



University of Novi Sad - Univerzitet u Novom Sadu  
Faculty of Agriculture - Poljoprivredni fakultet



# CONTEMPORARY AGRICULTURE *SAVREMENA POLJOPRIVREDA*

The Serbian Journal of Agricultural Sciences  
*Srpski časopis za poljoprivredne nauke*

**NOVI SAD**  
**Vol. 63**  
**No. 1-2**  
**2014.**

UDC: 63(497.1)(051)-"540.2"

ISSN: 0350 - 1205





University of Novi Sad - Univerzitet u Novom Sadu  
Faculty of Agriculture - Poljoprivredni fakultet



# **CONTEMPORARY AGRICULTURE** ***SAVREMENA POLJOPRIVREDA***

The Serbian Journal of Agricultural Sciences  
*Srpski časopis za poljoprivredne nauke*

**NOVI SAD**  
**Vol. 63**  
**No. 1-2**  
**2014.**

UDC: 63(497.1)(051)-"540.2"

ISSN: 0350 - 1205

ISSN: 0350-1205

UDC: 63(497.1)(051)-,540.2“

„CONTEMPORARY AGRICULTURE“  
The Serbian Journal of Agricultural Sciences

„SAVREMENA POLJOPRIVREDA“  
*Srpski časopis za poljoprivredne nauke*

**Editor-in-Chief / Glavni i odgovorni urednik**  
Prof. dr Milan Popović, dekan fakulteta.

**Editor / Urednik**  
Prof. dr Blagoje Stančić

**Assistant Editors / Pomoćnici urednika:**  
Prof. dr Vesna Rodić  
Prof. dr Đorđe Malenčić  
Prof. dr Niko Milošević  
Doc. dr Ivan Stančić

**Lectors for the English Language / Lektori za engleski jezik**  
Bojana Šobot, dipl. filolog  
Aleksandar Jagrović, dipl. filolog

**Editorship / Uredništvo**  
Prof. dr Petar Erić (Serbia), Prof. dr Branko Konstantinović (Serbia), Prof. dr Milenko Jovanović (Serbia), Prof. dr Zoran Keserović (Serbia), Prof. dr Milan Popović (Serbia), Prof. dr Stanimir Kovčičin (Serbia), Prof. dr Jelena Ninić-Todorović (Serbia), Doc. dr Radovan Savić (Serbia), Prof. dr Paul Pirsan (Romania), Prof. dr Vera Stojšin (Serbia), Robin C. Anderson, PhD (USA), Prof. dr Karaoglanidis George (Greece), Prof. dr Norbert Lukač (Slovakia).

**Editorial council / Izdavački savet**  
Prof. dr Radovan Pejanović (Serbia), Prof. dr Miroslav Malešević (Serbia), Dipl. ecc. Gordana Radović (Serbia), Prof. dr Vitomir Vidović (Serbia), Prof. dr Branka Gološin (Serbia), Prof. dr Saša Orlović (Serbia), Prof. dr Nedeljko Tica (Serbia), Prof. dr Nikola Đukić (Serbia), Prof. dr Dragan Glamočić (Serbia), Prof. dr Nada Korać (Serbia), Prof. dr Jovan Crnobarac (Serbia), Prof. dr Stanko Boboš (Serbia), Prof. dr Ljiljana Nešić (Serbia), Prof. dr Petar Sekulić (Novi Sad), Prof. dr Mirjana Milošević (Serbia), Prof. dr Cvijan Mekić (Serbia), Prof. MVD Juraj Pivko, DSc. (Slovakia), Prof. dr Šandor Šomodi (Hungary), Prof. dr Sava Bunčić (UK), Prof. dr Boris Stegny (Ukraine), Prof. dr Kole Popovski (Macedonia), Prof. dr Mircov Dragoslav-Vlad (Romania), Prof. Baruch Rubin, Ph.D. (Israel), Prof. dr habil. Imre Musci, CSc. (Hungary), Prof. dr Mark Gleason (USA), Roger B. Harvey, DVM (USA).

**Publisher / Izdavač:**  
UNIVERSITY of NOVI SAD, FACULTY of AGRICULTURE  
UNIVERZITET U NOVOM SADU, POLJOPRIVREDNI FAKULTET

**Address of editorship / Adresa uredništva:**  
FACULTY of AGRICULTURE, 21000 Novi Sad, Trg Dositeja Obradovića 8, R. Serbia  
POLJOPRIVREDNI FAKULTET, 21000 Novi Sad, Trg Dositeja Obradovića 8, R. Srbija

**Phones/Telefoni:**  
++381 21 450-355; ++381 21 6350-711; ++381 21 485-3482; Fax: ++021/459-761.  
<http://polj.uns.ac.rs/>, E-mail: [blagoje.stancic@stocarstvo.edu.rs](mailto:blagoje.stancic@stocarstvo.edu.rs)

Four issues in two volumes per year / Četiri broja u dva volumena godišnje.  
Circulation 200 copies / Tiraž 200 primeraka.

Print / Štampa:

## REVIEWERS / RECENZENTI

**Prof. dr Sanimir Dimitrov**, Department of Genetics, Selection and Reproduction,  
Tracia University, Agricultural Faculty, Student Campus, 6000 Stara Zagora, Bulgaria.  
E-mail: dimitrov@af.uni-sz.bg

**Prof. dr Norbert Lukač**, Department of Animal Physiology, Slovak University of Agriculture,  
Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, Nitra, SK-94976, Slovak Republic.  
E-mail: norolukac@gmail.com

**Prof. dr Lidija Perić**, Department for Animal Sciences, University of Novi Sad, Faculty of  
Agriculture,  
Trg D. Obradovića 8, 21000 Novi Sad, Republic of Serbia. E-mail: lidija.peric@stocarstvo.edu.rs

**Prof. dr Dragan Glamović**, Department for Animal Sciences, University of Novi Sad, Faculty of  
Agriculture,  
Trg D. Obradovića 8, 21000 Novi Sad, Republic of Serbia. E-mail: dragan.glamovic@stocarstvo.edu.rs

**Prof. dr Miroslav Plavšić**, Department for Animal Sciences,  
University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad,  
Republic of Serbia. E-mail: miroslav.plavsic@stocarstvo.edu.rs

**Anderson, PhD.**, USA Department of Agriculture, South Plains Agriculture Research Center,  
Food and Feed Safety Research Unit, 2882 F&B Road, College Station, TX 77845.  
E-mail: Robin.Anderson@ars.usda.gov

**Prof. dr Martin Wähner**, Hochschule Anhalt (FH), Fachbereich Landwirtschaft,  
Ökotrophologie, Landschaftsentwicklung, Strenzfelder Allee 28, 06406 Bernburg, Deutschland.  
E-mail: waehner@loel.hs-anhalt.de

**Roger B. Harvey, DVM, MS**, Veterinary Medicine Officer, FFSRU, SPARC, ARS, USDA,  
2881 F&B Road, College Station, TX 77845. E-mail: Roger.Harvey@ars.usda.gov

**Doc. dr Aleksandar Potkonjak**, Department of Veterinary Medicine,  
University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad,  
Republic of Serbia. E-mail: dvmed@polj.uns.ac.rs

**Prof. dr Stanko Boboš**, Department of Veterinary Medicine, University of Novi Sad, Faculty of  
Agriculture,  
Trg D. Obradovića 8, 21000 Novi Sad, Republic of Serbia. E-mail: bobos@polj.uns.ac.rs

**Prof. dr Dragan Gvozdić**, Faculty of Veterinary Medicine, University of Belgrade,  
Bulevar oslobođenja 18, 11000 Belgrade, R. Serbia. E-mail: stocarstvo@vet.bg.ac.rs

**Prof. dr Mirjana Vučković**, Department of Biology and Ecology, University of Novi Sad,  
21000 Novi Sad, R. Serbia. E-mail: dusanka.laketic@dbe.uns.ac.rs

**Prof. dr Slobodanka Stojković**, CSIRO/Monash University, School of Biological Sciences,  
Building 18, Clayton Campus, VIC-3800, Australia. E-mail: Slobodanka.stojkovic@csiro.au

**Dr Rania Ahmed Abd El-Wahab Mohammed**, Plant Protect Research Institute,  
7 Nadi Elasaïd St., Dokki, Giza, Egypt. E-mail: rania-proline@hotmail.com

**Prof. dr Margaret Loseby**, Dipartimento di Ecologia e Sviluppo Economico Sostenibile,  
Universita' Degli Studi Della Tuscia, Faculta di Agraria, 01100 Viterbo, Italy.  
E-mail: loseby@unitus.it

**Dr Oliver T. Neher**, Assistant Professor, Extension Plant Pathology, University of Idaho, USA,  
Twin Falls Research & Extension Center, CSI Evergreen Building, 315 Falls Avenue East  
Twin Falls ID 83303. E-mail: oneher@uidaho.edu; cellphone: (208) 320-0836; phone: (208) 736-3633;

**Doc. dr Sladana Krivokapić**, University of Montenegro, PMF, Biology, Podgorica, Montenegro.  
E-mail: sladjana@yahoo.com; Phone: +382 20 243 816.

**Prof. dr Radica Đedović**, University of Belgrade, Faculty of Agriculture, Beograd-Zemun.  
E-mail: genrad@agrif.bg.ac.rs.

**Prof. dr Dragana Bozic**, University of Belgrade, Faculty of Agriculture Institute for Phytomedicine,  
Nemanjina 6, 11080 Zemun, Serbia. E-mail: dbozic@agrif.bg.ac.rs.

## CONTENTS / SADRŽAJ

### A. Original scientific papers / *Originalni naučni radovi*

#### ASSESSMENT OF COPPER CONTENT IN SEMEN AND ITS EFFECT ON THE SPERMATOZOA MOTILITY

*ODREĐIVANJE KONCENTRACIJE BAKRA U SPERMI I NJEGOV EFEKT NA POKRETLJIVOST SPERMATOZOIDA*

**Z. Kňazhická, J. Lukáčová, A. Greň, G. Formicki, P. Massányi, N. Lukáč** ..... 1

#### INDUCTION OF FEATHERS ON ONE-YEAR-OLD APPLE TREES CULTIVAR GOLDEN DELICIOUS USING BENZYLADENINE AND GIBBERELINS 4+7

*FORMIRANJE PREVREMENIH GRANČICA KOD JEDNOGODIŠNJI SADNICA JABUKE SORTE ZLATNI DELIŠES PRIMENOM BENZILADENINA I GIBERELINA 4+7*

**M. Dorić, Z. Keserović, N. Magazin, B. Milić, M. Miodragović** ..... 13

#### PARENCHYMAL LEUKOCYTE INFILTRATION, SOMATIC CELL COUNT AND DUCTUS PAPILLARIS LENGTH IN DAIRY COWS UDDER

*LEUKOCITNA INFILTRACIJA PARENHIMA, SOMATSKE ĆELIJE MLEKA I DUŽINA DUCTUS PAPILLARIS U VIMENU MLEČNIH KRAVA*

**I. Davidov, M. Radinović, M. Erdeljan, Z. Kovačević, Ž. Jurakić, A. Božić** ..... 22

#### SOW FERTILITY AFTER THE INTRACERVICAL AI IN COOL AND WARM SEASONS USING CONVENTIONAL DOSES IN COMBINATION WITH SYNTHETIC SEMINAL PLASMA (PREDIL MR-A®)

*FERTILITET KRMAČA POSLE INTRACERVIKALNOG OSEMENJAVANJA U TOPLOJ I HLADNOJ SEZONI UPOTREBOM KONVENCIONALNIH DOZA KOMBINOVANIH SA SINTETIČKOM SEMENOM PLAZMOM (PREDIL MR-A®)*

**I. Stančić, I. Radović, M. Erdeljan, B. Stančić, T. Vasiljević, A. Božić, I. Žarković** ..... 29

#### YIELD RESPONSE TO ELEVATED SOIL BORON IN WHEAT CULTIVARS OF LOCAL AND FOREIGN ORIGIN

*PRINOS LOKALNIH I STRANIH SORTI PŠENICE GAJENIH NA ZEMLJIŠTU KOJE SADRŽI POVIŠENE KONCENTRACIJE BORA*

**M. Brdar-Jokanović, A. Kondić-Špika, B. Ljevnaić-Mašić, I. Maksimović** ..... 36

#### PROTECTIVE EFFECT OF 1-DEOXYNOJIRIMYCIN AND RESVERATROL AGAINST DIABETIC OXIDATIVE STRESS AND INFLAMMATION

*ZAŠTITINI EFEKT 1-DEOXYNOJIRIMYCINA I RESVERATROLA PROTIV DIABETIČKOG OKSIDATIVNOG STRESA I INFLAMACIJE*

**A. Greň, G. Formicki, N. Lukač, P. Massányi, R. Stawarz** ..... 44

<b>ZINC CONCENTRATION IN THE MILK SERUM OF COWS IN THE EARLY AND MID LACTATION</b> <i>KONCENTRACIJA CINKA U MLEČNOM SERUMU KRAVA U RANOJI SREDNJOJ LAKTACIJI</i> <b>Z. Kovačević, D. Stojanović, I. Davidov, G. Žugić, M. Radinović, M. Erdeljan</b> .....	55
<b>SCREENING TEST FOR DETECTION OF COLORADO POTATO BEETLE (<i>Leptinotarsa decemlineata</i> Say) SENSITIVITY TO ABAMECTIN</b> <i>PRIMENA SCREENING TESTA PRI DETEKCIJI OSETLJIVOSTI KROMPIROVE ZLATICE (<i>Leptinotarsa decemlineata</i> Say) NA ABAMEKTIN</i> <b>S. Vuković, D. Indić, S. Gvozdenac, S. Tanasković</b> .....	62
<b>VISITORS' SATISFACTION: THE CASE OF THE "ŠARGAN – MOKRA GORA" NATURE PARK</b> <i>ZNAČAJ ZADOVOLJSTVA POSETILACA PARKA PRIRODE "ŠARGAN - MOKRA GORA"</i> <b>Z.A. Ristić, I. Nađ, M. Matejević, M. Kovačević</b> .....	71
<b>EFFECT OF PGPR ON THE GERMINATION AND GROWTH OF ENGLISH RYEGRASS AND MICROBIOLOGICAL ACTIVITY IN ITS RHIZOSPHERE*</b> <i>UTICAJ PRIMENE PGPR NA KLIJAVOST I RAST ENGLESKOG LJULJA I NA MIKROBIOLOŠKU AKTIVNOST U RIZOSFERI BILJKE</i> <b>D. Stamenov, S. Đurić, T. Hajnal Jafari</b> .....	79
<b>THE IMPORTANCE OF LEGISLATION TO IMPROVING THE REPRODUCTIVE HEALTH OF PIGS</b> <i>ZNAČAJ ZAKONSKIH PROPISA ZA UNAPREĐENJE REPRODUKTIVNOG ZDRAVLJA SVINJA</i> <b>N. Stojanac, O. Stevančević, M. R. Cincović</b> .....	86
<b>CONCENTRATION OF BLOOD HSP70 AND ITS RELATION WITH LIPIDE MOBILISATION AND KETOGENESIS IN DAIRY COWS DURING PERIPARTURIENT PERIOD</b> <i>KONCENTRACIJA HSP70 U KRVI I NJEGOVA VEZA SA LIPIDNOM MOBILIZACIJOM I KETOGENEZOM KOD KRAVA U PERIPARTALNOM PERIODU</i> <b>M.R. Cincović, B. Belić</b> .....	92
<b>INSULIN RESISTANCE IN COWS DURING DRY PERIOD AND EARLY LACTATION</b> <i>INSULINSKA REZISTENCIJA KOD KRAVA U PERIODU ZASUŠENJA I RANOJ LAKTACIJI</i> <b>M.R. Cincović, B. Belić, R. Đoković, B. Toholj, T. Hristovska, B. Delić, M. Došenović</b> .....	98
<b>SERUM ACTIVITIES OF AST, ALT, GGT AND LDH IN CLINICALLY HEALTHY DAIRY COWS DURING TRANSITIONAL PERIOD AND MID LACTATION*</b> <i>SERUMSKE AKTIVNOSTI AST, ALT, GGT I LDH KOD KLINIČKI ZDRAVIH MLEČNIH KRAVA TOKOM TRANZICIJSKOG PERIODA I SREDINE LAKTACIJE</i>	

<b>R. Đoković, V. Kurčubić, Z. Ilić, M.R. Cincović, M. Petrović, B. Delić</b> . . . . .	106
<b>WHY ELIMINATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN SLOVENE FARMS FAILED?</b>	
<i>ZAŠTO ELIMINACIJA REPRODUKTIVNOG I RESPIRATORNOG SINDROMA (PRRS) SVINJA U SLOVENAČKIM UZGOJIMA SVINJA NIJE EFIKASNA?</i>	
<b>M. Štukelj</b> . . . . .	112
<b>SEROLOGICAL SURVEY ON SELECTED PATHOGENS ON 16 SMALL PIG FARMS IN SLOVENIA</b>	
<i>SEROLOŠKE PRETRAGE ODABRANIH UZROČNIKA NA 16 MANJIH UZGOJA SVINJA U SLOVENIJI</i>	
<b>I. Golinar Oven, M. Štukelj</b> . . . . .	122
<b>COMPOSITION AND GERMINATION CAPABILITY OF WEED SEED BANK IN THE SOIL UNDER MAIZE</b>	
<i>SASTAV I SPOSOBNOST KLIJANJA BANKE SEMENA KOROVA U ZEMLJIŠTU U USEVU KUKURUZA</i>	
<b>B. Konstantinović, M. Blagojević, B. Konstantinović sen., N. Samardžić</b> . . . . .	131
<b>PRODUCTIVE LIFE OF HIGH YIELDING DAIRY COWS</b>	
<i>PRODUKTIVNI ŽIVOT VISOKOMLEČNIH KRAVA</i>	
<b>Ž. Novaković, S. Trivunović, R. Jovanović, R. Beskorovajni, D. Ostojić-Andrić, N. Popović</b> . . . . .	139
<b>B. Review papers / Pregledni radovi</b>	
<b>WINE MARKET IN THE EUROPEAN UNION</b>	
<i>TRŽIŠTE VINA U EVROPSKOJ UNIJI</i>	
<b>B. Vlahović, A. Puškarić, M. Nacka</b> . . . . .	146
<b>ORGANIC DAIRY PRODUCTION – MOST COMMON ISSUES CONCERNING HEALTH CARE AND NUTRITION</b>	
<i>ORGANSKA PROIZVODNJA MLEKA – NAJČEŠĆA PITANJA OBZIROM NA ZDRAVSTVENU ZAŠTITU I ISHRANU</i>	
<b>A. Popović Vranješ, Ž. Jurakić, B. Belić, I. Davidov, A. Božić, M. Ostojić, G. Jež</b> . . . . .	153
<b>C. Book review / Prikaz knjige</b>	
<b>Review of Monography „EDUCATION, SCIENCE AND FOOD PRODUCTION“</b>	
<b>Author academic VASKRASIJA JANJIĆ</b>	
<i>PRIKAZ MONOGRAFIJE „OBRAZOVANJE, NAUKA I PROIZVODNJA HRANE“</i>	
<i>Autora akademika VASKRSIJA JANJIĆA</i>	
<b>B. Škundrić</b> . . . . .	162

