *Journal of Cell Science*, vol. 117, 2004, p. 738–798.
- XIAO, S. J. – JIA, X. D. – ZHONG, W. J. – JIN, X. P. – NORDBERG, G. 2002. Restorative effects of zinc and selenium on cadmium-induced kidney oxidative damage in rats. *Biomedical Environment Science*, vol. 5, 2002, p. 67–74.

*Short communication*

## THE EFFECT OF CURCUMA LONGA PLANT EXTRACT ON THE RABBIT EMBRYO DEVELOPMENT *IN VITRO*

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### ABSTRACT

The aim of this study was to evaluate the effect of *Curcuma longa* (CL) root extract on the rabbit embryo development *in vitro*. Totally 113 pronuclear stage zygotes were used in this experiment. Zygotes were divided into 4 groups: control (C; n = 28) and three experimental groups (E1, E2, E3; n = 31, 26 and 39, resp.) with addition of different concentrations of *Curcuma longa* extract to the culture medium (E1- 0.1 µg·ml<sup>-1</sup>; E2- 0.01 µg·ml<sup>-1</sup>; E3- 0.001 µg·ml<sup>-1</sup>). Zygotes were cultured up to the blastocyst stage (120 h) in 5 % CO<sub>2</sub> at 37.5 °C. At the end of culture period the blastocysts were stained with DAPI fluorochrome for the total cell number determination. Evaluation of embryo developmental potential showed, that higher blastocyst rate was observed in the E2 (61.5 %) and E3 (60 %) groups compared to the control group (46.4 %). In the group with highest CL concentration in culture (E1- 0.1 µg·ml<sup>-1</sup>) embryo development was stopped at the morula stage. In this group also the lowest (P < 0.001) number of cleaved embryos (19.4 %) compared to the control (60.7 %) and E3 group (82.1 %) was recorded. There were no differences in the blastocysts total cell number among the groups with lower CL concentrations (E2 77.81 ± 13.6; E3 89.25 ± 15.94) and control group (82.23 ± 21.75). On the basis of our results we suppose that *Curcuma longa* affects rabbit embryo development in a dose-dependent manner. Although lower concentrations showed positive effect, the highest concentration blocked embryo development at morula stage. It is necessary to determine since which concentration *Curcuma longa* may be toxic for normal embryonic development.

**Key words:** rabbit; embryos; *Curcuma longa* extract; *in vitro* development; DAPI staining

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### INTRODUCTION

Medical plants are widely used as a source of remedies for the treatment and prevention of many diseases as alternative therapeutic and medical tools (Kaur and Mondal, 2014). Natural products from some plants are used in pharmaceutical preparations either as pure compounds or as extracts (Araújo and Leon, 2001). One of them is *Curcuma longa* Linn. (*Zingiberaceae* family), well-known as turmeric, broadly grown in tropical areas of Asia and Central America (Ammon, 1991).

This rhizome in powder form is widely used as a food additive for impart flavour and a yellow colour (Miquel *et al.*, 2002). The major constituent, curcumin (diferuloylmethane) is the most important fraction of *Curcuma longa* (Araújo and Leon, 2001). It has been already demonstrated, that curcuminoids have anti-atherosclerotic (Olszanecki *et al.*, 2005), anti-diabetic (Nabavi *et al.*, 2015), anti-mutagenic, anti-cancer (Goel *et al.*, 2008), antioxidant (Huang *et al.*, 1994; Nishiyama *et al.*, 2005; Wei *et al.*, 2006; Kumar *et al.*, 2007), anti-bacterial (Park *et al.*, 2005), anti-inflammatory

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and anti-fertility (Mishra and Singh, 2009; Ammon and Wahl, 1991; Lantz *et al.*, 2005) activities. Garg *et al.* (1974; 1978) found out, that aqueous extracts of turmeric rhizome show complete inhibition of embryo implantation in rats when fed orally. Curcumin has the potential for the use in development of novel intravaginal contraceptive (Rithaporn *et al.*, 2003). Thakur *et al.* (2009) observed significant anti-fertilizing activity and decreasing of FSH and LH levels in blood plasma of albino rat females after oral administration of aqueous or ethanolic extracts from *Curcuma longa*. Many studies showed strong correlation between antioxidant activity and fertility (Ruder *et al.*, 2009), as well as between free radical accumulation and reduction in fertility (Behrman *et al.*, 2001). The aim of this study was to examine the effect of *Curcuma longa* Linn. on rabbit embryo development *in vitro*.