## ASSESSMENT OF COPPER CONTENT IN SEMEN AND ITS EFFECT ON THE SPERMATOZOA MOTILITY\*

ZUZANA KŇAŽICKÁ, JANA LUKÁČOVÁ, AGNIESZKA GREŃ,  
GRZEGORZ FORMICKI, PETER MASSÁNYI, NORBERT LUKÁČ<sup>1</sup>

*SUMMARY: The general objective of this in vitro study was at first to evaluate copper (Cu) content in the cell sediment and seminal plasma fraction and compare their relationship with basic motility characteristics and secondly expand the knowledge of its impact on the fertilization potential of the spermatozoa. Semen samples were collected from 12 breeding bulls. The motility analysis was carried out using the Computer Assisted Semen Analysis (CASA) system. The mean value for the percentage of motile spermatozoa (MOT) was  $91.24 \pm 5.17\%$  and the progressive motility of the spermatozoa (PROG) as  $88.58 \pm 5.85\%$ . Subsequently, the samples were centrifuged to obtain the seminal plasma fraction and cell sediment. The seminal plasma Cu concentrations were analyzed by UV/VIS spectrophotometry. The total Cu concentration of the seminal plasma was  $0.23 \mu\text{g/mL}$ . The analysis by the flame atomic absorption spectrophotometry (FAAS) showed that the average cell sediment Cu concentration was  $0.0014 \mu\text{g/mL}$ . The correlation analysis revealed a moderate positive correlations between MOT and seminal plasma Cu concentration ( $r_p = 0.504$ ;  $P > 0.05$ ) as well as between PROG and Cu content in the seminal plasma ( $r_p = 0.410$ ;  $P > 0.05$ ). Copper in the cell sediment positively affected both MOT ( $r_p = 0.265$ ) and PROG ( $r_p = 0.227$ ), however, no significant differences were found ( $P > 0.05$ ). Conclusions of this study clearly indicated that Cu is important for the preservation of spermatozoa motility. The obtained results proved higher level of Cu in the seminal plasma, which seems representative of cumulative exposures of this trace element. Based on these results, we can conclude that the evaluation of total content of Cu in the*

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> MSc. Zuzana Kňazická, PhD., MSc. Jana Lukáčová, PhD student, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovak Republic. MSc. Agnieszka Greń, PhD, teching assistant, Dr. Hab. Grzegorz Formicki, PhD, associated professor, Department of Animal Physiology and Toxicology, Faculty of Geography and Biology, Pedagogical University, Cracow, Poland. DVM. Peter Massányi, DrSc., professor, MSc. Norbert Lukáč, PhD, associated professor, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovak Republic.

Corresponding author: Norbert Lukáč, e-mail: norolukac@gmail.com, phone.: +421 37 641-4349.

\* The work was co-funded by European Community under project No. 26220220180: Building Research centre „AgroBioTech“ and Tatra Bank Foundation 2012/2013 (Slovak Republic).



*whole semen is an important factor for determining the fertilization potential of spermatozoa.*

**Key words:** *copper, bovine spermatozoa, semen parameters, cell sediment, seminal plasma, motility parameters.*

## INTRODUCTION

Exposure to heavy metals is a risk factor in the assessment of spermatogenesis (Semczuk and Kurpisz, 2006; Kabata-Pendias and Mukherjee, 2007), while certain trace elements have been shown to be essential for testicular development and spermatogenesis (Eidi et al., 2010; Akinloye et al., 2011). Mammalian semen is known to contain a big variety of chemical elements (Marzec-Wroblewska et al., 2012), whose influence on spermatozoa viability has been extensively studied in animals as well as in humans (Kanwal et al., 2000; Massanyi et al., 2003a, b; Eghbali et al., 2008; Atig et al., 2012). A large number of studies have proven that chemical elements are an essential component in the preservation of the fertilization capacity of spermatozoa (Tvrđá et al., 2012). Some of them are crucial for a proper sperm cell function (e. g. sodium, magnesium, calcium, potassium), others are required in relatively narrow limits (zinc, manganese, copper, iron, cobalt, selenium) (Massanyi et al., 2003a, b; 2004).

The possible influence of metals ions, and especially copper (Cu), on the male infertility is a matter of great interest. Copper is an important trace and essential element for the all organisms (Craig et al., 2009), because has a great positive role in physiological and regulatory processes (Dobrzanski et al., 1996; Tang, 2005). Moreover, it is a component of a number of metalloenzymes and metalloproteins (Agarwal et al., 1990), which are involved in energy and antioxidant metabolism (Haliwell and Gutteridge, 2000; Aydemir et al., 2006). Copper is a normal constituent of semen bound to the tail midpiece of spermatozoa (Manu, 1974) and present in seminal plasma, ampullar and seminal vesicular fluids and also released by other structures of the reproductive system (i.e. epididymis) (Valsa et al., 1994). Copper deficiency affects the development of sperm cells (Van Niekerk and Van Niekerk, 1989; Leonhard-Marek, 2000). On the other hand, the high doses of copper ions ( $\text{Cu}^{2+}$ ) have a toxic effect on the epididymis (Xu et al., 1985), testes, scrotum of mammals (Skandhan, 1992; Eidi et al., 2010), which may ultimately lead to a reduced fertility (Pesch et al., 2006). Increased levels of metal ions in semen (Umeyama et al., 1986) or seminal plasma (Stanwell-Smith et al., 1983) appear to be significantly and positively correlated with male infertility. Meeker et al. (2008) found evidence of an inverse association between high Cu levels and semen quality, which is consistent with a number of animal and human studies (Skandhan, 1992; Huang et al., 2000; Massanyi et al., 2004; Yuyan et al., 2007). Several experimental studies demonstrated the adverse effects of  $\text{Cu}^{2+}$  on spermatozoa motility (Wong et al., 2001; Machal et al., 2002; Roychoudhury and Massanyi, 2008; Roychoudhury et al., 2010; Knazicka et al., 2012a, c; Sakhaee et al., 2011). The incubation of spermatozoa in the presence of Cu had a negative effect on some of motility parameters (distance and velocity) examined by Computer Assisted Semen Analysis (CASA) system in studies by Roychoudhury et al. (2008). Our previous *in vitro* studies evaluated the negative effects

of a wide range of concentrations of Cu as a risk factor of environment on the motility of spermatozoa and subsequently pointed out a cytotoxic effect of Cu on the mitochondrial complex (Knazicka et al., 2012a, b).

Since, that Cu plays an essential role in spermatogenesis and fertility; this study was conducted to investigate the copper content in cell sediment and seminal plasma fraction and compare their relationship with basic motility characteristics. Furthermore, also expand the knowledge of its impact on the fertilization potential of the spermatozoa.

## MATERIAL AND METHODS

*Biological material.* Bovine semen samples were obtained from 12 adult breeding bulls (Slovak Biological Services, Nitra, Slovak Republic). The samples had to accomplish the basic quality criteria given for the corresponding breed. The semen was obtained on a regular collection schedule using an artificial vagina. After collecting the samples, they were stored in the laboratory at room temperature (22-25°C) and basic measurements were performed – volume (mL), pH, concentration ( $\times 10^9/\text{mL}$ ) and osmolality (mOsmol/kg) (Table 1). Each sample was diluted in physiological saline solution (PS) (sodium chloride 0.9% w/v, Bieffe Medital, Grosotto, Italia), using a dilution ratio of 1:40, depending on the original spermatozoa concentration.

*Spermatozoa motility analysis.* The spermatozoa motility was carried out using the Computer Assisted Semen Analysis (CASA) system – SpermVision™ program (MiniTüb, Tiefenbach, Germany) with the Olympus BX 51 phase contrast microscope (Olympus, Tokyo, Japan) equipped with heating plate (37°C). Each sample was placed into the Makler Counting Chamber (depth 10  $\mu\text{m}$ , Sefi-Medical Instruments, Haifa, Israel) and the following parameters were evaluated: MOT - percentage of motile spermatozoa (%; motility > 5  $\mu\text{m/s}$ ); PROG - percentage of progressive motile spermatozoa (%; motility > 20  $\mu\text{m/s}$ ); DAP - distance average path ( $\mu\text{m}$ ); DCL - distance curved line ( $\mu\text{m}$ ); DSL - distance straight line ( $\mu\text{m}$ ); VAP - velocity average path ( $\mu\text{m/s}$ ); VCL - velocity curved line ( $\mu\text{m/s}$ ); VSL - velocity straight line ( $\mu\text{m/s}$ ); STR – straightness (VSL:VAP); LIN – linearity (VSL:VCL); WOB – wobble (VAP:VCL); ALH - amplitude of lateral head displacement ( $\mu\text{m}$ ) and BCF – beat cross-frequency ( $\text{H}_z$ ). 1000-1500 cells were examined for each sample.

*Samples processing.* After measurements the samples were centrifuged (10 min, 9500 rpm, 4 °C) to obtain the cell sediment and seminal plasma fraction (supernatant). The fractions were separated and transferred into 1.5 mL tubes and kept frozen (-80°C) for further analysis.

*Analysis of cell sediment Cu concentration.* The cell sediment fractions were mineralized by adding 1 mL of  $\text{HNO}_3$  (65%; Sigma-Aldrich, St. Louis, MO, USA). The resulting solution was diluted to 3 mL with demineralised water. The Cu contents in sediment were determined by direct aspiration of the acidic sample into the flame atomic absorption spectrophotometry (FAAS). This complies with the specification for standardized FAAS quick procedure for metals when using the BUCK Model 200A atomic absorption spectrophotometer (Cole-Parmer International, Court Vernon Hills, Illinois, USA) equipped with a hollow cathode lamp. All measurements were performed at wavelength 324.80 nm. The quantification limit for Cu was 0.096 mg/L and for the detection limit 0.29 mg/L. Calibration Cu was delineated using suitable standard con-

centrations (0.125, 0.25, 0.50, 1.0 and 10.0 µg/g) by diluting standard (0.50% HNO<sub>3</sub>). Concentrations were converted to µg/mL.

*Analysis of seminal plasma Cu concentratio.* The analysis of Cu in the seminal plasma was performed BioLa Test commercial kit (PLIVA-Lachema, Brno, Czech Republic). The measurement was based on a colorimetric reaction between Cu(I) ions and bathocuproine (BCP) forming a stable orange coloured complex (Landers and Zak, 1958), which was easy to detect photometrically at 480 nm (Genesys 10 spectrophotometer, Thermo Fisher Scientific Inc., Madison, USA). Concentrations were expressed as µg/mL.

*Statistical analysis.* Statistical analysis of the results was carried out using the statistical program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (arithmetic mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. Pearson's correlation coefficient (two tailed) test was used to examine correlations between analyzed parameters of the semen. The level of significance was set at <sup>A</sup> (P<0.001); <sup>B</sup> (P<0.01); <sup>C</sup> (P<0.05).

## RESULTS

The results summarized in Table 1 indicate that the concentration of spermatozoa in semen was 3.15±0.96x10<sup>9</sup> per mL. The volume of the collected semen of bulls was 6.23±1.69 mL. The total Cu concentration of the seminal plasma was in the range 0.09-0.39 µg/mL with the average value 0.23 µg/mL. Results of the semen evaluation show, that the average cell sediment Cu concentration measured by the FAAS method was 0.0014 µg/mL.

Table 1 The basic parameters of analyzed bovine semen samples (n=12).

Parameters	x±S.D.
pH	6.56±0.20
Spermatozoa concentration (x10 <sup>9</sup> /mL)	3.15±0.96
Semen volume (mL)	6.23±1.69
Osmolarity (mOsmol/kg)	297.50±4.67
Seminal plasma copper concentration (µg/mL)	0.23
Cell sediment copper concentration (µg/mL)	0.0014

x – arithmetic mean; S.D. – standard deviation

The mean value for the percentage of motile spermatozoa was 91.24±5.17%. The CASA analysis showed 88.58±5.85% of progressive motile spermatozoa, the average path distance was 37.81±6.79 µm and the average path velocity was 89.53±16.79 µm/s. The other motility parameters with statistical differences are shown in the Table 2.

Table 2 The average values of spermatozoa motility parameters of analyzed bovine semen samples.

Parameters Motility	X	Minimum	Maximum	S.D.	Cv (%)
MOT (%)	91.24	76.08	99.39	5.17	5.67
PROG (%)	88.58	72.15	96.66	5.85	6.60
DAP (µm)	37.81	25.39	69.03	6.79	17.96
DCL (µm)	60.90	42.64	96.92	10.02	16.45
DSL (µm)	31.72	21.42	64.80	6.71	21.16
VAP (µm/s)	89.53	60.19	159.70	16.79	18.75
VCL (µm/s)	143.80	104.00	223.90	24.10	16.76
VSL (µm/s)	75.46	49.36	150.60	16.50	21.87
STR	0.83	0.74	0.96	0.04	4.88
LIN	0.52	0.38	0.68	0.06	12.45
WOB	0.62	0.46	0.74	0.06	9.36
ALH (µm)	4.72	2.99	6.14	0.72	15.33
BCF (Hz)	34.99	27.41	48.14	3.70	10.57

MOT – percentage of motile spermatozoa (%); PROG - percentage of progressive motile spermatozoa (%); DAP- distance average path (µm); DCL – distance curved line (µm); DSL – distance straight line (µm); VAP- velocity average path (µm/s); VCL – velocity curved line (µm/s); VSL – velocity straight line (µm/s); STR – straightness (VSL:VAP); LIN – linearity (VSL:VCL); WOB – wobble (VAP:VCL); ALH - amplitude of lateral head displacement (µm) and BCF – beat cross-frequency (Hz).

x – arithmetic mean, S.D. – standard deviation, CV (%) – coefficient of variation

In our study, the correlation analysis revealed a moderate positive correlations between percentage of motile spermatozoa and seminal plasma Cu concentration ( $r_p=0.504$ ;  $P>0.05$ ) as well as between progressive of motile spermatozoa and Cu content in the seminal plasma ( $r_p=0.410$ ;  $P>0.05$ ). Copper in the cell sediment positively affected both motility ( $r_p=0.265$ ) and progressive motility ( $r_p=0.227$ ), however, no significant differences were found ( $P>0.05$ ). Results of correlation analysis are listed in Table 3, 4.

Table 3 The Pearson's coefficient of correlations ( $r_p$ -values) for relationship between seminal plasma Cu concentration and selected spermatozoa motility parameters.

	Cu	MOT	PROG
Cu	1		
MOT	0.504	1	
PROG	0.410	0.963 <sup>A</sup>	1

The correlation analysis was based on the value of the correlation coefficient:  $\pm 0.111$  to  $\pm 0.333$ : *low correlation*;  $\pm 0.334$  to  $\pm 0.666$ : *moderate correlation*;  $\pm 0.667$  to  $\pm 0.999$ : *high correlation*.

MOT – percentage of motile spermatozoa (%); PROG - percentage of progressive motile spermatozoa (%); <sup>A</sup> $P<0.001$ ; <sup>B</sup> $P<0.01$ ; <sup>C</sup> $P<0.05$

Table 4 The Pearson's coefficient of correlations ( $r_p$ -values) for relationship between cell sediment Cu concentration and selected spermatozoa motility parameters.

	Cu	MOT	PROG
Cu	1		
MOT	0.265	1	
PROG	0.227	0.586	1

The correlation analysis was based on the value of the correlation coefficient:  $\pm 0.111$  to  $\pm 0.333$ : *low correlation*;  $\pm 0.334$  to  $\pm 0.666$ : *moderate correlation*;  $\pm 0.667$  to  $\pm 0.999$ : *high correlation*.

MOT – percentage of motile spermatozoa (%); PROG - percentage of progressive motile spermatozoa (%); <sup>A</sup>P<0.001; <sup>B</sup>P<0.01; <sup>C</sup>P<0.05

## DISCUSSION

Essential trace minerals (ETMs) are among important factors in maintaining and recovering health (Hostetler et al., 2003); however their requirement for reproduction has not been as extensively studied (Lu et al., 2009). Despite the complex relationship between semen analysis and ETMs, we have attempted evaluate a content of trace element - Cu in the whole semen (i.e. seminal plasma versus cell sediment/fraction) and expand the knowledge concerning its relationship with spermatozoa motility.

Semen volume, pH, concentration, viability and motility of spermatozoa as well as composition of the seminal plasma are common parameters to assess spermatozoa quality (Alavi and Cosson, 2006). These factors are directly related to the fertilization success (Bozkut et al., 2009). Our results of basic semen parameters showed that all observed characteristics were at physiological rates. The semen has a very high buffering capacity, much higher than that of most other fluids in the body (Meacham, 2002). The bovine semen maintains a slightly acidic pH (Gamcik, 1992), which was in accordance with our results (pH of 6.56). The semen is notable also for its high osmolarity, which is substantially higher than that of blood plasma. The osmolarity of semen depends greatly on the concentration of sugars and other organics concentrations as well as ionic salt concentrations (Mandal and Bhattacharyya, 1987; Owen and Katz, 2005). In our experiment, semen has a target osmolarity of 297.50 mOsmol/kg. Some researchers have noted that osmolarity increases measurably with semen aging (Velazquez et al., 1977).

Currently, there are little studies dealing with issue of analysis of trace elements (Cu) in the cell sediment or only in the seminal plasma alone. Therefore, this study is the first, which completely evaluate total content of Cu in the whole semen. Our observation showed higher level of Cu in the seminal plasma (0.23  $\mu\text{g/mL}$ ) in comparison with the cell sediment (0.0014  $\mu\text{g/mL}$ ). Besides, we found a weak positive correlations between cell sediment Cu concentration and the percentage of motile spermatozoa ( $r_p=0.265$ ;  $P>0.05$ ) as well as progressive motility of the spermatozoa ( $r_p=0.227$ ;  $P>0.05$ ).

Regarding of seminal plasma makes up about 95% of the total volume of whole semen (Gamcik, 1992) and contains a variety of biochemical components, some of which are relatively specific for the regulation of spermatozoa function (Asadpour, 2012). Besides, the seminal plasma is a reliable biological marker for evaluating vitality, sperm metabolism, motility and others relevant semen parameters (Maxwell et al., 1996; Asadpour, 2012). Several studies have shown its relationships with volume, pH,

concentration, vitality and morphology (Massanyi et al., 2005; Shinohara et al., 2005; Akinloye et al., 2011). The experimental study by Machal et al. (2002) confirmed a positive coefficient of correlation ( $r_p=0.360$ ;  $P<0.01$ ) between the Cu concentration in seminal plasma and the mean volume of bulls semen. A positive significant ( $P<0.05$ ) correlation between seminal plasma Cu and semen volume was reported also in the human study by Akinloye et al. (2011). Shinohara et al. (2005) found significant correlations between Cu concentration in semen and sperm concentration, semen volume and abnormal morphology. Eidi et al. (2010) examined seminal plasma levels of Cu and its relationship with human semen parameters. Their study demonstrated significant negative correlation between seminal plasma Cu concentration and pH ( $r_p=-0.173$ ;  $P<0.05$ ) as well as sperm concentration ( $r_p=-0.114$ ;  $P<0.05$ ) and motility ( $r_p=-0.399$ ;  $P<0.01$ ). Subsequently, they confirmed that high concentration of Cu is related to lowering pH of seminal plasma, acidic pH, with changing condition of seminal plasma due to decrease motile or alive percent of spermatozoa. The excess Cu in seminal plasma is detrimental for male reproductive capacity by reducing spermatozoa count, motility, vitality and morphology. Katayose et al. (2004) demonstrated that higher concentrations of Cu had significant adverse effects on spermatozoa motility. Equally, Rebrelo et al. (1996) confirm that the high concentration of this essential element in seminal plasma is correlated with reduced spermatozoa motility. In our case, the mean value for the percentage of motile spermatozoa (quantity of movement) was  $91.24\pm 5.17\%$  and the progressive motility of the spermatozoa (quality of movement) as  $88.58\pm 5.85\%$ . The correlation analysis revealed a moderate positive correlation between percentage of motile spermatozoa and seminal plasma Cu concentration ( $r_p=0.504$ ;  $P>0.05$ ) as well as between progressive of motile spermatozoa and Cu content in the seminal plasma ( $r_p=0.410$ ;  $P>0.05$ ), which is in agreement with the report of Tvrdá et al. (2012). Eghbali et al. (2008) recorded, that spermatozoa motility was  $92.24\pm 0.51\%$  in excellent group,  $81.66\pm 0.62\%$  in good group and moderate group  $71.66\pm 1.05\%$ , which were significantly different. In this study demonstrated a positive correlation between seminal plasma Cu concentration and bovine (*Bubalus bubalis*) spermatozoa motility with viability. Machal et al. (2002) reported a statistically significant ( $P<0.05$ ) positive coefficients of correlation between the Cu concentration in seminal plasma and spermatozoa motility ( $r_p=0.330$ ) and the total number of sperm cells with progressive motility ( $r_p=0.280$ ). These their results correspond with the studies of Dhami et al. (1994) and Leonhard-Marek (2000). Wong et al (2001) also recorded a weak but significant positive correlation between blood Cu concentrations and spermatozoa motility. In a similar study, Jockenhövel et al. (1990) showed significant correlation between seminal plasma Cu concentrations and spermatozoa count, motility and normal morphology. The findings of other authors (Eidi et al., 2010; Akinloye et al., 2011) are however controversial in the comparison with our results.

The differences in opinion concerning the Cu content in seminal plasma of different species of animals were detected. The mean total Cu value of the buffalo seminal plasma in the study of Eghbali et al. (2008) was recorded as  $2.51\pm 0.04$  mg/kg wet weight. Comparing these results with other authors we found out that according to Massanyi et al., (2003a, b) the seminal plasma Cu concentration was significantly higher ( $P<0.01$ ) in the rams ( $2.49\pm 0.18$  mg/kg), fox ( $2.16\pm 0.53$  mg/kg) than that in the bulls ( $1.64\pm 0.21$  mg/kg), boars ( $1.64\pm 0.28$  mg/kg) and stallions ( $0.86\pm 0.10$  mg/kg). The concentration of Cu in rabbit semen was assessed Lukac et al., (2009) on the level  $20.10\pm 4.09$  mg/kg wet weight, while rabbit semen is characterized by very high Cu concentration. Machal et

al. (2002) state that the mean Cu level in seminal plasma of bulls was 38.17  $\mu\text{M/L}$ , which in comparison with our results is too high a concentration. Skandhan and Mazundar (1979) stressed that an enhanced seminal plasma concentration of Cu may be one of the direct factors attributed to oligoasthenospermia and asthenospermia. Excess levels of monovalent and divalent Cu ions in solution should result in lipid peroxidation in sperm plasma membrane, an effect that may render spermatozoa immotile (Rebrelo et al., 1996; Wong et al., 2001). It is reported that Cu acts as a catalyst in the formation of reactive oxygen species (ROS) that can lead to oxidative stress development (Stohs and Bagchi, 1995). Therefore, it is important to systematically evaluate its content in tissues and body fluids in relation to antioxidant capacity, in order to prevent complications resulting from its bilateral role in the organism (Tvrđá et al., 2012).

## CONCLUSION

The data obtained from this *in vitro* study proved that the copper is necessary component of bovine semen and is needed for a proper spermatozoa function. Conclusions of this study clearly indicated that there is a relationship between a copper concentration in the semen and basic motility characteristics (motility, progressive motility), which also are affected by many factors. The obtained results proved higher level of copper in the seminal plasma, which seems representative of cumulative exposures of this trace element. Additionally, we can conclude that the evaluation of total content of copper in the whole semen (i.e. seminal plasma and cell sediment) is an important factor for determining the fertilization potential of spermatozoa.

## REFERENCES

- AGARWAL, K., SHARMA, A., TALUKDER, G.: Clastogenic effects of copper sulphate on the bone marrow chromosomes of mice *in vivo*. *Mutation Research*, 243:1–6, 1990.
- AKINLOYE, O., ABBIYESUKU, F.M., OQUNTIBEJU, O.O., AROWOJOLU, A.O., TRUTER, E.J.: The impact of blood and seminal plasma zinc and copper concentrations on spermogram and hormonal changes in infertile Nigerian men. *Reprod. Biol.*, 11(2):83–89, 2011.
- ALAVI, S.M.H. COSSON, J.: Sperm motility in fishes: (II) effects of ions and osmotic pressure. *Cell. Biol. Int.*, 30:1–14, 2006.
- ASADPOUR, R.: Relationship between mineral composition of seminal plasma and semen quality in various ram breeds. *Acta Scientiae Veterinariae*, 40(2):1027–1035, 2012.
- ATIG, F., RAFFA, M., BEN-ALI, H., KERKENI, A., SAAD, A., MOUNIR, A.: Impact of seminal trace element and glutathione levels on semen quality of Tunisian infertile men. *BMC Urology*, 12:6–14, 2012.
- AYDEMIR, B., KIZILER, A.R., ALICI, B., OZKARA, H., AKYOLCU, M.C.: Impact of Cu and Fe concentrations on oxidative damage in male infertility. *Biol. Trace Elem. Res.*, 112:193–203, 2006.
- BOZKUT, Y., OGRETMEN, F., SERTEL SECER, F., ERCIN, U.: Relationship between seminal plasma composition and spermatological parameters in Scaly Carp (*Cyprinus carpio*). *J. Anim. Vet. Adv.*, 8(12):2745–2749, 2009.
- CRAIG, P.M., GALUS, M., WOOD, CH.M., McCLELLAND, G.B.: Dietary iron alters

waterborne copper-induced gene expression in soft water acclimated zebrafish (*Danio rerio*). *Regulatory Physiology*, 296(2)362-373, 2009.

DHAMI, A.J., SAHNI, K.L., MOHAN, G., TRIPATHI, R.P.: Comparative – evaluation of initially static and motile semen ejaculates from Friesian and Murrah buffalo bulls for physicomorphological, biochemical, enzymatic and mineral constituents of seminal plasma. *Indian J. Anim. Sci.*, 64:926-932, 1994.

DOBZANSKI, Z., KOŁACZ, R., BODAK, E.: Heavy metals in animal environment. *Medycyna Weterynaryjna*, 52:570-574, 1996 (in Polish with English summary).

EIDI, M., EIDI, A., POUYAN, O., SHAHMOHAMMADI, P., FAZAELI, R., BAHAR, M.: Seminal plasma levels of copper and its relationship with seminal parameters. *Iran J. Reprod. Med.*, 8(2)60-65, 2010.

EGHBALI, E., ALAVI-SHOUSHTARI, S.M., ASRI REZAI, S.: Effects of copper and superoxide dismutase content of seminal plasma on buffalo semen characteristics. *Pakistan Journal of Biological Sciences*, 11(15)1964-1968, 2008.

GAMCIK, P., KOZUMOLIK, J., MESAROS, P., SCHVARC, F., VLCEK, Z., ZIBRIN, M.: Andrológia a inseminácia hospodárskych zvierat. Bratislava: Príroda, 1992 (in Slovak).

HALLIWELL, B., GUTTERIDGE, J.M.C.: *Free Radicals in Biology and Medicine*, 3rd ed. London, Oxford University Press, 2000.

HOSTETLER, C.E., KINCAID, R.L., MIRANDO, M.A.: The role of essential trace elements in embryonic and fetal development in livestock. *Vet. J.*, 166:125-139, 2003.

HUANG, Y.L., TSENG, W.C., CHENG, S.Y. - LIN, T.H.: Trace elements and lipid peroxidation in human seminal plasma. *Biol. Trace Elem. Res.*, 76:207-215, 2000.

JOCKENHÖVEL, F., BALS-PRATSCH, M., BERTRAM, H.P., NIESCHLAG, E.: Seminal lead and copper in fertile and infertile men. *Andrologia*, 22:503-511, 1990.

KABATA-PENDIAS, A., MUKHERJEE, A.B.: *Trace Elements from Soil to Human*. Springer, Heidelberg, Germany, 2007.

KANWAL, M.R., REHMAN, N.U., AHMAD, N., SAMAD, H.A., REHMAN, Z.U., AKHTAR, N., ALI, S.: Bulk cations and trace elements in the Nili-Ravi buffalo and crossbred cow bull semen. *International Journal of Agriculture and Biology*, 2:302-305, 2000.

KATAYOSE, H., SHINOHARA, A., CHIBA, M., YAMADA, H., TOMINAGA, K., SATO, A., YANAGIDA, K.: Effects of various elements in seminal plasma on semen profiles. *Journal of Mamalian Ova Research*, 21:141-148, 2004.

KNAZICKA, Z., TVRDA, E., BARDOS, L., LUKAC, N.: Dose- and time-dependent effect of copper ions on the viability of bull spermatozoa in different media. *Journal of Environmental Science and Health, Part A*, 47(9)1294-1300, 2012a.

KNAZICKA, Z., LUKAC, N., GREN, A., FORMICKI, G., MASSANYI, P.: In vitro effects of copper on the motility and viability of spermatozoa. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(6)1529-1539, 2012b.

KNAZICKA, Z., LUKACOVA, J., TVRDA, E., HASHIM, F., MASSANYI, P., LUKAC, N.: Impact of copper to the chelation effect of bovine serum albumin and spermatozoa motility parameters in vitro. *Contemporary Agriculture Savremena poljoprivreda*, 61(1/2)15-28, 2012c.

LANDERS, J.W., ZAK, B.: Determination of serum copper and iron in a single small sample. *Am. J. Clin. Pathol.*, 29(5):590-592, 1958.

LEONHARD-MAREK, S.: Why do trace elements have an influence on fertility? Tier-



arztliche Praxis Ausgabe Grobtiere Nutztiere, 28:60-65, 2000.

LU, L., WANG, CH., GAO, X., XU, P., WANG, J., WANG, Q., CHENG, J., XIAO, H.: Effects of copper on T-Type Ca<sup>2+</sup> channels in mouse spermatogenic cells. *J. Membrane Biol.*, 227:87-94, 2009.

LUKAC, N., MASSANYI, P., KROCKOVA, J., NAD, P., SLAMECKA, J., ONDRUSKA, L., FORMICKI, G., TRANDZIK, J.: Relationship between trace element concentrations and spermatozoa quality in rabbit semen. *Slovak J. Anim. Sci.*, 42(1):46-50, 2009.

MACHAL, L., CHLADEK, G., STRAKOVA, E.: Copper, phosphorus and calcium in bovine blood and seminal plasma in relation to semen quality. *J. Anim. Feed Sci.*, 11:425-435, 2002.

MANDAL, A., BHATTACHARYYA, A.K.: Differences in osmolality, pH, buffering capacity, superoxide dismutase and maintenance of sperm motility in human ejaculates according to the degree of coagulation. *Int J Androl.* 11:45-51, 1987.

MANU, T.: Secretary function of the prostate, seminal vesicle and other male accessory organs of reproduction. *Journal of Reproduction and Fertility*, 37:179-188, 1974.

MARZEC-WRÓBLEWSKA, U., KAMIŃSKI, P., ŁAKOTA, P.: Influence of chemical elements on mammalian spermatozoa. *Folia Biologica*, 58:7-15, 2012.

MASSANYI, P., TRANDZIK, J., NAD, P., SKALICKA, M., KORENEKOVA, B., LUKAC, N., FABIS, M., TOMAN, R.: Seminal concentration of trace elements in fox and relationships to spermatozoa quality. *J. Environ. Sci. Health Part A*, 40:1097-1105, 2005.

MASSANYI, P., TRANDZIK, J., NAD, P., KORENEKOVA, B., SKALICKA, M., TOMAN, R., LUKAC, N., HALO, M., STRAPAK, P.: Concentration of copper, iron, zinc, cadmium, lead, and nickel in bull and ram semen and relation to the occurrence of pathological spermatozoa. *J. Environ. Sci. Health Part A*, 39:3005–3014, 2004.

MASSANYI, P., TRANDZIK, J., NAD, P., TOMAN, R., SKALICKA, M., KORENEKOVA, B.: Seminal concentrations of trace elements in various animals and their correlations. *Asian J. Androl.*, 5(2) 101-104, 2003a.

MASSANYI, P., TRANDZIK, J., NAD, P., KORENEKOVA, B., SKALICKA, M., TOMAN, R., LUKAC, N., STRAPAK, P., HALO, M., TURCAN, J.: Concentration of copper, iron, zinc, cadmium, lead and nickel in boar semen and relation to the spermatozoa quality. *J. Environ. Sci. Health Part A*, 38: 2643–2651, 2003b.

MAXWELL, W.M.C., WELCH, G.R., JOHNSON, L.A.: Viability and membrane integrity of spermatozoa after dilution and flow cytometric sorting in the presence or absence of seminal plasma. *Reprod Fertil Dev*, 8:1165-1178, 1996.

MEACHAM, R.: Perspectives and editorials: from Androlog. *J Androl.*, 23:330-331, 2002.

MEEKER, J.D., ROSSANO, M.G., PROTAS, B., DIAMOND, M.P., PUSCHECK, E., DALY, D., PANETH, N., WIRTH, J.J.: Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. *Environ. Health Perspect.*, 116:1473-1479, 2008.

OWEN, D.H., KATZ, D.F.: A review of the physical and chemical properties of human semen and the formulation of a semen stimulant. *J Androl*, 26:459-469, 2005.

PESCH, S., BERGMANN, M., BOSTEDT, H.: Determination of some enzymes and macro- and microelements in stallion seminal plasma and their correlations to semen quality. *Theriogenology*, 66:307-313, 2006.

REBRELO, L., GUADARRAMA, A., LOPEZ, T., ZEGERS, H.F.: Effect of Cu ion on the motility, viability, acrosome reaction and fertilizing capacity of human spermatozoa

in vitro. *Reprod. Fertil. Dev.*, 8:871-874, 1996.

ROYCHOUDHURY, S., MASSANYI, P., BULLA, J., CHOUDHURY, M.D., STRAKA, L., LUKAC, N., FORMICKI, G., DANKOVA, M., BARDOS, L.: In vitro copper toxicity on rabbit spermatozoa motility, morphology and cell membrane integrity. *J Environ. Sci. Health Part A*, 45:1482-1491, 2010.

ROYCHOUDHURY, S., MASSANYI, P.: In vitro copper inhibition of rabbit spermatozoa motility. *J. Environ. Sci. Health Part A*, 43:658-663, 2008.

ROYCHOUDHURY, S., SLIVKOVA, J., BULLA, J., MASSANYI, P.: Copper administration alters fine parameters of spermatozoa motility in vitro. *Folia Vet.*, 52:64-68, 2008.

SAKHAEI, E., EMADI, L., ABSHENAS, J., KHEIRANDISH, R., AZARI, O., AMIRI, E.: Evaluation of epididymal sperm quality following experimentally induced copper poisoning in male rats. *Andrologia*, 44(1)1-7, 2011.

SEMCZUK, M., KURPISZ, M.: *The Andrology*. Wyd. Lek. PZWL, Warszawa. 2006 (in Polish).

SHINOHARA, A., CHIBA, M., TAKEUCHI, H., KINOSHITA, K., INABA, Y.: Trace elements and sperm parameters in semen of male partners of infertile couples. *Nippon Eiseigaku Zasshi*, 60:418-425, 2005.

SKANDHAN, K.P., MAZUMDAR, B.N.: Semen copper in normal and infertile subjects. *Experientia*, 35:877-878, 1979.

SKANDHAN, K.P.: Review on copper in male reproduction and contraception. *Rev. Fr. Gynecol. Obstet.*, 87(12)594-608, 1992.

STANWELL-SMITH, R., THOMSON, S.G., HAINES, A.P.: A comparative study of zinc, copper, cadmium, and lead levels in fertile and infertile men. *Fertil Steril*, 40:670-677, 1983.

STOHS, S.T., BAGCHI, D.: Oxidative mechanisms in the toxicity of metals. *Free Radic Biol Med*, 18:321-326, 1995.