## MATERIAL AND METHODS

### Animals and superovulation

The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 1488/06-221/3a. Sexually mature New Zealand White rabbit does from the Department of Small Farm Animals, APRC Nitra were used in this experiment. Superovulation of rabbit does was induced by intramuscular application of 50 IU PMSG (SERGON,

Bioveta, a. s. Ivanovice na Hané, Czech Republic) and after 48 hours by 100  $\mu$ l of HCG (Supergestran, Nordic Pharma s.r.o. Jesenice, Czech Republic) per doe. Before the HCG injection, all rabbit does were artificially inseminated by heterospermic dose of rabbit semen (0.5 ml/doe).

### Egg recovery, culture and staining

At 19-20 h *post-coitum*, rabbit does were humanely slaughtered by the electrical stunning (Relco, Gewiss, Milano, Italy, alternating current 0.3 A/female, frequency 50 Hz, exposition 4s) and reproductive organs were expertly dissected. The pronuclear stage eggs were flushed from the oviducts with PBS (Gibco, Auckland, New Zealand) and subsequently morphologically evaluated. The selected eggs were placed into 4-well dishes (Nunc, Roskilde, Denmark) containing 500  $\mu$ l of k-DMEM medium (Gibco) supplemented with three different concentrations of *Curcuma longa* (CL) extract (E1- 0.1  $\mu$ g.ml<sup>-1</sup> CL; E2- 0.01  $\mu$ g.ml<sup>-1</sup>; E3- 0.001  $\mu$ g.ml<sup>-1</sup>) and cultured up to 120 hours *post-coitum* in 5 % CO<sub>2</sub> at 37.5 °C (the time point to reach the blastocyst stage). After the culture, embryos were washed in PBS with polyvinylpyrrolidone (PBS-PVP, 4 mg.ml<sup>-1</sup>) for 3 x 5 min, stained with 4  $\mu$ l of Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) and mounted between the microslide and coverslip. Total cell number was counted under a Zeiss fluorescence microscope equipped with a specific wave-length filter (Fig. 1).

A



B

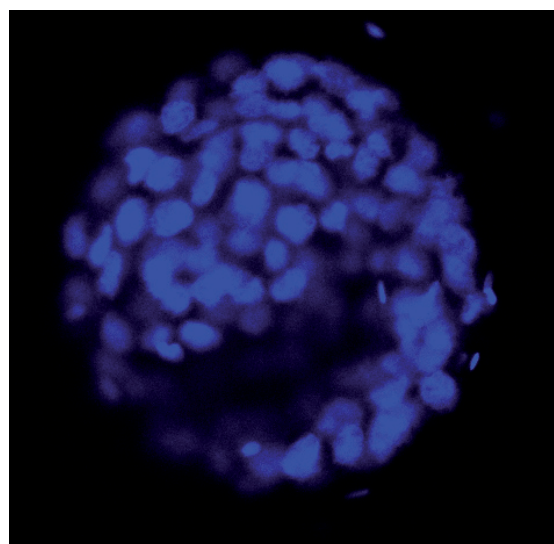


Fig. 1: Representative image of the embryo cultured with the lowest concentration (0.001  $\mu$ g.ml<sup>-1</sup>) of *Curcuma longa* extract. A: light microscopy; B: fluorescence microscopy (cells stained by DAPI fluorochrome)

**Statistical analysis**

The data were analysed by Pearson's Chi-square test.

**RESULTS AND DISCUSSION**

To examine the effect on embryo development we cultured rabbit pronuclear stage eggs in the medium enriched with three different concentrations of *Curcuma longa*.

The highest concentration of *CL* (E1 group) had negative effect on the embryo development, as was shown by decreasing ( $P < 0.001$ ) cleavage rate (19.4 %) compared to the control and the other experimental groups (C: 60.7 %, E2: 69.2, E3: 82.1 %).

The lowest concentration of *CL* (E3 group) increased ( $P < 0.001$ ) cleavage rate (82.1 %) when compared to control. Development to the blastocyst stage was completely stopped in the E1 group, whereas blastocyst rates in the E2 (61.5 %), E3 (60 %) and C (46.4 %) groups were not statistically different (Chi-square test).

Negative effect of *Curcuma longa* on reproduction was already reported on granulosa cells of porcine ovary, where it inhibited proliferation (accumulation of PCNA) and induced apoptosis (accumulation of bax) (Kádasi *et al.*, 2012; Voznesenska *et al.*, 2010; Bhaumik *et al.*, 1999). In our study, the highest concentration (0.1  $\mu\text{g}\cdot\text{ml}^{-1}$ ) of *CL* in the culture stopped embryo development at the morula stage. Possible explanation could be the stimulation of apoptotic process due to the toxicity of mentioned concentration in culture. At the blastocyst stage apoptosis is responsible for the elimination of undesirable cells during the normal embryonic development (Hardy *et al.*, 2003). However, increased occurrence of apoptosis before or during the blastocyst stage probably removes important

cell lineages, what might negatively affect embryonic development and lead to embryo degeneration (Long *et al.*, 2000). Although, the objective of our study was not an evaluation of apoptosis incidence, the similar total cell number in each group indicates that there is no a developmental delay and increased apoptosis incidence. A similar conclusion was reported by Chen *et al.* (2010), who applied *Curcuma longa* to mouse embryo culture. On the basis of the blastocyst development evaluation by differential staining, the authors found that higher concentration (24  $\mu\text{M}\cdot\text{ml}^{-1}$ ) of the *CL* extract induced apoptosis in the ICM but not in trophoblastic cells. Nevertheless, lower concentrations (6 and 12  $\mu\text{M}\cdot\text{ml}^{-1}$ ) did not affect the apoptosis incidence or cell number. Likewise, in our study similar blastocyst cell numbers in the groups with lower *CL* concentration (E2:  $77.81 \pm 13.6$ ; E3:  $89.25 \pm 15.94$ ) and control group ( $82.23 \pm 21.75$ ) were found. Because none of the embryos were developed to blastocyst stage in the group with the highest concentration, counting of the total cell number in this group was not performed.

**CONCLUSION**

According to our results we can conclude that the highest concentration of *Curcuma longa* root extract added to culture medium negatively affects embryo cell number and terminates embryo development at the morula stage.

**ACKNOWLEDGEMENT**

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**Table 1: Effect of different *Curcuma longa* concentrations on rabbit embryo development**

Group	Number of embryos (n)	Cleavage rate (%)	Morula rate (%)	Blastocyst rate (%)
C	28	17/60.7 <sup>b</sup>	4/14.3 <sup>c</sup>	13/46.4 <sup>f</sup>
E1	31	6/19.4 <sup>a</sup>	6/19.4 <sup>c</sup>	0/0 <sup>e</sup>
E2	26	18/69.2 <sup>b</sup>	2/7.7 <sup>d</sup>	16/61.5 <sup>f</sup>
E3	39	32/82.1 <sup>b</sup>	7/17.9 <sup>c</sup>	23/60.0 <sup>f</sup>

Level of significance:  $P < 0.001$  a:b and e:f;  $P < 0.01$  c:d

C- control group, E1- 0.1  $\mu\text{g}\cdot\text{ml}^{-1}$  of *Curcuma longa* in the culture, E2- 0.01  $\mu\text{g}\cdot\text{ml}^{-1}$  of *Curcuma longa* in the culture,