TANG, S.H.: Study of interaction between gelatin and copper (II) using synchronous fluorescence spectroscopy. *J. Photographic. Sci. Photochem.*, 23:102-107, 2005.

TVRDÁ, E., KŇAŽICKÁ, Z., LUKÁČOVÁ, J., SCHNEIDGENOVÁ, M. – MASSANYI, P. – GOC, Z. – STAWARZ, R. – LUKÁČ, N.: Relationships between iron and copper content, motility characteristics and antioxidant status in bovine seminal plasma. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(2)536-547, 2012.

UMEYAMA, T., ISHIKAWA, H., TAKESHIMA, H.: A comparative study of seminal trace elements in fertile and infertile men. *Fertil Steril*, 46:494-499, 1986.

VALSA, J., GUSANI, P.H., SKANDHAN, K.P., MODI, H.T.: Copper in split and daily ejaculates. *The Journal of Reproductive Medicine*, 39:725-728, 1994.

VAN NIEKERK, F.E., VAN NIEKERK, CH.: The influence of experimentally induced copper deficiency on the fertility of rams. Semen parameters and peripheral plasma androgen concentration. *J. S Afr. Vet. Assoc.*, 60:28-31, 1989.

VELAZQUEZ, A., PEDRON, N., DELGADO, N.M., ROSADO, A.: Osmolality and conductance of normal and abnormal human seminal plasma. *Int J Fertil.*, 22:92-97, 1977.

WONG, W.Y., FLIK, G., GROENEN, P.M., SWINKELS, D.M., THOMAS, C.M., COPIUS-PEEREBOOM, J.H., MERKUS, H.M., STEEGERS-THEUNISSEN, R.P.: The impact of calcium, magnesium, zinc and copper in blood and seminal plasma on semen parameters in men. *Reproductive Toxicology*, 15:131-136, 2001.

XU, Y., XIAO, F.L., XU, N., QIAN, S.Z.: Effect of intra-epididymal injection of copper particles on fertility, spermatogenesis and tissue copper levels in rats. Int. J. Androl., 8(2)168-174, 1985.

YUYAN, L., JUNQING, W., WEI, Y., WEIJIN, Z., ERSHENG, G.: Are serum zinc and copper levels related to semen quality? Fertil. Steril., 89:1008–1011, 2007.

## ODREĐIVANJE KONCENTRACIJE BAKRA U SPERMI I NJEGOV EFEKT NA POKRETLJIVOST SPERMATOZOIDA

ZUZANA KŇAŽICKÁ, JANA LUKÁČOVÁ, AGNIESZKA GREŃ,  
GRZEGORZ FORMICKI, PETER MASSÁNYI, NORBERT LUKÁČ

### Izvod

Cilj ovog istraživanja je bio da se, *in vitro*, odredi sadržaj Bakra (Cu) u sedimentu frakciju ćelija i semene plazme i uporedit ovaj odnos sa osnovnim karakteristikama motiliteta spermatozoida i, drugo, proširi znanje o njegovom uticaju na oplodni potencijala spermatozoida. Uzorci semen su sakupljeni od 12 priplodnih bikova. Analiza motilitet je izveden korišćenjem računarskog sistema Assisted Semen Analiza (CASA). Srednja vrednost za procenat pokretnih spermatozoida (MOT) je  $91.24 \pm 5.17$  %, apogresivna pokretljivost (PROG)  $88,58 \pm 5,85$  %. Nakon toga, uzorci su centrifugirani da se odvoji frakcija spermatozoida i frakcija plazme. Koncentracije CU u plazmi je analizirani pomoću UV/VIS spektrofotometrije. Ukupna Cu koncentracija u semenoj plazmi bila je  $0.23 \text{ ug / ml}$ . Analiza plamena atomske apsorpcione spektrofotometrije (FAAS) pokazala je da prosečna koncentracija Cu u ćelijama sedimenta iznosi  $0.0014 \text{ ug/mL}$ . Ustanovljena je umerena pozitivna korelacija između MOT i koncentracije Cu u semenoj plazmi (  $RP = 0,504$  ;  $p > 0,05$  ), kao i između PROG i sadržaja Cu u semenoj plazmi (  $RP = 0,410$  ;  $p > 0,05$  ). Prisustvo Cu u ćelijama sedimenta, pozitivno je uticao na MOT (  $rp = 0,265$  ) i PROG (  $rp = 0,227$  ), ali pve razlike nisu statistički značajne (  $p > 0,05$  ). Rezultati ove studije jasno pokazuju da je Cu važan za očuvanje motiliteta spermatozoida. Na osnovu dobijenih rezultata, može se zaključiti da je procena ukupnog sadržaja Cu u celom ejakulatu, važan faktor za određivanje stepena oplodnog potencijal spermatozoida.

**Ključne reči:** bakar, spermatozoidi bika, parametri sperme, ćelijski sediment, semena plazma, parametri pokretljivosti.

Received / Primljen: 07.12.2013.

Accepted / Prihvaćen: 27.12.2013.

## INDUCTION OF FEATHERS ON ONE-YEAR-OLD APPLE TREES CULTIVAR GOLDEN DELICIOUS USING BENZYLADENINE AND GIBBERELINS 4+7

MARKO DORIĆ, ZORAN KESEROVIĆ, NENAD MAGAZIN,  
BISERKA MILIĆ, MAJA MIODRAGOVIĆ<sup>1</sup>

*SUMMARY: In the production of one-year apple trees some cultivars exhibit poor feathering. The Golden Delicious cultivar has a relatively good tendency to grow feathers but under standard technologies in the production of unbranched trees it forms very few feathers at 62-64 cm above ground. Phytohormones BA and BA+GA<sub>4+7</sub> significantly affected the formation of feathers on apple trees. In this research the solutions of BA and BA+GA<sub>4+7</sub> in the concentrations from 200 to 450 µl/l of active ingredient BA were applied three times at 7-day intervals during vegetation. At the end of vegetation, the following parameters were measured: the total number of feathers, the total feather length, the mean feather length and tree height. Increasing concentrations of BA and BA+ GA<sub>4+7</sub> to a certain limit leads to an increase in the number and total length of feathers. The Golden Delicious cultivar tends to form feathers of different lengths in a nursery. A significant variation was found in the mean feather length with different treatments. The application of BA phytohormone in higher concentrations can negatively affect the tree height.*

**Key words:** feathers, benzyladenine, gibberellins, one-year-old trees, nursery, Golden Delicious.

### INTRODUCTION

For a high-quality tree, the presence of a good number of feathers is desirable because they form flower buds in the second year of nursery production and enable the tree to bear fruit in the first year (Sadowski et al., 2007). Also, feathered trees enable an earlier formation of a canopy structure. Apple cultivars vary greatly in their tendency

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Marko Dorić, MSc, research assistant, Zoran Keserović, PhD, Full Professor, Nenad Magazin, PhD, Assistant Professor, Biserka Milić, MSc, Teaching Assistant, Maja Miodragović, MSc, PhD student, University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia.

Corresponding author: Marko Dorić, e-mail: doric@polj.uns.ac.rs

to form feathers in the nursery (Wertheim and Webster, 2003). In the production of one-year-old apple trees, some cultivars exhibit poor feathering (Volz et al. 1994; Hrotko et al. 2000; Elfving and Visser, 2006; Sazo and Robinson, 2011). Important factors in feathering are apical dominance (Cline 1991; Volz et al. 1994; Sazo and Robinson, 2011), nutrient availability (Tromp 1996) and ecological conditions (Tromp 1996; Tromp and Boertjes, 1996). Apical dominance is a term referring to the control that the terminal bud exerts over the development of lateral buds (Cline, 1997). This dominance establishes certain branching patterns characteristic of each woody plant species (Wilson, 2000). There are several hypotheses which try to explain the mechanism of apical dominance: the hormonal hypothesis, the photosynthetic hypothesis, and the hypothesis of water and mineral nutrient transport (Wilson, 2000). The hormonal hypothesis stresses the importance of auxins in the apical growth dominance and the importance of cytokinins in overcoming apical dominance (Cline, 1991; Wang et al., 1994; Cook et al., 2001). With apples, benzyladenine (BA) and the combination of BA and gibberellins 4+7 ( $GA_{4+7}$ ) play an important role in overcoming apical dominance and in the production of well-feathered apple nursery trees (Volz et al., 1994; Hrotko et al., 2000; Rossi et al., 2004; Elfving and Visser 2006; Sazo and Robinson, 2011; Dorić et al., 2013b).

The Golden Delicious and its mutants are still one of the most important apple cultivars in Europe (WAPA, 2012). The objective of this paper is to determine the effects of BA and BA +  $GA_{4+7}$  on feather formation on one-year-old Golden Delicious apple trees in a nursery with standard production methods of one-year-old unbranched trees.

## MATERIAL AND METHODS

The experiment was carried out in 2011 and 2012 in a commercial nursery with one-year-old Golden Delicious apple trees on M9 T337 rootstock. The nursery used in the experiment is situated in Kanjiža.

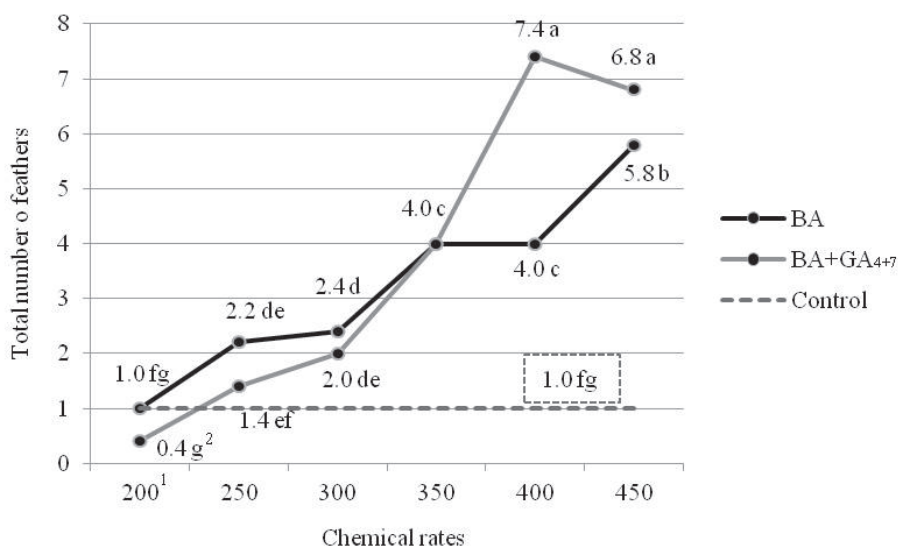
The experiment was set up using a randomized block trial with five replications, where each replication is represented with two plants (total of 10 per treatment). The rootstocks were planted in March, at a 1.1 x 0.1 m distance. In August, during the first vegetation, the plants were grafted by chip budding at 20 cm above the ground. At the time of the second vegetation, during the growth of the main shoot, all feathers below 62-64 cm were removed. During the second vegetation, the apical section of the main shoot was sprayed. Spraying was done with a hand sprayer. Three spray treatments were performed at 7-day intervals. The first treatment was applied in mid-June, when the growth of the main shoot was around 78-80 cm. Irrigation was carried out as needed by utilizing a sprinkler system.

The following treatments were used: Gerba 4 LG, containing 4% BA and Progerbalin LG, containing 1.8% BA and 1.8%  $GA_{4+7}$  ("L-Gobbi", Italy). The applied concentrations were 450, 400, 350, 300, 250 and 200  $\mu\text{l/l}$  of active ingredient (a.i.) BA. To each treatment a surfactant, Trend® 90 ("Du-Pont", USA), was added at the rate of 500  $\mu\text{l/l}$ .

At the end of vegetation, the following parameters were measured: the total number of feathers, the total feather length, the mean feather length and tree height. The data were statistically processed by the analysis of variance (ANOVA) and mean values were compared with Duncan's multiple range test ( $P < 0.05$ ) with Statistica 12 (StatSoft Inc., Tulsa, USA).

## RESULTS AND DISCUSSION

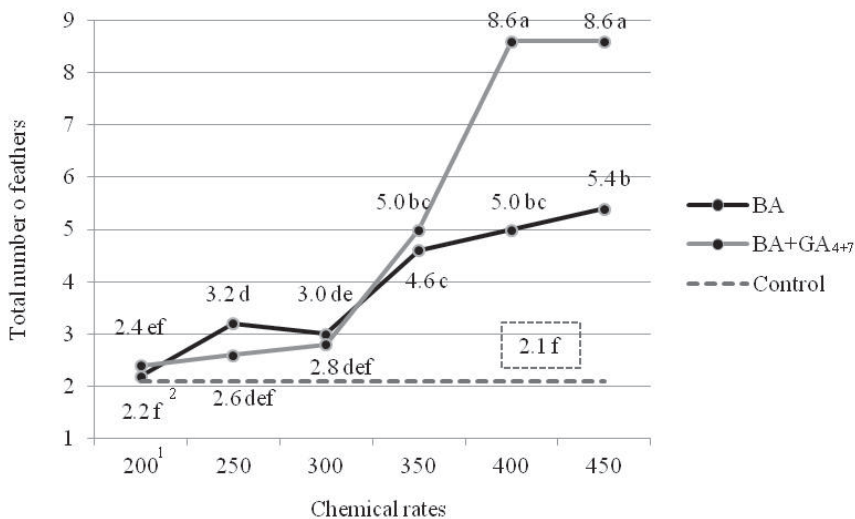
Graphs 1 and 2 present the effects of BA and BA+GA<sub>4+7</sub> on the total number of feathery branches. On the basis of these results, it is evident that in both experimental years the control had very few feathery branches. In 2011 the control had 1 feathery branch per tree on average, and in 2012 it had 2.1. These trees have practically no feathery branches because such a small number of feathery branches (fewer than three) have to be removed at planting in order to avoid the formation of an unbalanced tree in the orchard (Palmer et al., 2011). The treatments with BA and BA+GA<sub>4+7</sub> significantly affected the formation of feathery branches. The total number of feathery branches increased with an increase in the concentration of BA and BA+GA<sub>4+7</sub>. In 2011, the highest number of feathery branches was obtained with BA+GA<sub>4+7</sub> 400 (7.4) treatment while in 2012 it was obtained with BA+GA<sub>4+7</sub> 450 and 400 (8.6) treatment. Other studies show that increasing concentrations of BA and BA+ GA<sub>4+7</sub> to a certain limit leads to an increase in the number of feathery branches (Rossi et al., 2004; Jacyna and Barnard, 2008).



Graph 1. Effects of BA and BA+GA<sub>4+7</sub> applications on total number of feathery branches in Golden Delicious one-year-old apple trees (year 2011)

<sup>1</sup> Number indicates concentration of active ingredient BA µl/l

<sup>2</sup> Means with different letters are significantly different (P<0.05)



Graph 2. Effects of BA and BA+GA<sub>4+7</sub> applications on total number of feathers in Golden Delicious one-year-old apple trees (year 2012)

<sup>1</sup> Number indicates concentration of active ingredient BA μl/l

<sup>2</sup> Means with different letters are significantly different (P<0.05)

The results show that in both years the BA+GA<sub>4+7</sub> treatments, at higher concentrations, were more effective than BA treatments when the total feather numbers are compared. Benzyladenine is responsible for overcoming apical dominance and enhancing feathering (Müller and Layser, 2011), while the main role of GA is to elongate feathers (Volz et al., 1994; Hrotko et al., 2000). Also, when applied on its own, GA is capable of enhancing feathering in pear trees (Palmer et al., 2011). The results show positive effects of GA<sub>4+7</sub> in combination with BA on the total number of feathers in treatments BA+GA<sub>4+7</sub> 400 and 450.

The Golden Delicious has a good branching potential but it is characterized by feathers of varying lengths (Cvetković et al., 2010). In standard technologies in the production of unbranched trees, which is still widespread in Serbian nurseries, this cultivar forms a small number of feathers if untreated with phytohormones. An important factor which affects feather formation is agroecological conditions (Tromp, 1996; Tromp and Boertjes, 1996). Of great importance in the production of feathered trees is also the production method, primarily nutrition and irrigation. In tree production in Serbia irrigation is performed only when absolutely necessary, in order to avoid drought effects. In the production of feathered trees, however, it is necessary to irrigate the plants more frequently than in the production of unfeathered trees because feathered trees have a higher number of shoots and more leaf area and consequently higher demands for water if an adequate growth rate is to be maintained. Maintaining an adequate growth rate of the main shoot is essential since feathering in apples mainly occurs when the growth rate of the main shoot is the highest (Tromp, 1996).

In 2012, the research results were significantly better than in 2011. A possible reason for this may lie in more favourable environmental conditions in 2012. Soil tempera-

ture, air humidity and air temperature are important factors affecting feather formation and growth on an apple tree (Tromp 1996; Tromp and Boertjes, 1996). The second year, 2012, was warmer than 2011, which possibly facilitated a better growth and development of the trees. Average air temperatures in June, July and August 2011 were 21.4, 22.2 and 23.3 °C, respectively, and in 2012 they were 22.3, 25.0 and 23.9 °C (RHSS 2012). Another important factor in the variable quality of plants is the soil. In order to avoid apple tree diseases, nurserymen often rent new land so different soil types may have an effect too (Palmer et al., 2011).

Tables 1 and 2 contain the following parameters: feather length, tree height and trunk diameter. A parameter that is also important in determining the quality of the trees is the feather length. Sadowski et al. (2007) noted that there is a relationship between the length of laterals and productivity of trees in the orchard. The highest values for the total feather length were recorded in 2011 with BA+GA<sub>4+7</sub> 450 (172.6 cm) and 400 (164.3 cm) treatments and in 2012 with BA+GA<sub>4+7</sub> 450 (199.6 cm) treatment. Based on the results of the research, it is safe to conclude that increasing BA and BA+GA<sub>4+7</sub> concentrations leads to an increase in the total feather length.