E3- 0.001  $\mu\text{g}\cdot\text{ml}^{-1}$  of *Curcuma longa* in the culture

## REFERENCES

- AMMON, H. P. – WAHL, M. A. 1991. Pharmacology of *Curcuma longa* L. *Planta Medica*, vol. 57 (1), 1991, p. 1–7.
- ARAÚJO, C. A. C. – LEON, L. L. 2001. Biological activities of *Curcuma longa* L. *The Memórias do Instituto Oswaldo Cruz*, vol. 96 (5), 2001, p. 723–728.
- BEHRMAN, H. R. – KODAMAN, P. H. – PRESTON, S. L. – GAO, S. 2001. Oxidative stress and the ovary. *Journal of Society for Gynecologic Investigation*, vol. 8 (1), 2001, p. 40–42.
- BHAUMIK, S. – ANJUM, R. – RANGARAJ, N. – PARDHASARADHI, B. V. – KHAR, A. 1999. Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. *FEBS Letters*, vol. 456 (2), 1999, p. 311–314.
- GARG, S. K. 1974. Effect of *Curcuma longa* (rhizomes) on fertility in experimental animals. *Planta Medica*, vol. 26 (3), 1974, p. 225–227.
- GARG, S. K. – MATHUR, V. S. – CHAUDHURY, R. R. 1978. Screening of Indian plants for antifertility activity. *Indian Journal of Experimental Biology*, vol. 16 (10), 1978, p. 1077–1079.
- GOEL, A. – KUNNUMAKKARA, A. B. – AGGARWAL, B. B. 2008. Curcumin as “Curecumin”: from Kitchen to clinic. *Biochemical Pharmacology*, vol. 75 (4), 2008, p. 787–809.
- HARDY, K. – STARK, J. – WINSTON, R. M. 2003. Maintenance of the inner cell mass in human blastocysts from fragmented embryos. *Biology of Reproduction*, vol. 68 (4), 2003, p. 1165–1169.
- HUANG, M. T. – LOU, Y. R. – MA, W. – NEWMARK, H. L. – REUHL, K. R. – CONNEY, A. H. 1994. Inhibitory effects of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. *Cancer Research*, vol. 54 (22), 1994, p. 5841–5847.
- CHEN, CH. CH. – HSIEH, M. S. – HSUW, Y. D. – HUANG, F. J. – CHAN, W. H. 2010. Hazardous Effects of Curcumin on Mouse Embryonic Development through a Mitochondria-Dependent Apoptotic Signaling Pathway. *International Journal of Molecular Science*, vol. 11 (8), 2010, p. 2839–2855.
- KAUR, S. – MONDAL, P. 2014. Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. *Journal of Microbiology and Experimentation*, vol. 1 (1), 2014, p. 1–6.
- KÁDASI, A. – SIROTKIN, A. V. – MARUNIAKOVA, N. – KOLESÁROVÁ, A. – BULLA, J. – GROSSMANN, R. 2012. The effect of curcumin on secretory activity, proliferation and apoptosis of the porcine ovarian granulosa cells. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2 (1), 2012, p. 20349–20357.
- KUMAR, P. – PADI, S. S. – NAIDU, P. S. – KUMAR, A. 2007. Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. *Methods and Findings in Experimental a Clinical Pharmacology*, vol. 29 (1), 2007, p. 19–25.
- LANTZ, R. C. – CHEN, G. J. – SOLYOM, A. M. 2005. The effect of turmeric extracts on inflammatory mediator production. *Phytomedicine*, vol. 12 (6-7), 2005, p. 445–452.
- LONG, L. H. – CLEMENT, M. V. – HALLIWELL, B. 2000. Artifacts in cell culture: Rapid generation of hydrogen peroxide on addition of (-)-epigallocatechin, (-)-epigallocatechingallate, (+)-catechin, and quercetin to commonly used cell culture media. *Biochemical and Biophysical Research Communications*, vol. 273 (1), 2000, p. 50–53.
- MIQUEL, J. – BERND, A. – SEMPRES, J. M. – DÍAZ-ALPERI, J. – RAMÍREZ, A. 2002. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. *Archives of Gerontology and Geriatrics*, vol. 34 (1), 2002, p. 37–46.
- MISHRA, R. K. – SINGH, S. K. 2009. Reversible antifertility effect of aqueous rhizome extract of *Curcuma longa* L. in male laboratory mice. *Contraception*, vol. 79 (6), 2009, p. 479–487.
- NABAVI, S. F. – THIAGARAJAN, R. – RASTRELLI, L. – DAGLIA, M. – SOBARZO-SANCHEZ, E. – ALINEZHAD, H. – NABAVI, S. M. 2015. Curcumin: A Natural Product for Diabetes and its Complications. *Current topics in medicinal chemistry*, vol. 15 (23), 2015, p. 2445–2455.
- NISHIYAMA, T. – MAE, T. – KISHIDA, H. – TSUKAGAWA, M. – MIMAKI, Y. – KURODA, M. 2005. Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. *Journal of Agricultural and Food Chemistry*, vol. 53 (4), 2005, p. 959–963.
- OLSZANECKI, R. – AWIEŃ, J. – GAJDA, M. – MATEUSZUK, L. – GEBSKA, A. – ORABIOWSKA, M. – CHŁOPICKI, S. – KORBUT, R. 2005. Effect of curcumin on atherosclerosis in apoE/LDLR-double knockout mice. *Journal of Physiology and Pharmacology*, vol. 56 (4), 2005, p. 627–635.
- PARK, B. S. – KIM, J. G. – KIM, M. R. 2005. *Curcuma longa* L. constituents inhibit sortase A and staphylococcus aureus cell adhesion to fibronectin. *Journal of Agricultural and Food Chemistry*, vol. 53 (23), 2005, p. 9005–9009.

- 
- RITHAPORN, T. – MONGA, M. – RAJASEKHARAN, M. 2003. Curcumin: a potential vaginal contraceptive. *Contraception*, vol. 68 (3), 2003, p. 219–223.
- RUDER, E. H. – HARTMAN, T. J. – GOLDMAN, M. B. 2009. Impact of oxidative stress on female fertility. *Current Opinion in Obstetrics and Gynecology*, vol. 21 (3), 2009, p. 219–222.
- THAKUR, S. – BAWARA, B. – DUBEY, A. – NANDINI, D. – CHAUHAN, N. S. – SARAF, D. K. 2009. Effect of *Carum carvi* and *Curcuma longa* on hormonal and reproductive parameter of female rats. *International Journal of Phytomedicine*, vol. 1 (1), 2009, p. 31–38.
- VOZNESENSKA, T. I. – BRYZHINA, T. M. – SUKHINA, V. S. – MAHKOHON, N. V. – ALEKSIEIEVA, I. M. 2010. Effect of NF-kappaB activation inhibitor curcumin on the oogenesis and follicular cell death in immune ovarian failure in mice. *Fiziologichnyĭ zhurnal*, vol. 56 (4), 2010, p. 96–101.
- WEI, Q. Y. – CHEN, W. F. – ZHOU, B. – YANG, L. – LIU, Z. L. 2006. Inhibition of lipid peroxidation and protein oxidation in rat liver mitochondria by curcumin and its analogues. *Biochimica Biophysica Acta*, vol. 1760 (1), 2006, p. 70–77.



*Short communication*

## THE EFFECT OF GREEN TEA ADDITION TO DIET ON WEIGHT GAINS OF RABBIT FEMALES

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### ABSTRACT

The aim of present study was to evaluate the effect of different concentrations of green tea plant (*Camellia sinensis*) addition to the diet on the weight gains of rabbits. Rabbit females (n = 31) of New Zealand White breed were used in the experiment. Rabbit does in the control group (C; n = 12) were fed with a commercially available feed. In the experimental groups, 5 g (E1; n = 9) and 20 g (E2; n = 10) of green tea dried powder were added to 100 kg of commercially available feed. The lower weight gains per week (g) were observed in both experimental groups (E1; 229.7 ± 21.95 and E2; 223.09 ± 24.00 g, respectively) when compared to control (C; 242.58 ± 19.76 g). Total average weight gain was the highest in control (C; 2668.3 ± 97.61 g) when compared to the both experimental groups (E1, 2526.67 ± 79.64 g; E2, 2454.0 ± 118.09 g).

In conclusion, addition of green tea powder to the commercial diet for rabbit does had negative effect on the weight gains (g) per week and on the total average weight gains (g) during the fattening period.

**Key words:** rabbit females; green tea; weight gains

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### INTRODUCTION

Nowadays, biologically active substances and extracts are used worldwide particularly in terms of their stimulation and therapeutic effects (Park *et al.*, 2014).

More suitable composition of feed mixture or administration of natural additives at an appropriate concentration might be beneficial in livestock farming without negative effect on the environment and the animals as an individual (Abdel-Wareth *et al.*, 2014). Plant polyphenols are natural antioxidants and most of their pharmacological properties are considered to be due to their antioxidant action (Ames *et al.*, 1995). The most important polyphenolic compounds in green tea are catechins: epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECG),

epigallocatechin (EGC), catechin and galocatechin (GC) (Wang *et al.*, 2013). Green tea contains also high levels of other bioactive phenols, such as caffeine. EGCG, the most abundant catechin in green tea, accounts for 65 % of the total catechin content. A cup of green tea may contain 100–200 mg of EGCG. Catechin and galocatechin are present in trace amounts (Chu and Juneja, 1997).

Green tea polyphenols (GTPP) are known for their preventive, antibacterial and therapeutic effects, anticancer and apoptosis inducing-properties. These molecules prevent neural cell death and induce chromosomal damage in lymphoblastoid cell lines (Reznichenko *et al.*, 2005; Sugisawa and Umegaki, 2002).

Some epidemiological and clinical studies have shown the health benefits of EGCG on obesity

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and diabetes (Rains *et al.*, 2011) and the underlying mechanisms involve modulations of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism (Chacko *et al.*, 2010).

The studies have suggested that the extent of absorption of dietary polyphenols in the small intestine is relatively small (10–20 %). The majority of ingested polyphenols will reach the large intestine where they encounter the colonic microflora (Spencer, 2003). The colon contains microorganisms having an enormous catalytic and hydrolytic potential. This enzymatic degradation of flavonoids by the colonic microflora results in a huge array of new metabolites. These polyphenols do not interfere with the microflora of the colon (Zdunczyk *et al.*, 2002).