Table 1. Effects of BA and BA+GA<sub>4+7</sub> applications on feather length and tree height in Golden Delicious one-year-old trees (year 2011)

Treatment	Total feather length (cm)	Average feather length (cm)	Tree height (cm)
BA+GA <sub>4+7</sub> 450 <sup>1</sup>	172.6a <sup>2</sup>	25.8bcd	143.2d
BA+GA <sub>4+7</sub> 400	164.3a	22.2cd	151.0cd
BA+GA <sub>4+7</sub> 350	99.0c	25.8bcd	158.5abc
BA+GA <sub>4+7</sub> 300	40.8e	20.4cd	155.0abc
BA+GA <sub>4+7</sub> 250	23.4e	17.9de	159.6ab
BA+GA <sub>4+7</sub> 200	32.5e	32.5ab	155.8abc
BA 450	137.3b	23.6cd	159.4ab
BA 400	105.8c	26.0bcd	157.0abc
BA 350	99.2c	27.1bc	153.3bc
BA 300	41.2e	18.8cde	159.0ab
BA 250	71.5d	35.0a	153.8abc
BA 200	18.0e	11.2e	154.7abc
Control	25.0e	19.9cd	161.4a
F test	*	*	*

<sup>1</sup> Number indicates concentration of active ingredient BA µl/l

<sup>2</sup> Means with different letters are significantly different (P<0.05)



Table 2. Effects of BA and BA+GA<sub>4+7</sub> applications on feather length and tree height in Golden Delicious one-year-old trees (year 2012)

Treatment	Total feather length (cm)	Average feather length (cm)	Tree height (cm)
BA+GA <sub>4+7</sub> 450 <sup>1</sup>	199.6a <sup>2</sup>	23.1ab	145.7e
BA+GA <sub>4+7</sub> 400	167.7b	19.4b	151.3d
BA+GA <sub>4+7</sub> 350	129.5cd	19.1b	159.2b
BA+GA <sub>4+7</sub> 300	73.0e	25.7a	155.0bcd
BA+GA <sub>4+7</sub> 250	62.1e	24.0ab	157.1bc
BA+GA <sub>4+7</sub> 200	50.6ef	22.1ab	155.8bc
BA 450	132.4c	24.5a	144.9e
BA 400	126.8cd	25.4a	145.9e
BA 350	109.0d	23.4ab	154.3cd
BA 300	73.0e	24.5a	158.0bc
BA 250	70.2e	22.8ab	157.2bc
BA 200	32.2fg	12.8c	159.0b
Control	23.9g	12.3c	169.6a
F-test	*	*	*

<sup>1</sup> Number indicates concentration of active ingredient BA µl/l

<sup>2</sup> Means with different letters are significantly different (P<0.05)

Mean feather lengths varied significantly in both years. In 2011 they ranged from 11.2 to 35.0 cm and in 2012 from 12.3 to 25.7 cm. The highest values of the mean feather length parameter were obtained in 2011 with BA 250 (35.0 cm) and in 2012 with BA+GA<sub>4+7</sub> 300 (25.7 cm), BA 400 (25.4 cm), BA 450 (24.5 cm) and BA 300 (24.5 cm). In the course of the 2011 research it was observed that some trees treated with BA+GA<sub>4+7</sub> 200 and BA 250, which had a relatively small number of feathers (fewer than 4), formed feathers of greater lengths, even longer than 50 cm. The trees treated with BA+GA<sub>4+7</sub> 200 and BA 250 in 2011 formed longer feathers probably because the nutrients were used for the growth of a smaller number of shoots. As the main shoot and feathers are in competition for water and nutrients (Cline, 1991), an increase in the number of feathers enables variation in mean feather length (Dorić et al., 2013a).

The highest values of the tree height parameter in both research years were obtained with the control (161.4; 169.6 cm). A negative effect of phytohormones was observed in 2011 with BA+GA<sub>4+7</sub> 450 (143.2 cm) treatment and in 2012 with BA+GA<sub>4+7</sub> 450 (145.7 cm) and BA 450 (144.9 cm) and 400 (145.9 cm) treatment. With the other treatments the trees were of satisfactory height. The application of BA affects the flow of auxins (Müller and Layser, 2011) and impedes the growth of the main shoot for a short time (Sazo and Robinson, 2011), which in some cases can lead to a significant decrease of tree height (Hrotko et al., 2000; Sazo and Robinson, 2011). When planting apple orchards it is necessary for the trees to be of an adequate height (150 cm at least) in order to achieve a good position of the lowest tier of branches in the nursery and to enable the

formation of the second tier of branches in the orchard. In the present research we found that the treated trees were lower than the control.

## CONCLUSION

One-year-old Golden Delicious apple trees untreated with phytohormones formed a small number of feathers at 62-64 cm above ground in a standard nursery production method for one-year-old unbranched trees. Phytohormones BA and BA+GA<sub>4+7</sub> significantly affected the formation of feathers. Increasing concentrations of BA and BA+GA<sub>4+7</sub> to a certain limit leads to an increase in the number and total length of feathers. In agroecological conditions prevalent in Serbia, and with standard planting technologies, it is necessary to use BA and BA+GA<sub>4+7</sub> in concentrations of 400 µl/l a.m. BA so that a sufficient number of feathers is formed. If used in higher concentrations, the result may be trees shorter than 150 cm due to a negative effect of BA. A possible method of obtaining an adequate number of feathers with the application of BA and BA+GA<sub>4+7</sub> while neutralizing their negative effect on tree height is intensive irrigation accompanied with the provision of nutrients during the period of intensive growth of the main shoot, which should maintain an adequate growth rate.

## ACKNOWLEDGEMENTS

This research was supported and funded by the Provincial secretariat for science and technological development of Autonomous Province of Vojvodina, Republic of Serbia as one of the research topics in the project “Using bioregulators in apple production”, number 114-451-3765/2013-01.

## REFERENCES

- CLINE, M.G.: Apical dominance. *Botanical review*, 57:318-358, 1991.
- CLINE, M.G.: Concepts and Terminology of Apical Dominance. *American Journal of Botany*, 84(9): 1064-1069, 1997.
- COOK, N.C., Bellstedt, D.U., Jacobs, G.: Endogenous cytokinin distribution patterns at budburst in Granny Smith and Braeburn apple shoots in relation to bud growth. *Scientia Horticulturae*, 87:53-63, 2001.
- CVETKOVIĆ, M., MIĆIĆ, N., ĐURIĆ, G., STANKOVIĆ, B.: Karakteristike i obrasci grananja sadnica jabuke proizvedenih okuliranjem. Zbornik naučnih radova XXV savetovanja – Unapređenje proizvodnje voća i grožđa. Institut PKB Agroekonomik, Beograd, 16(5) 69-77, 2010.
- DORIĆ, M., KESEROVIĆ, Z., MAGAZIN, N., MILIĆ, B.: Formiranje prevremenih grančica kod jednogodišnjih sadnica trešnje. *Voćarstvo*, 47(181-182)47-53, 2013a.
- DORIĆ, M., KESEROVIĆ, Z., MAGAZIN, N., MILIĆ, B.: Formiranje prevremenih grančica na jednogodišnjim sadnica jabuke sorte Idared primenom BA i GA<sub>4+7</sub>. *Voćarstvo*, 47(183-184) 87-93, 2013b.
- ELFVING, D., VISSER, D.: Timing cyclanilide and cytokinin applications in the nursery to obtain desired lateral branch height in apple and sweet cherry trees. *HortScience*, 41: 1238-1242, 2006.

HROTKO, K., MAGYAR, L., RONAY, Z.: Improved feathering on apple nursery trees by BA application. *Acta Horticulturae*, 514:113-119, 2000.

JACYNA T., BARNARD J.: Modification of branching behavior in apical-dominant apple trees with plant growth regulators and their residual effects on tree growth after transplanting. *Journal of the American Pomological Society*, 62(4)160-172, 2008.

MÜLLER, D., LEYSER, O.: Auxin, cytokinin and the control of shoot branching. *Annals of Botany*, 107(7)1203-1212, 2011.

PALMER, J.W., SEYMOUR, S.M., DIACK, R.: Feathering of 'Doyenné du Comice' pear in the nursery using repeat sprays of benzyladenine and gibberellins. *Scientia Horticulturae*, 130:393-397, 2011.

ROSSI, A.D., RUFATO, L., GIACOBBO, C.L., GOME, F.R.C., FACHINELLO, J.C.: Use of promalin® on one-year old trees of the apple cv. 'Catarina'. XXVI International Horticultural Congress: Key Processes in the Growth and Cropping of Deciduous Fruit and Nut Trees. *Acta Horticulturae*, 636:145-149, 2004.

SADOWSKI, A., MACKIEWCZ, M., DZIUBAN, R.: Growth and early bearing of apple trees as affected by the type of nursery trees used for planting. *Acta Horticulturae*, 732:447-455, 2007.

SAZO, M., ROBINSON, T.: The use of plant growth regulators for branching of nursery trees in NY state. *New York Fruit Quarterly*, 19:5-9, 2011.

THE WORLD APPLE AND PEAR ASSOCIATION (WAPA), European apple and pear crop forecast 2012 – summary. <http://www.wapa-association.org>, 2012.

TROMP, J., BOERTJES, B.C.: The effect of air temperature in successive periods of the growing season on sylleptic shoot formation in young apple trees *Plant Growth Regulation*, 19:177-182, 1996.

TROMP, J.: Sylleptic Shoot Formation in Young Apple Trees Exposed to Various Soil Temperature and Air Humidity Regimes in Three Successive Periods of the Growing Season. *Annals of Botany*, 77:63-70, 1996.

VOLZ, R.K., GIBBS, H.M., POPENOE, J.: Branch induction on apple nursery trees: Effects of growth regulators and defoliation. *New Zealand Journal of Crop and Horticultural Science*, 22:277-283, 1994.

WANG, S.Y., FAUST, M., LINE, M.J.: Apical Dominance in Apple (*Malus domestica* Borkh): The Possible Role of Indole-3-Acetic Acid (IAA). *Journal of the American Society for Horticultural Science*, 119:1215-1221, 1994.

WERTHEIM, S.J., WEBSTER, A.D.: Propagation and nursery tree quality. In: *Apples : botany, production, and uses* (D.C. Ferree and I.J. Warrington, eds.). CAB International, Wallingford, pp.125-151, 2003.

WILSON, B.: Apical control of branch growth and angle in woody plants. *American Journal of Botany*, 87:601-607, 2000.

# FORMIRANJE PREVREMENIH GRANČICA KOD JEDNOGODIŠNJI SADNICA JABUKE SORTE ZLATNI DELIŠES PRIMENOM BENZILADENINA I GIBERELINA 4+7

MARKO DORIĆ, ZORAN KESEROVIĆ, NENAD MAGAZIN,  
BISERKA MILIĆ, MAJA MIODRAGOVIĆ

## Izvod

U proizvodnji jednogodišnjih sadnica jabuke kod nekih sorti je otežano formiranje prevremenih grančica. Sorta Zlatni Delišeš ima relativno dobru sklonost ka formiranju prevremenih grančica, ali pri standardnoj tehnologiji gajenja koja se primenjuje kod proizvodnje sadnica bez prevremenih grančica veoma slabo obrazuje bočne poraste na 62-64 cm visine od zemlje. Fitohormoni BA i BA+GA<sub>4+7</sub> značajno utiču na formiranje prevremenih grančica kod sadnica jabuke. U ovom istraživanju korišćeni su preparati na bazi BA i BA+GA<sub>4+7</sub> u koncentracijama od 200 do 450 µl l<sup>-1</sup> aktivne materija BA tri puta tokom vegetacije u razmaku od 7 dana. Na kraju vegetacije mereni su parametri: ukupan broj prevremenih grančica, ukupna dužina prevremenih grančica, prosečna dužina prevremenih grančica i visina sadnice. Povećavanjem koncentracije BA i BA+GA<sub>4+7</sub> do određene granice povećava se i broj i ukupna dužina prevremenih grančica. Sorta Zlatni Delišeš ima sklonost ka formiranju prevremenih grančica različite dužine u rasadniku. U tretmanima je zabeleženo značajno variranje parametra prosečna dužina prevremenih grančica. Primena fitohormona BA može negativno da utiče na visinu sadnice pri primeni viših koncentracija.

**Ključne reči:** prevremene grančice, benziladenin, giberelini, jednogodišnje sadnice, rasadnik, Zlatni Delišeš.

Received / Primljen: 21.11.2013.

Accepted / Prihvaćen: 17.12.2013.

## PARENCHYMAL LEUKOCYTE INFILTRATION, SOMATIC CELL COUNT AND DUCTUS PAPILLARIS LENGTH IN DAIRY COWS UDDER

IVANA DAVIDOV, MIODRAG RADINOVIĆ, MIHAJLO ERDELJAN, ZORANA  
KOVAČEVIĆ, ŽELJKA JURAKIĆ, ALEKSANDAR BOŽIĆ<sup>1</sup>

*SUMMARY: Parenchyma leukocyte infiltration is characterized by physical, chemical and bacteriological changes in the glandular tissue of the udder. Also that have affect on the quality and quantity of milk, too. Somatic cell count is a useful predictor of intramammary infection. Ductus papillaris is first defense line of udder. The aim of experiment was to determine relation between ductus papillaris length, somatic cell count and leukocyte infiltration in mammary gland parenchyma. During three years, 26 dairy cows were excluded from production and send to slaughterhouse. A week before sending cows to slaughterhouse, milk samples were took to determine somatic cell count. Udders from slaughtered cows were taken for measuring ductus papillaris length and for histopathology. From 104 mammary glands, 36 (52,94%) glands were with length of ductus papillaris less 5 mm, with parenchyma leukocyte infiltration between 50-75% and with milk somatic cell count above 400.000/ ml. According to statistical correlation test, show positive correlation between ductus papillaris length, leukocyte infiltration in parenchyma and somatic cell count. Also, to statistical test ANOVA, all results are less 0.05 that means there is a statistically significant difference between leukocyte infiltration and milk somatic cell count.*

**Key words:** *parenchyma, ductus papillaris, somatic cell count, dairy cow.*

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Ivana Davidov, PhD DVM, assistant professor, Miodrag Radinović, PhD DVM, assistant professor, Mihajlo Erdeljan, DVM, teaching assistant, Zorana Kovačević, MSc Pharm, teaching assistant, MSc Željka Jurakić, teaching assistant, Aleksandar Božić, Phd, professor. University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad, Serbia.

Corresponding author: Ivana Davidov, .e-mail: [ivana.davidov@polj.edu.rs](mailto:ivana.davidov@polj.edu.rs); [davidov.ivana@nsveterina.edu.rs](mailto:davidov.ivana@nsveterina.edu.rs)

## INTRODUCTION

Udder is a skin organ and belongs to tubuloalveolar type of glands (Pobrić 1990; Boboš and Vidić 2005). Glandular parenchyma was pink-gray in color, firmer consistency as opposed to fatty tissue that surrounds the gland. Parenchyma is a complex character and consists of glandular tubes with alveolar enlargement (Boboš and Vidić 2005; Sordillo and Nickerson 1988; Davidov et al. 2011). Cow teats are cylindrical and slightly inclined towards the ventrocranial. Their function is the secretion of milk into the environment, as well as suckling, which is the only way of feeding calves. Their shape and size depend on the shape and size of udder and milk production. Cow teat has only one channel -ductus papillaris with one hole-ostium papillae and it is the link between the internal system of milk secretion and the environment. Ductus papillaris is the main barrier to infection (Boboš and Vidić 2005; Davidov et al., 2011). Length of ductus papillaris and proper length of papillae varies and increases with increasing number of lactation. Most authors (Davidov et al., 2011; Sordillo and Nickerson 1988; Pobrić et al., 1998), reported that the length of the ductus papillaris is about 10 mm, but varies from 3 to 18 mm (McDonald, 1973; Hamann, 1987; Geishauser and Querengasser, 2000; Paurtud and Rasmussen, 2004; Paurtud et al., 2004), depending on the breed, and even the stage of lactation (McDonald, 1979). Ductus papillaris in last teats are 5-10% longer than the ductus papillaris on frontal teats (Paurtud et al., 2004; Pobrić, 1990). In dairy cows, udder has a simple defense system consisting of teat and udder. When pathogenic microorganisms penetrate this barrier, they are in the parenchyma of the udder and produce the toxin, leading to damage the wall, causing inflammation and tissue injury. Mc Donald (1975) argued that the length of the ductus papillaris is not associated with the emergence of new infections because udder quarters that were infected have not a longer ductus papillaris than quarters who were infected. Opposite him, Gulyas i Ivancsics (2000) suggested that all biological characteristics (udder-morphologic, pigmentation of teats and length of *ductus papillaris*) together with those influencing somatic cell count are to be taken into account in the selection aimed towards decreasing somatic cell count. Milking microorganisms located in the immediate vicinity papilla take the opportunity to penetrate the ductus papillaris, causing trauma and damage to the keratin layer or even mucosal channels (Capuco et al., 1992). Ductus papillaris may remain partially open for 1-2 hours after milking and during this period microorganisms can get in it. Pathogenic microorganisms are able to enter through an open ostium papilla, avoiding antibacterial activity (Khan and Khan, 2006). If microorganisms pass the first line of defense and penetrate into the tank, they get to the second line of defense which is consisting of polymorphonuclear leukocytes and macrophages the main phagocytic cells of the udder. Macrophages are more dominate leukocytes in uninfected and infected udder tissue (Davidov et al. 2011).

The aim of experiment was to determine relation between ductus papillaris length, somatic cell count and leukocyte infiltration in mammary gland parenchyma.

## MATERIAL AND METHOD

During three years (2010-2012), 26 dairy Holstein-Friesian cows were excluded from production and send to slaughterhouse. A week before sending cows to slaughterhouse, milk samples were took to determine somatic cell count. Udders from slaugh-

tered cows were taken for measuring ductus papillaris and for histopathology. All samples were fixed in buffered 10% formalin, and dehydrated through a series of growing concentrations of ethanol and xylol treatment infused as a medium for the introduction of paraffin wax-molding means. Made paraffin molds were cut on microtome, at a thickness of 5 µm. Stained with hematoxylin oesin and all were performed microscopic with light microscope. Histological analysis was performed on the Leica microscope. Chakly's quantitative method described by Mayer and Klein (1961) was used for assessment of the degree of impairment of the alveolar epithelium, lumen and alveolar stroma. The degree of leukocyte infiltration was determined based on the presence of inflammatory response cell in the visual field. Several neutrophil granulocytes and lymphocytes revealed leukocyte infiltration from 0% to 25%. A significant number of neutrophils with few cells of lymphocytes are leukocyte infiltrate from 25.1% to 50%. A massive infiltration of lymphocytes, a significant number of macrophages and rare eosinophils are leukocyte infiltration of 50.1% to 75%, and massive infiltration of lymphocytes and macrophages with a few plasma cells and eosinophils are leukocyte infiltration of 75.1% to 100%. Milk from all four quarters was taken before morning milking and whole milk samples were taken with milk meter for somatic cell count. When quarter milk samples were taken the teat ends were disinfected. Milk samples for somatic cell count were analyzed by the fluoro-optoelectronic method (Fossomatic; Foss Electric, Hillerod, Denmark).

For statistical analysis we used test of correlation and ANOVA test by Microsoft Excel 2007.

## RESULTS AND DISCUSSION

Comparing the ductus papillaris length, and leukocyte infiltrate in the mammary parenchyma, are shown in table 1. It is observe dominant presence leukocyte infiltrate in those complex with the mammary ductus papillaris length less 5 mm and 5 to 10 mm.

Table 1. Parenchyma leukocyte infiltrate, ductus papillaris length and somatic cell count

Length of ductus papillaris	Leukocyte infiltrate				Total
	0-25%	25,1-50%	50,1-75%	75,1-100%	
<b>≤ 5mm</b>					
No. of mammary parenchymal	5	8	36	18	68
% of mammary parenchymal	4,81	8,65	52,94	17,31	65,38
Somatic cell count	420.000	450.000	550.000	470.000	
<b>5-10 mm</b>					
No. of mammary parenchymal	3	4	12	8	28
% of mammary parenchymal	2,88	4,81	11,54	7,69	26,92
Somatic cell count	390.000	410.000	450.000	450.000	
<b>&gt; 10 mm</b>					

No. of mammary parenchymal	1	1	3	3	8
% of mammary parenchymal	0,96	0,96	2,89	2,89	7,70
Somatic cell count in milk	330.000	330.000	340.000	330.000	
Total No. of mammary parenchymal	9	13	51	29	104
Total, %	8,65	14,43	49,04	27,88	100

Ductus papillaris length and proper length of papillae varies and increases with increasing number of lactation. Most authors (Davidov et al., 2011; Sordillo and Nickerson 1988; Pobrić et al., 1998) reported that the length of the ductus papillaris is about 10 mm and it is similar to results in this study. From a total of 104 mammary gland parenchyma, in 68 (65.38%) mammary complexes with the length of the ductus papillaris less 5 mm was found mass of leukocyte infiltration and with milk somatic cell count above 400.000/ ml. Mass of leukocyte infiltration of 50.1 to 75% was present in 36 (52.94%) parenchyma shown in table 1.

Table 2. Correlation test between ductus papillaris length, milk somatic cell count and mammary gland parenchyma

	Somatic cell count	No. of parenchyma
Ductus papillaris length $\leq$ 5mm	420.000	5
	450.000	8
	550.000	36
	470.000	18
Correlation test	0.986998	
Ductus papillaris length 5-10mm	390.000	3
	410.000	4
	450.000	12
	450.000	8
Correlation test	0.904992	
Ductus papillaris length $>$ 10mm	330.000	1
	330.000	1
	340.000	3
	330.000	3
Correlation test	0.57735	

Results from statistical correlation test, show positive correlation between ductus papillaris length, leukocyte infiltration in parenchyma and somatic cell count, which indicate that ductus papillaris length has no influence on leukocyte infiltration and milk somatic cell count.



Table 3. ANOVA test between ductus papillaris length, milk somatic cell count and mammary gland parenchyma

	Somatic cell count	No. of parenchyma
Ductus papillaris length $\leq$ 5mm/	420.000	5
	450.000	8
	550.000	36
	470.000	18
ANOVA test	0.048386	
Ductus papillaris length 5-10mm	390.000	3
	410.000	4
	450.000	12
	450.000	8
ANOVA test/ ANOVA test	0.008461	
Ductus papillaris length $>$ 10mm/	330.000	1
	330.000	1
	340.000	3
	330.000	3
ANOVA test	0.038091	

According to statistical ANOVA test all results are less 0.05 and it means that there is a statistically significant difference between leukocyte infiltration and milk somatic cell count and no statistical significant in ductus papillaris length.

## CONCLUSION

Ductus papillaris length has no indicate as first line or barrier to the udder infection. Presence of different percentage leukocyte infiltration in mammary gland parenchyma has influence on somatic cell count in milk.

## REFERENCE

- BOBOŠ, S., AND VIDIĆ, B.: Mlečna žlezda preživara. Monografija, Univerzitet u Novom Sadu – Poljoprivredni fakultet, Naučni Institut za veterinarstvo “Novi Sad” – Novi Sad, Novi Sad, pp. 41-47, 2005.
- CAPUCO, A.V., BRIGHT, S.A., PANKEY, J.W., WOOD, D.L., MILLER, R.H., AND BITMAN, J.: Increased susceptibility to intramammary infection following removal of teat canal keratin. *J. Dairy Sci.*, 75:2126-2132, 1992.
- DAVIDOV, I., BOBOŠ, S., RADINOVIĆ, M., AND ERDELJAN, M.: Effect of different length Ductus Papillaris on pathomorphological changes in udder parenchyma. *Contemporary Agriculture*, 60(1-2)139-144, 2011.
- GEISHAUSER, T., AND QUERENGASSER, K.: Investigation on teat canal length in teats with milk flow disturbance. *J. Dairy Sci.*, 83:1976-1980, 2000.
- GULYÁS, L., AND IVÁNCSICS, J.: The relationship between the somatic cell count and certain udder-morphologic traits. *Állattenyésztés és Takarmányozás*, 49(4)331-339, 2000.
- HAMANN, J.: Effects of machine milking on teat end condition- a literature review.

IDF Bulletin, 215: 33-53, 1987.

KHAN, M. Z., AND KHAN, A.: Basic facts of mastitis in dairy animals. A review. Pakistan Vet. J., 26:204-208, 2006.

MAYER, G., AND KLEIN, M.: Histology and cytology of the mammary gland. Page 55 in Milk: The mammary gland and its secretion. Vol. 1 S.K. Kon and A.T. Cowie, ed. Academic Press, New York, NY, 1961.

McDONALD, J. S.: Radiographic method for anatomic study of the teat canal: characteristics related to resistance to new intramammary infection during lactation and the early dry period. Cornell Vet., 65: 492-497, 1979.

McDONALD, J.S.: Radiographic method for anatomic study of the teat canal: changes within the first lactation. American Journal of Veterinary Research, 34:169-171, 1973.

McDONALD, J.S.: Radiographic method for anatomic study of the teat canal: changes between milking periods. American Journal of Veterinary Research, 36:1241-1242, 1975.

PAURLUD, C.O., AND RASMUSSEN, M.D.: How teat canal depends on the length and diameter of the teat canal in dairy cows. Journal of Dairy Research, 71:253-255, 2004.

PAURLUD, C.O., THAMSBORG, S.M., ENEVOLDSEN, C., AND RASMUSSEN, M.D.: Effect of three different liners on heifer teat canal keratin. Journal of Dairy Research, 72: 255-257, 2004.

POBRIĆ, H.: Makro i mikromorfološka istraživanja vrha sise i stanje keratina u odnosu na hronično-kataralna oboljenja mlečne žlezde kod krava. Veterinaria Sarajevo, 39:32-38, 1990.

POBRIĆ, H., BABIĆ, LJ., FILIPOVIĆ-PANIĆIĆ, M., PODŽO, M., AND FEJZIĆ, N.: Patološka građa sisinog kanala s obzirom na debljinu keratina i njegova uloga u nastanku oboljenja vimena krava. Veterinaria Sarajevo, 47:89-101, 1998.

SORDILLO, L.M., AND NICKERSON, S.C.: Morphometric changes in the bovine mammary gland during involution and lactogenesis. Amer. J. Vet. Res., 49:1112-1116, 1988.

## **LEUKOCITNA INFILTRACIJA PARENHIMA, SOMATSKE ČELIJE MLEKA I DUŽINA DUCTUS PAPILLARIS U VIMENU MLEČNIH KRAVA**

**IVANA DAVIDOV, MIODRAG RADINOVIĆ, MIHAJLO ERDELJAN,  
ZORANA KOVAČEVIĆ, ŽELJKA JURAKIĆ, ALEKSANDAR BOŽIĆ**

### **Izvod**

Do leukocitne infiltracije parenhima vimena mlečnih krava dolazi zbog fizičkih, hemijskih i bakterioloških promene u žlezdanom tkivu. Ove promene u parenhimau vimena utiču na kvalitet i kvantitet mleka. Broj somatskih ćelija u mleku je koristan indikator intramamarnih infekcije. Ductus papillaris je prva linija odbrane vimena mlečnih krava. Cilj eksperimenta je da se utvrdi odnos između ductus papillaris-a, somatskih ćelija u mleku i broja leukocitne infiltracije u parenhimu mlečne žlezde. Tokom

tri godine, 26 krave su isključeni iz proizvodnje i poslate na ekonomsko iskorišćavanje. Nedelju dana pre slanja krava na ekonomsko iskorišćavanje, uzimani su uzorci mleka da bi se utvrdio broj somatskih ćelija u mleku. Vimena zaklanih krava su uzeta da bi se izmerila dužina ductus papillaris papila i da bi se uradila patohistološka analiza. Od 104 mlečne žlezde, 36 (52,94 %) žlezda su bile sa dužinom ductus papillaris manje od 5 mm i sa leukocitnom infiltracijom parenhima između 50-75%. Broj somatskih ćelija u mleku tih krava je bio iznad 400.000/ml. Statističkim testom korelacije, uočava se pozitivna korelacija između dužine ductus papillaris-a, infiltracije leukocita u parenhimu i broja somatskih ćelija u mleku. ANOVA testom su dobijene vrednosti manje od 0,05, koje ukazuje na statističku značajnost između leukocitne infiltracije parenhima mlečne žlezde i broja somatskih ćelija u mleku.

**Ključne reči:** parenhim, ductus papillaris, somatske ćelije, mlečne krave.

Received / *Primljen*: 04.11.2013.

Accepted / *Prihvaćen*: 18.12.2013.

**SOW FERTILITY AFTER THE INTRACERVICAL AI  
IN COOL AND WARM SEASONS USING CONVENTIONAL  
DOSES IN COMBINATION WITH SYNTHETIC  
SEMINAL PLASMA (PREDIL MR-A®)\***

IVAN STANČIĆ, IVAN RADOVIĆ, MIHAJLO ERDELJAN, BLAGOJE STANČIĆ,  
TEODORA VASILJEVIĆ, ALEKSANDAR BOŽIĆ, IVAN ŽARKOVIĆ<sup>1</sup>

*SUMMARY: A significant reduction in the boar sperm quality during the warmer season of the year is a well-known phenomenon which directly contributes to a considerable decrease in the fertility of sows artificially inseminated (AI) during the period. The purpose of this study is to determine whether the conventional intracervical AI combined with the synthetic seminal plasma Predil MR-A® can increase the fertility of sows inseminated in warm seasons. The obtained results show that the insemination with Predil MR-A® significantly increases the farrowing rate (82%) in the warm periods of the year in comparison with the control sows (72%). Although an increasing trend in the average number of live born piglets per litter was recorded in the sows inseminated with Predil-MRA®, this increase was not statistically significant ( $p < 0.05$ ) both within or between the observed seasons (ranging from 14.65 to 15.41 piglets per litter). The obtained results can increase the total efficiency of boar reproductive exploitation as well as the fertility of inseminated sows.*

**Key words:** artificial insemination, season, seminal plasma, Predil MR-A®, fertility, sow.

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Ivan Stančić, DVM, PhD, Assistant Professor, Ivan Radović, PhD, Associated Professor, Mihajlo Erdeljan, DVM, MS, Teaching Assistant, Blagoje Stančić, PhD, Full Professor, Aleksandar Božić, PhD, Full Professor and Ivan Žarković, PhD student, University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad, Serbia. Teodora Vasiljević, dipl. vet. spec., Napredak, a.d., Golubinački put bb, Stara Pazova, Serbia.

Corresponding author: Ivan Stančić, e-mail: dr.ivan.stancic@gmail.com, phone: +381 21 485-3496.

\* The paper is a part of the research work on the project "Increasing of boar reproductive efficiency at farms of AP Vojvodina", file No. 114-451-1311/2013-04, financed by the Provincial Secretariat for Science and Technological Development (AP Vojvodina, Serbia).

## INTRODUCTION

Sperm transport from the site of deposition (cervix, natural mating or conventional artificial insemination) through the uterine horns up to the utero-tubal junction, which serve as a sperm reservoir (Hunter, 1981), is believed to be a passive process in which intrinsic sperm cell motility plays no part (Langendijk et al., 2005). However, this passive transport is quite fast due to the fact that the sufficient population of spermatozoa is established in the caudal isthmus of the oviducts within 30 minutes following natural mating, and they are able to fertilize a high proportion of the oocytes (Hunter, 1990). Adequate antiperistaltic uterine contractions are the main factor for passive sperm transport (Scott, 2000). It has been shown that estrogens oxytocin and prostaglandin F<sub>2a</sub>, which contain the natural seminal plasma, play an important role in the stimulation of the myometrial contraction (Roseboom et al. 2000). The inadequate sperm transport within the uterus results in decreasing the sow fertility (Langendijk et al., 2005).

The reproductive performance of artificially inseminated sows is often lower, in farm practice, than that achievable with natural breeding (Spronk et al., 1997; Stančić, 2000). It is often the result of inadequate myometrial stimulation due to a small dose volume, a high dilution rate of native ejaculate, an inadequate stimulation of the sow by the boar presence and the absence of mechanical stimulation of the cervix (Langendijk et al., 2003; Beham and Watson, 2005; Kemp et al., 2005; Mezalira et al., 2005; Stančić et al., 2006; Stančić et al., 2013). Furthermore, abnormal myometrial contractions can be one of the factors for the summer sow infertility (Almond and Bilkie, 2005; Stančić et al., 2011). In order to stimulate myometrial contractions and thus enhance the sperm transport through the uterine horns, oxytocin, estrogens, prostaglandins and the synthetic seminal plasma Predil MR-A® can be added to the AI dose (Levis, 2002; Castaneda Morreno, 2002; Dimitrov, 2012; Stančić et al., 2013). It has been shown that a two-phase insemination in combination with the synthetic seminal plasma Predil MR-A® increases sow fertility parameters (the farrowing rate and litter size) (Martin Rillo et al., 1996; Lyczynski, et al., 2000; Garcia Ruvalcaba et al., 2008; Garcia Ruvalcaba et al., 2009).

The purpose of this study is to investigate the effects of a two-phase insemination in combination with the synthetic seminal plasma Predil MR-A® on the sows fertility in the cool and warm season of the year.

## MATERIAL AND METHODS

The study was conducted on a commercial pig farm during the cool and warm seasons of 2012/2013. The sows in the experiment were F1-generation Yorkshire x Danish Landrace. The total of 200 sows (100 in the cool and 100 in the warm season) were included in the experiment. In each season, 50 sows were intracervically inseminated with conventional doses ( $4 \times 10^9$  spermatozoa in 100mL dose volume) + 30 mL of the synthetic seminal plasma (Predil MR-A®, Kubus S.A, Madrid, Spain), whereas the experimental group and another 50 sows were inseminated without the Predil MR-A® addition – the control group.

Predil MR-A® is a replacement of natural seminal plasma which gives the female genital tract salts, buffer and antibiotics that improve reproductive results. The synthetic

seminal plasma serves as the spermatozoa transportation medium, and contains organic and inorganic components which stimulate the sperm transport. It also enhances the insemination process of gilts and sows decreasing the backflow and improving the fertilization due to an increment of spermatozoa concentration. The use of synthetic seminal plasma before insemination introduces substances and components which are important for the viability of spermatozoa and ovum fertilization, improving fertility and litter size in gilts and sows. It stimulates uterine contractions and gives dilatation effect in the cervix (According to Kubus S.A, Madrid, Spain).

The semen was collected twice per week from high fertile boars. Each ejaculate was diluted with BTS1 for the short-term storage of the liquid diluted by boar semen extender (Minitüb, Germany) and packaged in 100 mL plastic bottles. The diluted sperm was stored in a termo-box at 17°C until used within 24 hours after collection. The estrus detection was performed twice a day (in the morning and evening with a 12-hour interval), starting on the second day after the sow weaning. Only the sows in estrus within the first 7 days after weaning were used in the experiment. The gilts were not used in the experiment. Conventional intracervical artificial insemination was performed about 3h to 4h after estrus detection, and the sows were reinseminated about 24h later. Sterile disposable catheters (Foamtip safeblue®, Minitüb, Germany), were used for AI. A two-phase insemination was performed in the experimental sows: 30 mL Predil-MRA® followed by conventional semen doses. The farrowing rate (calculated as a percentage of inseminated females that farrowed) and the litter size at farrowing (live born, stillborn and total born piglets per litter) were observed.

The data were processed by the *Statistica 10* software.

## RESULTS AND DISCUSSION

The insemination with Predil-MRA did not have significant effects ( $p>0.05$ ) on the sow farrowing rate in the cool season. However, in the warm season, the farrowing rate increased significantly ( $p<0.05$ ) after the insemination combined with Predil-MRA (82%) in comparison with the control sows (72%). The farrowing rate after Predil-MRA insemination in the warm season was lower (82%), but not significantly ( $p>0.05$ ) in comparison with the farrowing rate obtained after the inseminations performed in the cool season both in the sows inseminated with Predil-MRA and in the control sows (Table 1).

Table 1. Farrowing rate and litter size in sows treated during the cool and warm season (aver.  $\pm$  SD)

		Cool season		Warm season	
		Predil-MRA	Control	Predil-MRA	Control
Sows inseminated (n)		50	50	50	50
Farrowing rate (%)		88% <sup>ax</sup> (44/50)	84% <sup>ax</sup> (42/50)	82% <sup>ax</sup> (41/50)	72% <sup>by</sup> (36/50)
Average litter size at farrowing (n)	Live born	15.18 $\pm$ 3.18 <sup>ax</sup>	14.79 $\pm$ 3.32 <sup>ax</sup>	15.41 $\pm$ 1.75 <sup>ax</sup>	14.65 $\pm$ 2.80 <sup>ax</sup>
	Stillborn	1.09 $\pm$ 1.72 <sup>ax</sup>	0.98 $\pm$ 1.77 <sup>ax</sup>	1.31 $\pm$ 1.30 <sup>ax</sup>	1.65 $\pm$ 1.30 <sup>ay</sup>
	Total	16.27 $\pm$ 2.93 <sup>ax</sup>	15.76 <sup>ax</sup> $\pm$ 2.23	16.71 <sup>ax</sup> $\pm$ 1.82	16.30 <sup>ax</sup> $\pm$ 3.13

Values with different superscripts within the rows, differ ( $p < 0.05$ );

<sup>a,b</sup> Comparison within the same season, <sup>x,y</sup> Comparison between the cool and warm season.