Multiple *in vitro* studies on catechins report mechanisms consistent with protection against degenerative diseases (Crespy and Williamson, 2004). Green tea catechins (GTC) have been reported to possess multiple properties, such as cancer prevention, hypotensive effects, anti-viral and antioxidant properties, inhibition of plaque formation, anti-allergic potential and blood glucose-lowering effects (Katiyar and Mukhtar, 1996; Matsumoto *et al.*, 1993).

Green tea has not been tested in rabbits yet, so the objective of this study was to evaluate the effect of different concentration of green tea powder addition to the diet on the total and average weight gains of the rabbit does.

## MATERIAL AND METHODS

### Animals

Two months old clinically healthy rabbit does of the New Zealand White line (NAFC Nitra, SR) were used in this experiment. The animals were housed in individual cages, under a constant photoperiod of 14 hours of light. Temperature and humidity in the building were recorded continuously by means of a thermograph positioned at the same level as the cages (average relative humidity and temperature during the year was maintained at  $60 \pm 5$  % and  $17 \pm 3$  °C). The rabbits were fed *ad libitum* and water was provided *ad libitum* with nipple drinkers.

Rabbit does ( $n = 31$ ) were divided into three groups: control (C;  $n = 12$ ) and two experimental groups (E1;  $n = 9$  and E2;  $n = 10$ ). The does in the control group were fed with a commercially available complete feed mixture. Green tea (right loose green tea, made in China and distributed by Oxalis, Czech Republic) was added at two different concentrations (E1: 5 g; E2: 20 g) to the 100 kg of the complete feed mixture in both experimental groups. The animals were fed for 77 days and weighted weekly.

The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 1488/06-221/3a.

### Statistical analysis

All values are expressed as means  $\pm$  S.E.M. Differences between the control and experimental groups were evaluated by one-way ANOVA test using the SigmaPlot 11 software (Systat Software Inc., Erkrarth, Germany).

## RESULTS AND DISCUSSION

In our study the effect of different concentrations of green tea plant powder added to the diet on the total and average weight gains (g) of the rabbit does was evaluated.

The highest average weight gain of rabbit does per week (g) was recorded in the control group (C;  $242.58 \pm 19.76$  g) when compared to the experimental (E1;  $229.7 \pm 21.95$  and E2;  $223.09 \pm 24.00$ , respectively) groups (Figure 1). Similarly, total average weight gain (g) was the highest in the control group (C;  $2668.3 \pm 97.61$  g) compared to the experimental (E1;  $2526.67 \pm 79.64$  and E2;  $2454.0 \pm 118.09$  g, respectively) groups, although these differences were not statistically significant (Figure 2).

Decreasing of weight in other animal species fed with green tea was also reported. In particular, in the study of Hamdaoui *et al.* (2003) the weight gains were evaluated in rats fed a commonly consumed Tunisian meal 'bean seeds ragout' (BSR), with or without beef and with black or green tea decoction. Both, black and green teas significantly reduced the weight gains, where the black tea decoction had the strongest effect.

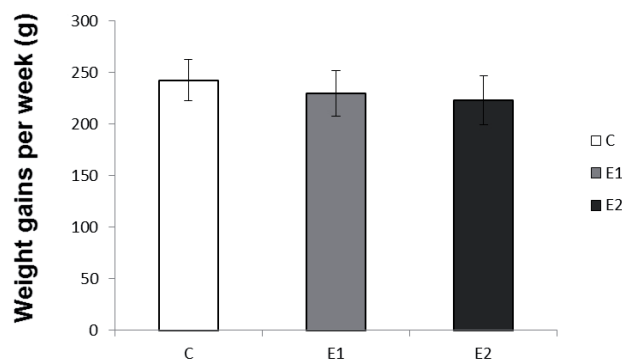
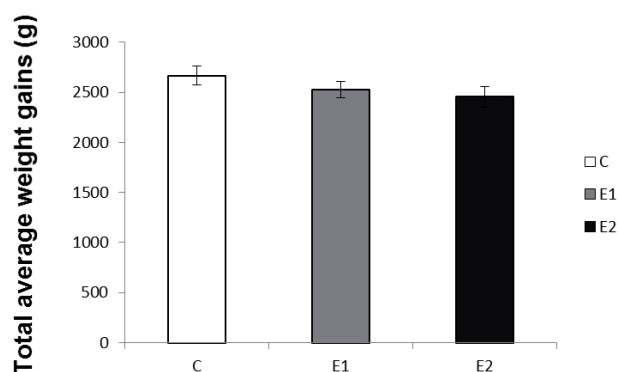


Fig. 1: Average weight gains (g) per week of rabbit does fed with green tea plant added into complete feed mixture



**Fig. 2: Total average weight gains (g) of rabbit does fed with green tea plant added into complete feed mixture**

Ito *et al.* (2008) found out that the administration of 0.5 % catechins decreased the body fat accumulation and levels of serum cholesterol and bile acids. These results indicate that green tea catechins modulate lipid metabolism in obese, but also in the non-obese subjects.