The number of live born piglets after the insemination with MRA in both seasons (15.18 in the cool and 15.41 in the warm season) was slightly higher than in the control sows (14.79 in the cool and 14.65 in the warm season), but these differences were not statistically significant ( $p > 0.05$ ). The number of stillborn piglets was significantly ( $p < 0.05$ ) higher in the control sows in the warm season (1.65) in comparison with the Predil-MRA (1.09) or conventionally inseminated sows (0.98) in the cool season (Table 1).

Seasons of the year greatly affect the variation of native semen quality parameters. The elevated ambient temperature during summer months is the main factor of reducing the boar semen quality. This result in decreasing the boar reproduction exploitation on the one hand, and the sow fertility rate in the warmer season on the other (Corcuera et al., 2002; Stančić et al., 2003; Okere, 2003; Suriyasomboon et al., 2004; Stančić et al., 2013). The results obtained in this study clearly show that the fertility of sows in the warm period of the year may significantly increase if conventional insemination is combined with synthetic seminal plasma (Predil-MRA<sup>®</sup>). Namely, the farrowing rate within the warm season was significantly higher in the sows inseminated with Predil-MRA<sup>®</sup> addition (82%) in comparison with the control (untreated) sows (72%). Reduced sperm number, progressive motility and morphologically normal sperm, increased number of abnormal and dead sperm in the ejaculate, as well as the reduction in the concentration of some natural bioactive substances in seminal plasma after semen dilution are the factors which decrease sow fertility in the warm season of the year (Roseboom et al., 2000; Ramirez Ovalle, 2002; Rekiel and Sujka, 2007; Stančić et al., 2011; Stančić et al., 2012; Stančić et al., 2013). The results of other authors (Martin Rillo et al., 1996, Lyczynski, et al., 2000, Garcia Ruvalcaba et al., 2008, Garcia Ruvalcaba et al., 2009; Dimitrov, 2012) also show positive effects of the application of synthetic seminal plasma (Predil-MRA<sup>®</sup>) on the sow fertility after intracervical or postcervical insemination.

## CONCLUSION

The two-phase conventional intracervical insemination in combination with synthetic seminal plasma (Predil MR-A<sup>®</sup>) significantly increases the farrowing rate in the sows within the warm season of the year.

The usage of synthetic seminal plasma Predil MR-A<sup>®</sup> can be recommended as a method of improving the fertilization capacity of boar sperm in the warmer period of the year. This would increase the overall boar reproductive exploitation efficiency and, consequently the total sow fertility.

## REFERENCES

- ALMOND, P.K., BILKEI, G.: Seasonal infertility in large pig production units in a Eastern-European climate. *Australian Veterinary Journal*, 83(6)344-346, 2005.
- ALMOND, P.K., BILKIE, G.: Seasonal infertiliti in large pig production units in Eastern-European climate. *Australian Veterinary Journal*, 83(6)344-346, 2005.
- BEHAM, J.R., WATSON, P.F.: The effect of managed boar contact in the post-weaning

period on the subsequent fertility and fecundity of sows. *Anim. Reprod. Sci.*, 88:319-324, 2005.

CASTANEDA MORRENO, J.: Effect of sexual stimulus applied during artificial insemination on reproductive performance in pigs. Thesis, University of Colima, 2002.

DIMITROV, S.: Postcervical artificial insemination of sows in combination with synthetic seminal plasma (Predil MR-A®). *Contemporary Agriculture*, 61(3-4)169-174, 2012.

DIMITROV, S.: Postcervical artificial insemination of sows in combination with synthetic seminal plasma (Predil MR-A®). *Contemporary Agriculture*, 61(3-4)169-174, 2012.

GARCIA RUVALCABA, J.A., PALLAS ALONSO, R., HERNANDEZ-GIL, R. and DIMITROV, S.: The use of synthetic seminal plasma (Predil MR-A®) as a method to facilitate procedures with cervical and post-cervical artificial insemination of sows, *Agricultural science and technology*, 1(1)2-7, 2009.

GARCIA RUVALCABA, J.A., PHAM DUY, P.: Effect of transcervical infusion of synthetic seminal plasma (Predil MR-A®) prior to insemination in sows as a method to improve reproductive results. XIII<sup>th</sup> AAAP Congr., Hanoi, Vietnam, 2008. P. 332.

KEMP, B., SOEDE, N.M., LANGENDIJK, P.: Effect of boar contact and housing conditions on estrus expression in sows. *Theriogenology*, 63(2)643-656, 2005.

LANGENDIJK, P., BOUWMAN, E.G., SCHAMS, D., SOEDE, N.M., KEMP, B.: Effects of different sexual stimuli on oxytocin release, uterine activity and receptive behavior in estrus sows. *Theriogenology*, 59(3-4)849-861, 2003.

LANGENDIJK, P., SOEDE, N.M., KEMP, B.: Uterine activity, sperm transport, and the role of boar stimuli around insemination in sows. *Theriogenology*, 63:500–513, 2005.

LEVIS, D.G.: Use of additives to a dose of boar semen. Ohio Pork Ind. Center. The Ohio State Univ., Columbus, 46-53, 2002.

LYCZYNSKI, A., SOCZYWKO, T., MARTIN RILLO, S., DE ALBA ROMERO, C.: The effect of Predil MR-A synthetic seminal plasma used to inseminate sows and gilts on their reproductive efficiency. Proceedings of IV<sup>th</sup> International Conference on Boar Semen Preservation. Beltsville, MD, Allen Press, Inc., Lawrence, KS, 2000. Pp. 250 (Abs.).

MARTIN RILLO, S., LAPUENTE, S., HERNANDEZ-GIL, R., GARCIA RUVALCABA, J.A., GARCIA ARTIGA, C.: Improvement of fertility results by means of usage of synthetic seminal plasma before artificial insemination. Proceedings 14<sup>th</sup> International Pig Veterinary Society Congress, Bologna, Italy, 1996. Pp 605 (Abs.).

MEZALIRA, A., DALLANORA, D., BERNARDI L.M., WENTZ, I., BORTOLOZZO, P.F.: Influence of Soerm Cell Dose and Post-insemination Backflow on Reproductive Performance of Intrauterine inseminated Sows. *Reprod. Dom. Anim.*, 40:1-5, 2005.

RAMIREZ OVALLE, F.: Aplicación de semen muerto y del plasma seminal sintético en el estro anterior a la primera inseminación en nulíparas para evaluar su respuesta reproductiva, Doctoral Thesis. Universidad Mayor, Escuela Medicina Veterinaria, Santiago de Chile, 2002.

REKIEL, A., SUJKA, E.: Wpływ stymulacji dróg rodnych syntetyczna plazma nasienia na wyniki rozrodu loszek i loch, *Medycyna Wet.*, 63(6)7-3-707, 2007.

ROZEBOOM, K.J., TROEDSSON, M.H., HODSON, H.H., SHURSON, G.C., CRABO, B.G.: The importance of seminal plasma on the fertility of subsequent artificial inseminations in swine. *J. Anim. Sci.*, 78:443-448, 2000.



- SCOTT, M.A.: A glimpse at sperm function in vivo: sperm transport and epithelial interaction in the female reproductive tract. *Animal Reproduction Science*, 60–61:337-348, 2000.
- SPRONK, G.D., KERKAERT, B.R., BOBB, J.D., KENNEDY, G.F.: Managing the breeding herd. *International Pig Topics*, 12(7)7-11, 1997.
- STANČIĆ, B., BOŽIĆ, A., STANČIĆ, I., RADOVIĆ, I., DRAGIN, S.: Sow seasonal infertility (a review). *Contemporary Agriculture*, 60(1-2)195-203, 2011.
- STANČIĆ, B., BOŽIĆ, A., STANČIĆ, I., DRAGIN, S., RADOVIĆ, I., ERDELJAN, M.: Effect of Season and Boars Breed on Ejaculate Quality. *Contemporary Agriculture*, 62(1-2)8-13, 2013.
- STANČIĆ, B., BOŽIĆ, A., STANČIĆ, I., DRAGIN, S., RADOVIĆ, I., PETROVIĆ, M.: Effect of warm and cold period of the year on boar semen quality parameters. *Contemporary Agriculture*, 61(3-4)163-168, 2012.
- STANČIĆ, B., BOŽIĆ, A., STANČIĆ, I., RADOVIĆ, I., DRAGIN, S.: Sow seasonal infertility. *Contemporary Agriculture*, 60(1-2)195-203, 2011.
- STANČIĆ, B., RADOVIĆ, I., GRAFENAU, P., KUBOVIČOVA, E., PIVKO, J.: Uticaj rase i godišnje sezone na kvalitet native sperme nerastova u Vojvodini. *Savremena poljop.*, 52(3-4)257-262, 2003.
- STANČIĆ, B., RADOVIĆ, I., STANČIĆ, I., KRAGIĆ, S.: The influence of cervix stimulation before and after insemination on the sows fertility. *Contemporary Agriculture*, 55(5)8-12, 2006.
- STANČIĆ, B.: Contemporary principles in pig artificial insemination (a review). *Proc. 3<sup>rd</sup> Symposium »Breeding and pig health protection«. Vršac (Serbia), 21. do 23. june, 2000. Pp. 35-41*
- STANČIĆ, I., APIĆ, I., APIĆ, J., KRAGIĆ, S.: The influence of cervix stimulation and boar presence at artificial insemination on sows fertility. *Contemporary Agriculture*, 62(1-2)21-27, 2013.
- STANČIĆ, I., APIĆ, I., STANČIĆ, B., STOJANAC, N.: Sows fertility after oxytocin addition in semen dose or vulvar injection to stimulate myometrial activity around insemination. *Contemporary Agriculture*, 62(1-2)21-27, 2013.
- STANČIĆ, I., STANČIĆ, B., DRAGIN, S., RADOVIĆ, I., BOŽIĆ, A.: Sows fertility after intracervical or postcervical artificial insemination (AI) in warm and cold season. *Journal of Microbiology, Biotechnology and Food Sciences*, 2 (Special issue on BQRMF)1592-1601, 2013.
- STANČIĆ, I., STANČIĆ, B., DRAGIN, S., RADOVIĆ, I., BOŽIĆ, A.: Sows fertility after intracervical or postcervical artificial insemination (AI) in warm and cold season. *Journal of Microbiol. Biotechnol. and Food Sci.*, 2 (Special issue) 1592-1601, 2013.

#### ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to the company Kubus S.A, Madrid, Spain, which has provided a sufficient amount of the Predil-MRA® preparation for this research.

**FERTILITET KRMAČA POSLE INTRACERVIKALNOG  
OSEMENJAVANJA U TOPLOJ I HLADNOJ SEZONI UPOTREBOM  
KONVENCIONALNIH DOZA KOMBINOVANIH SA  
SINTETIČKOM SEMENOM PLAZMOM (PREDIL MR-A®)**

IVAN STANČIĆ, IVAN RADOVIĆ, MIHAJLO ERDELJAN, BLAGOJE STANČIĆ,  
TEODORA VASILJEVIĆ, ALEKSANDAR BOŽIĆ, IVAN ŽARKOVIĆ

**Izvod**

Značajno redukovan kvalitet sperme nerastova, tokom toplije godišnje sezone, dobro je poznat fenomen. On ima direktan uticaj na značajno smanjenje fertiliteta krmača, osemenjenih u ovom periodu godine. Cilj ovog rada je bio da se ustanovi da li konvencionalno veštačko osemenjavanje, kombinovano sa sintetičkom spermalnom plazmom (Predil MR-A®), može povećati parametre fertiliteta krmača (% prašenja i veličina legla), osemenjenih u toplom periodu godine. Pokazalo se da je vrednost prašenja bila statistički značajno ( $p < 0.05$ ) veća kod krmača osemenjenih kombinacijom sa Predil MR-A® (82%), od one kod kontrolnih krmača (72%). Iako je ustanovljena tendencija povećanja prosečnog broja živo rođene prasadi u leglima krmača osemenjenih kombinacijom sa Predil MR-A®, ovo povećanje nije bilo statistički značajno ( $p > 0.05$ ) ni unutar ni između ispitivanih godišnjih sezona (kretalo se između 14.65 i 15.41 prasadi po leglu). Ovi rezultati pružaju mogućnost povećanja ukupne efikasnosti reproduktivne efikasnosti nerastova, kao i povećanja fertiliteta osemenjenih krmača.

**Ključne reči:** veštačko osemenjavanje, sezona, spermalna plazma, Predil MR-A®, fertilitet, krmača.

Received / *Primljen*: 17.01.2014.

Accepted / *Prihvaćen*: 30.01.2014.

## YIELD RESPONSE TO ELEVATED SOIL BORON IN WHEAT CULTIVARS OF LOCAL AND FOREIGN ORIGIN

MILKA BRDAR-JOKANOVIĆ, ANKICA KONDIĆ-ŠPIKA,  
BRANKA LJEVNAIĆ-MAŠIĆ, IVANA MAKSIMOVIĆ<sup>1</sup>

*SUMMARY: The two-year field study was aimed to investigate the effect of increasing soil boron treatments (3.3, 6.7 and 13.3 g H<sub>3</sub>BO<sub>3</sub>/m<sup>2</sup>) on eight wheat cultivars, as well as to estimate the rate in which yield decreases followed the increase in soil boron. A strong positive linear relationship was found between the treatments and soil hot water extractable boron. On average, wheat yield response to elevated soil boron was decrease of 7.7%. Cultivars of local origin over-yielded foreign cultivars at all levels of boron supply. The estimated yield loss was 1.8% per increase in soil boron of 0.1 ppm.*

**Key words:** wheat cultivars, yield, boron tolerance.

### INTRODUCTION

Macro- and micronutrients are elements essential for healthy growth and development of vascular plants. Both nutrient deficiency and toxicity are disorders that may significantly decrease yield of agricultural plants. Nutrient deficiencies can be ameliorated by fertilizer application; however, toxicity-related problems are more difficult to manage. For the past few decades, elevated amounts of micronutrient boron (B) have received broad attention as possible yield-limiting factor in cereals. This is especially true for arid and semiarid environments, as well as for saline soils, which occupy approximately 200,000 ha in Vojvodina.

Soil boron concentration affecting plants strongly enough to cause significant

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Milka Brdar-Jokanović, PhD, Research Associate, Ankica Kondić-Špika, PhD, Senior Research Associate, Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad, Serbia. Branka Ljevnaić-Mašić, PhD, Assistant Professor, Ivana Maksimović, PhD, Professor, University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 821000 Novi Sad, Serbia.

Corresponding author: Milka Brdar-Jokanović, e-mail: milka.brdar@nsseme.com, phone: +381 21 780 365.

\*This article results from the project TR31066 (2011-2014), supported by Ministry of Education, Science and Technological Development of Republic of Serbia

yield decrease is not easy to determine. It depends on plant species (e.g. monocotyledonous plants are generally more sensitive when compared to dicotyledonous), soil type, texture, moisture, temperature, pH etc. Since great variability in boron tolerance has been reported within plant species, breeding tolerant cultivars has been proposed as the most promising approach for solving the problem (Miljković, 1960; Matoh, 1997; Nable et al., 1997; Yau and Ryan, 2008; Reid, 2010; Mertens et al., 2011; Wimmer and Goldbach, 2012; Brdar-Jokanović et al., 2013).

This study was undertaken to assess the effect of elevated soil boron on high-yielding wheat (*Triticum aestivum* L.) cultivars of different origin. The second aim of this research was to estimate the rate in which wheat yield decreases following the increase in soil boron.

## MATERIAL AND METHODS

Four high-yielding wheat (*Triticum aestivum* L.) cultivars of local origin (Arija, Astra, Kantata, Oda – NS cultivars, Novi Sad, Serbia) and four high-yielding cultivars originating from breeding institutions in the region (Fundulea 4 – Romania, Magdalena – Hungary, Trakija – Bulgaria, Žitarka – Croatia) have been included in two-year (2005-06, 2006-07) field study comprising control and three boron treatments, replicated three times. The trial was set at the Rimski Šančevi experimental field (Institute of Field and Vegetable Crops, Novi Sad, Serbia, 45°20' N, 19°51' E, 84 m altitude). Soil was fertile chernozem.

Official reports of Republic Hydrometeorological Service of Serbia have been used for weather data analysis (Table 1.).

Table 1. Meteorological data for the two wheat growing seasons and 30-year average, Rimski Šančevi, Novi Sad, Serbia  
*Tabela 1. Meteorološki podaci za dve sezone u kojima je izvođen ogled i tridesetogodišnji prosek, Rimski Šančevi, Novi Sad, Srbija*

Parameter / Parametar	2005-06	2006-07	1981-2010
Average daily temperature (°C) / Srednja dnevna temperatura (°C)	9.2	10.6	8.6
Minimum temperature (°C) / Minimalna temperatura (°C)	-14.0	-6.0	-3.1
Maximum temperature (°C) / Maksimalna temperatura (°C)	34.0	36.0	28.1
Sum of temperatures (°C) / Suma temperatura (°C)	2465.0	2599.5	2317.1
Sum of precipitation (mm) / Suma padavina (mm)	498.4	390.9	465.3

Main plot (1.2 m<sup>2</sup>) consisted of six rows, with intra and inter spacing of 2 and 20 cm, respectively. Sowing was performed at optimum time (mid-October), and harvesting when plants reached maturity (mid-July and third decade of June for the first and second growing season, respectively).