Snoussi *et al.* (2014) found that chronic administration of green tea decoction (GTD) in rat fed high-fat diet reduced body weight gain, circulating triglycerides and cholesterol and improved glucose tolerance. On the other hand, administration of GTD did not affect food intake, suggesting that the reduced feed intake does not correspond to the reduction in weight gain.

Morita *et al.* (2009) administered green tea catechins to pregnant rats at two concentrations (2000 mg.kg<sup>-1</sup> and 600 mg.kg<sup>-1</sup> day) and observed the reduction in the weight gain ( $P < 0.05$ ). However, heat-sterilized green tea catechin (GTC-H) administration did not affect mean gravid uterine weights or intrauterine growth and survival.

The results of Sayama *et al.* (2000) indicated that lipid metabolism in mice was suppressed by the administration of green tea powder and, thereby, the fatty accumulation and body weight increase was suppressed.

In mice fed a high-fat diet (60 % energy as fat), supplementation with dietary EGCG treatment (3.2 g.kg<sup>-1</sup> diet) for 16 weeks reduced body weight gain, body fat percentage and visceral fat weight ( $P < 0.05$ ) compared to mice without EGCG treatment (Bose *et al.*, 2008). Their results indicate that long-term EGCG treatment attenuated the development of obesity, symptoms associated with the metabolic syndrome and fatty liver. Short-term EGCG treatment appeared to reverse pre-existing high-fat-induced metabolic pathologies in obese mice. These effects may be mediated by decreased lipid absorption and decreased inflammation.

Similarly, Lu *et al.* (2012) reported beneficial effects of green tea polyphenols (GTP) on body weight via regulating obesity-related genes, anti-inflammation, anti-oxidant capacity and estrogen-related actions in high-fat-induced obese rats.

Weight gains observed in our experiment were slightly lower in the experimental groups compared to the control group, but the differences were not statistically significant. It can be explained by variability between females (very high S.E.M. values).

Similarly as in our study, Juśkiewicz *et al.* (2008) found out, that supplementation of a diet with green tea extract had no significant influence on elevated food intake and body weight loss.

## CONCLUSION

In conclusion, addition of green tea powder to the commercial diet for rabbit does can potentially decrease the weight gains per week and the total average weight gains during the fattening period.

## ACKNOWLEDGEMENT

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## REFERENCES

- ABDEL-WARETH, A. A. A. – HAMMAD, S. – AHMED, H. 2014. Effects of Khaya Senegalensis levae on performance, carcass traits, haematological and biochemical parameters in rabbits. *Experimental and Clinical Sciences Journal*, vol. 13, 2014, p. 502–512.
- AMES, B. N. – GOLD, L. S. – WILLETT, W. C. 1995. The causes and prevention of cancer. *Proceedings of National Academy of Sciences, USA* 1995, 92, p. 5258–5265.
- BOSE, M. – LAMBERT, J. D. – JU, J. – REUHL, K. R. – SHAPSES, S. A. – YANG, C. S. 2008. The major green tea polyphenol, (–)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat fed mice. *Journal of Nutrition*, vol. 138 (9), 2008, p. 1677–1683.
- CRESPY, V. – WILLIAMSON, G. 2004. A review of the health effects of green tea catechins in *in vivo* animal models. *Journal of Nutrition*, vol. 134 (12), 2004, p. 3431–3440.

- HAMDAOUI, M. H. – CHABCHOUB, S. – HÉDHILI, A. 2003. Iron bioavailability and weight gains to iron-deficient rats fed a commonly consumed Tunisian meal bean seeds ragout with or without beef and with green or black tea decoction. *Journal of Trace Elements*, vol. 17 (3), 2003, p. 159–164.
- CHACKO, S. – THAMBI, P. – KUTTAN, R. – NISHIGAKI, I. 2010. Beneficial effects of green tea: a literature review. *Chinese Medicine*, vol. 5, 2010, p. 13.
- CHU, D. C. – JUNEJA, L. R. 1997. General chemical composition of green tea and its infusion. In: Juneja, L.R., Chu, D.-C., Kim, M. (Eds.), *Chemistry and Applications of Green Tea*. CRC Press, Boca Raton, 1997, pp. 13–22.
- ITO, Y. – ICHIKAVA, T. – MOROHOSHI, Y. – NAKAMURA, T. – SAEGURA, Y. – ISHIHARA, K. 2008. Effect of teacatechins on body fat accumulation in rats fed a normal diet. *Biomedical Research*, vol. 29 (1), 2008, p. 27–32.
- JUŚKIEWICZ, J. – ZDUŃCZYK, Z. – JURGOŃSKI, A. – BRZUZAN, Ł. – GODYCKA –KŁOS, I. – ŻARY-SIKORSKA, E. 2008. Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutrition Research*, vol. 28 (5), 2008, p. 343–349.
- KATIYAR, S. K. – MUKHTAR, H. 1996. Tea in chemoprevention of cancer: epidemiologic and experimental studies (Review). *International Journal of Oncology*, vol. 8, 1996, p. 221–238.
- LU, CH. – ZHU, W. – SHEN, CH. – GAO, W. 2012. Green Tea Polyphenols Reduce Body Weight in Rats by Modulating Obesity-Related Genes. *Plos One* [online], vol. 7 (6), 2012, DOI: 10.1371/journal.pone.0038332.
- MATSUMOTO, N. – ISHIGAKI, F. – ISHIGAKI, A. – IWASHIMA, H. – HARA, Y. 1993. Reduction of blood glucose levels by tea catechin. *Bioscience, Biotechnology and Biochemistry*, vol. 57 (4), 1993, p. 525–527.
- MORITA, O. – KNAPP, J. F. – TAMAKI, Y. – STUMP, D. G. – MOORE, J. S. – NEMEC, M. D. 2009. Effects of green tea catechin on embryo/fetal development in rats. *Food and Chemical Toxicology*, vol. 47 (6), 2009, p. 1296–1303.
- PARK, J. H. – KANG, S. N. – CHU, G. M. – JIN, S. K. 2014. Growth performance, blood cell profiles, and meat quality properties of broilers fed with *Saposhnikovia divaricata*, *Lonicera japonica* and *Chelidonium majus* extracts. *Livestock Science*, vol. 165, 2014, p. 87–94.
- RAINS, T. M. – AGARWAL, S. – MAKI, K. C. 2011. Antiobesity effects of green tea catechins: a mechanistic review. *The Journal of Nutritional Biochemistry*, vol. 22, 2011, p. 1–7.
- REZNICHENKO, L. – AMIT, T. – YOUNG, M. B. H. – MANDEL, S. 2005. Green tea polyphenol (–)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. *Journal of Neurochemistry*, vol. 93, 2005, p. 1157–1167.
- SAYAMA, K. – LIN, S. – ZHENG, G. – OGUNI, I. 2000. Effects of green tea on growth, food utilization and lipid metabolism in mice. *In vivo*, vol. 14 (4), 2000, p. 481–484.
- SNOUSSI, C. – DUCROC, R. – HAMDAOUI, M. H. – DHAOUADI, K. – ABAIDI, H. – CLUZEAUD, F. – NAZARET, C. – LE GALL, M. – BADO, A. 2014. Green tea decoction improves glucose tolerance and reduces weight gain of rats fed normal and high-fat diet. *Journal of Nutritional Biochemistry*, vol. 25 (5), 2014, p. 557–564.
- SPENCER, J. P. E. 2003. Metabolism of tea flavonoids in the gastrointestinal tract. *Journal of Nutrition*, vol. 133, (10), 2003, p. 3255–3261.
- SUGISAWA, A. – UMEGAKI, K. 2005. Physiological concentrations of (–)-epigallocatechin-3-O-gallate (EGCg) prevent chromosomal damage induced by reactive oxygen species in WIL2-NS cells. *Journal of Nutrition*, vol. 132, 2005, p. 1836–1839.
- WANG, Z. G. – FU, CH. – YU, S. 2013. Green tea polyphenols added to IVM and IVC media affect transcript abundance, apoptosis, and pregnancy rates in bovine embryos. *Theriogenology*, vol. 79 (1), 2013, p. 186–192.
- ZDUŃCZYK, Z. – FREJNAGEL, S. – WRÓBLEWSKA, M. – JUŚKIEWICZ, J. – OSZMIAŃSKI, J. – ESTRELLA, I. 2002. Biological activity of polyphenol extracts from different plant sources. *Food Research International*, vol. 35 (2-3), 2002, p. 183–186.

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