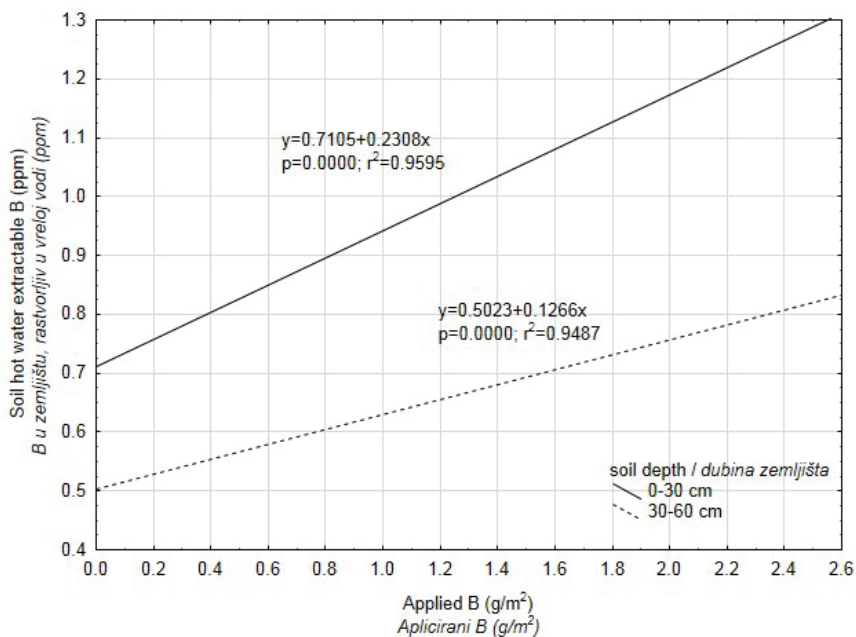
Plots were treated immediately after sowing with boric acid (H<sub>3</sub>BO<sub>3</sub>) dissolved in distilled water at the rates of 3.3 (B1), 6.7 (B2) and 13.3 (B3) g per m<sup>2</sup>. Besides for boron treatments, standard agronomic procedures have been applied. Soil sampling (depth 0-30 and 30-60 cm) was performed at wheat heading and hot water extractable boron concentration was determined by atomic absorption spectrophotometer.

Data was processed by analysis of variance. Polynomial regression analysis was

performed in order to estimate the rate in which wheat yield decreases as a function of increasing soil boron concentrations. STATISTICA 12 software package (StatSoft, Tulsa, OK, USA, University license, Novi Sad) was used for data analysis.

## RESULTS AND DISCUSSION

In order to confirm that the increasing boron treatments applied at wheat sowing gradually increase boron concentration in the soil and that those effects last throughout the plant life cycle, soil sampling was performed at heading and hot water extractable boron was determined. Strong positive linear relationship was found between the applied treatments and soil boron concentration (Graph 1.), which was true for soil depth of both 0-30 and 30-60 cm. The increase in soil boron was more pronounced in 0-30 than in the 30-60 cm soil layer (32.5 and 25.2% of the initial concentration, for every g of added B/m<sup>2</sup> of soil, respectively). This pattern was expected, since the plots were treated by watering soil surface with boric acid dissolved in distilled water. Opposite, the increase in boron concentration with soil depth was noted for soils naturally laden with the element, with the point of maximum concentration depending on soil type, texture, humidity etc. (Jelenić et al., 1973; Brennan and Adcock, 2004).



Graph 1. The effect of increasing boron treatments on hot water extractable soil boron  
*Grafik 1. Uticaj tretmana borom na koncentraciju bora u zemljištu, rastvorljivog u vreloj vodi*

Analysis of variance detected significant ( $p < 0.01$ ) yield variation among the analyzed wheat cultivars, seasons and boron treatments (Table 2). In addition, all the interactions were significant (cultivar/season, cultivar/treatment, season/treatment and cultivar/season/treatment). Therefore; the applied treatments affected wheat yield and these effects were not the same for all cultivars and for the two seasons of the experi-

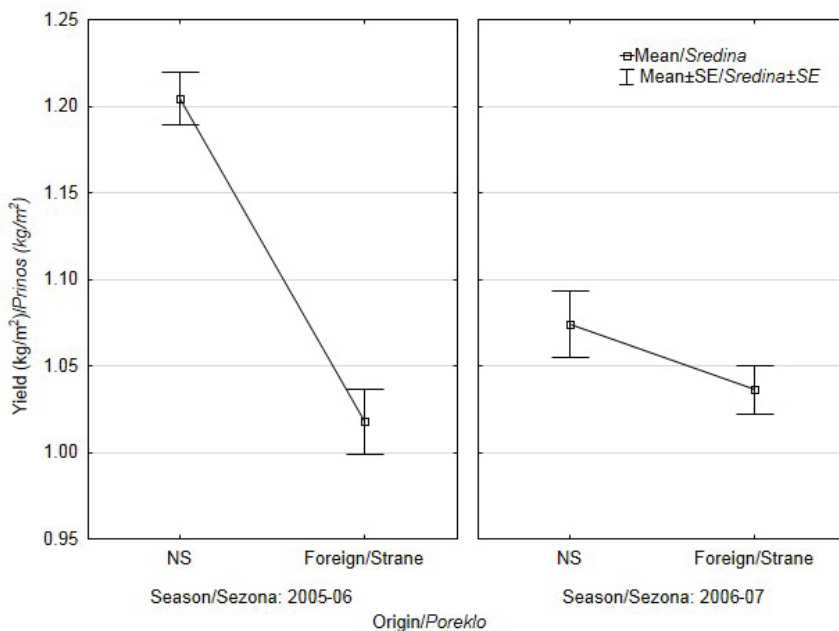
ment. Differences among wheat cultivars in yield response to elevated soil boron have been also documented by other authors (Nable et al., 1997; Kalayci et al., 1998; Yau and Ryan, 2008; Prabhakar et al., 2013). In our trial, yield response to boron treatments ranged from the reduction of 19.1% (Žitarka, 2005-06) to the increase of 5.0% (Kantata, 2006-07). On two-year average, the highest level of boron tolerance exhibited cultivar Kantata (increase of 1.4%) and the lowest Žitarka (decrease of 13.2%). Average yield decrease due to boron treatments of 7.7% was somewhat lower than expected according to literature data, which might be due to both soil chemical properties and choice of wheat cultivars included in the analyses.

Table 2. Mean squares from analysis of variance for wheat yield  
*Tabela 2. Sredine kvadrata iz analize varijanse za prinos pšenice*

Effect / <i>Efekat</i>	df	Mean squares / Sredine kvadrata	p
Cultivar / <i>Sorta</i> (C)	7	0.1744	0.0000
Season / <i>Sezona</i> (S)	1	0.1506	0.0000
Treatment / <i>Tretman</i> (T)	3	0.2800	0.0000
(C) / (S)	7	0.1288	0.0000
(C) / (T)	21	0.0108	0.0000
(S) / (T)	3	0.0085	0.0000
(C) / (S) / (T)	21	0.0076	0.0000
Error / <i>Pogreška</i>	128	0.0006	

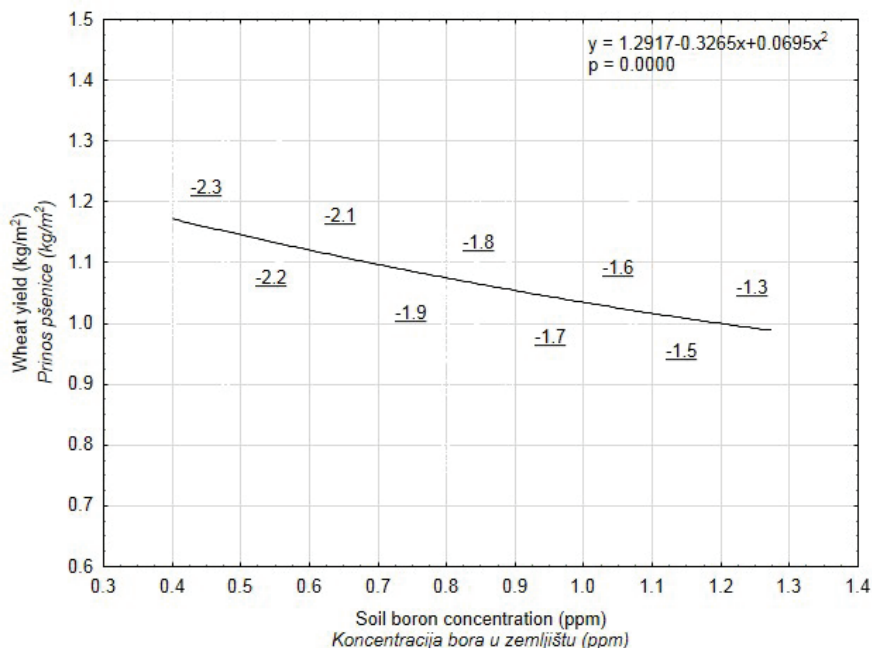
df – degrees of freedom / *stepeni slobode*

Although both local and foreign cultivars included in the experiment are high-yielding, cultivars of local origin (NS) over-yielded foreign cultivars at all levels of boron supply (Graph 1). This was somewhat expected, since they have been constructed with the aim to achieve high yields in local environmental conditions. Lower average yields that have been noted for all cultivars in 2006-07 in comparison to 2005-06 may be attributed to higher temperatures and lower sum of precipitation characterizing the season (Table 1).



Graph 2. Yield of local (NS) and foreign wheat cultivars (average of all levels of boron supply)  
 Grafik 2. Prinos lokalnih (NS) i stranih sorti pšenice (sredina kontrole i svih tretmana)

In order to investigate the rate in which wheat yield decreases in response to increasing soil boron concentration, data was subjected to polynomial regression analysis and Graph 3 was constructed. The constructed polynomial curve covers the observed range from 0.4 to 1.3 ppm hot water extractable soil boron and the corresponding wheat yields. The estimates of yield losses due to soil boron increase (underlined) are given along the curve in steps of 0.1 ppm. As seen in the graph, the average wheat yield decrease was about 1.8% per 0.1 ppm increase in soil boron concentration. The estimated percentage of yield response to boron gradually decreases along the curve, which would primarily be attributed to the mathematical model applied for its construction.



Graph 3. Wheat yield decrease following the increase in soil boron concentration

*Grafik 3. Redukcija prinosa pšenice kod rastuće koncentracije bora u zemljištu*

The estimated yield losses are high and confirm the conclusions of other authors that even a slight increase in soil boron concentration may exhibit detrimental effects on crops. According to Miljković (1964), Yau et al. (1994) and Shorrocks (1997) soil hot water extractable boron in the range of only 0.7 to few ppm may be toxic to susceptible plant species and varieties. The fact that Ubavić et al. (1993) examined in detail 1609 samples of Vojvodina agricultural soils (0-30 cm depth) and reported boron range of 0.0 to 15.9 ppm, with average value of 3.49 ppm, highlights the importance of research on boron tolerance in major agricultural crops, primarily the sensitive ones such as cereals. In addition, our trial was performed on fertile chernozem using appropriate agronomic procedures, which minimized other soil constraints that could lead to yield reduction. Therefore, higher yield decreases are expected on less fertile soils and those containing high levels of other elements or compounds, such as saline soils.

## CONCLUSION

Between the applied boron treatments and soil hot water extractable boron was found a strong positive linear relationship. The increase in soil boron following the increasing treatments was 32.5 and 25.2% of the initial concentration for every g of added B/m<sup>2</sup> of soil (for soil depth of 0-30 and 30-60 cm, respectively). Wheat yield in response to elevated soil boron decreased 7.7% in average, ranging from 13.2% decrease in Žitarka to 1.4% increase in Kantata. Cultivars of local origin over-yielded foreign cultivars at all levels of boron supply. The estimated yield loss was 1.8% per increase in soil boron of 0.1 ppm.



## REFERENCES

- BRENNAN, R.F., ADCOCK, K.G.: Incidence of boron toxicity in spring barley in Southwestern Australia. *J. Plant Nutr.*, 27(3)411-425, 2004.
- BRDAR-JOKANOVIĆ, M., MAKSIMOVIĆ, I., KRALJEVIĆ-BALALIĆ, M., ZEREMSKI-ŠKORIĆ, T., KONDIĆ-ŠPIKA, A., KOBILJSKI, B.: Boron concentration vs content as criterion for estimating boron tolerance in wheat. *J. Plant Nutr.*, 36(3)470-480, 2013.
- JELENIĆ, Đ., DŽAMIĆ, R., JOVANOVIĆ, M.: Količinski odnos makro- i mikroelemenata u zemljištu voćnjaka podignutih na černozeu i gajnjači. *Zemljište i biljka*, 22:359-373, 1973.
- KALAYCI, M., ALKAN, A., ÇAKMAK, I., BAYRAMOĞLU, O., YILMAZ, A., AYDIN, M., OZBEK, V., EKIZ, H.: Studies on differential response of wheat cultivars to boron toxicity. *Euphytica*, 100(1-3)123-129, 1998.
- MATOH, T.: Boron in plant cell walls. *Plant Soil*, 193(1-2)59-70, 1997.
- MERTENS, J., VAN LAER, L., SALAETS, P., SMOLDERS, E.: Phytotoxic doses of boron in contrasting soils depend on soil water content. *Plant Soil*, 342(1)73-82, 2011.
- MILJKOVIĆ, N.: Karakteristike vojvođanskih slatina i problem bora u njima. Doktor-ska disertacija, Univerzitet u Novom Sadu, Poljoprivredni fakultet, Srbija, Novi Sad, 1960.
- MILJKOVIĆ, N.: Problem bora u slatinama Panonske nizije u Jugoslaviji. *Zemljište i biljka*, 13:319-326, 1964.
- NABLE, R.O., BAÑUELOS, G.S., PAULL, J.G.: Boron toxicity. *Plant Soil*, 193(1-2)181-198, 1997.
- PRABHAKAR, M., SAHA, B., SAHA, S., SINGH, A.P.: Screening of wheat genotypes for their differential response to boron application in calcareous soil. *Annals of Agri Bio Research*, 18(3)339-343, 2013.
- REID, R.: Can we really increase yield by making crop plants tolerant to boron toxicity? *Plant Sci.*, 178(1)9-11, 2010.
- SHORROCKS, V.M.: The occurrence and correction of boron deficiency. *Plant Soil*, 193(1-2)121-148, 1997.
- UBAVIĆ, M., BOGDANOVIĆ, D., DOZET, D., HADŽIĆ, V., ČIROVIĆ, M., SEKULIĆ, P.: Sadržaj teških metala u zemljištima Vojvodine. *Zbornik radova Instituta za ratarstvo i povrtarstvo*, 21:49-58, 1993.
- WIMMER, M.A., GOLDBACH, H.E.: Boron-and-salt interactions in wheat are affected by boron supply. *J. Plant Nutr. Soil Sci.*, 175(2)171-179, 2012.
- YAU, S.K., HAMBLIN, J., RYAN, J.: Phenotypic variation in boron toxicity tolerance in barley, durum and bread wheat. *Rachis*, 13:20-25, 1994.
- YAU, S.K., RYAN, J.: Boron toxicity tolerance in crops: a viable alternative to soil amelioration. *Crop Sci.*, 48(3)854-865, 2008.

# PRINOS LOKALNIH I STRANIH SORTI PŠENICE GAJENIH NA ZEMLJIŠTU KOJE SADRŽI POVIŠENE KONCENTRACIJE BORA

MILKA BRDAR-JOKANOVIĆ, ANKICA KONDIĆ-ŠPIKA,  
BRANKA LJEVNAIĆ-MAŠIĆ, IVANA MAKSIMOVIĆ

## Izvod

Dvogodišnji poljski ogled je postavljen sa ciljem ispitivanja uticaja tretmana bocom (3.3, 6.7 and 13.3 g H<sub>3</sub>BO<sub>3</sub>/m<sup>2</sup>) na osam sorti pšenice, kao i procene intenziteta kojim se prinos smanjuje usled rastuće koncentracije ovog elementa u zemljištu. Utvrđena je jaka pozitivna linearna veza između primenjenih tretmana i zemljišnog bora rastvorljivog u vreloj vodi. Prosečna redukcija prinosa pšenice na tretmanima iznosila je 7,7%. Kod lokalnih sorti je zabeležen viši prinos u odnosu na strane, bez obzira na tretman. Procenjeno je da porast koncentracije zemljišnog bora od 0,1 ppm za posledicu ima redukciju prinosa od 1,8%.

**Ključne reči:** sorte pšenice, prinos, tolerancija na bor.

Received / Primljen: 21.11.2013.

Accepted / Prihvaćen: 07.02.2014.

## PROTECTIVE EFFECT OF 1-DEOXYNOJIRIMYCIN AND RESVERATROL AGAINST DIABETIC OXIDATIVE STRESS AND INFLAMMATION

AGNIESZKA GREŃ, GRZEGORZ FORMICKI, NORBERT LUKAČ,  
PETER MASSÁNYI, ROBERT STAWARZ<sup>1</sup>

*SUMMARY: Diabetes mellitus (DM) was induced in groups II, V, and VI. Animals III experimental group was administered 1-deoxynojirimycin (DNJ) in dose 0,75 mgK<sup>-1</sup>g<sup>-1</sup> body weight/day for 14 days. Mice IV experimental group was administered resveratrol (RSV) in dose 30 mgK<sup>-1</sup>g<sup>-1</sup> body weight/day for 14 days. Animals group V: diabetic group receiving DNJ, group VI: diabetic group receiving RSV. Thirty minutes after the last injection animals were anaesthetized and decapitated. Serum level of interleukin-6 (IL-6) was assessed as surrogate markers of inflammation. The livers levels of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activity were measured as surrogate markers of oxidative stress. Serum level of insulin and plasma glucose levels were assessed as surrogate markers of diabetes. The administration of DNJ or RSV significantly restored glucose, insulin, IL-6, GSH levels and SOD, CAT, GPx activity in the diabetic mice to near control. The results demonstrated that DNJ and RSV with its antidiabetic, antiinflammation and antioxidant properties could be a potential herbal medicine in treating diabetes and hepatic problems.*

**Keywords:** oxidative stress, inflammation, 1-deoxynojirimycin, resveratrol, diabetes.

### INTRODUCTION

Diabetes mellitus (DM) is characterized by chronic hyperglycemia, resulting from defects in insulin secretion, action, or both, leading to disturbance in carbohydrate, lipid and protein metabolism. This state induces immediate oxidative stress (Jakus, 2000;

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Agnieszka Greń, PhD, Assistant Professor, Grzegorz Formicki, Associate Professor, Robert Stawarz, Associate Professor, Faculty of Geography and Biology, Pedagogical University, Kraków, Poland, Norbert Lukač, Professor, Peter Massányi, Professor, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovakia.

Corresponding author: Agnieszka Greń, e-mail: [agrenagren@gmail.com](mailto:agrenagren@gmail.com), phone: +48 12 6626715.

King and Loeken, 2004). One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes,  $\alpha$ -amylase and  $\alpha$ -glucosidases, in the digestive system organs (Deshpande et al., 2009). Renewed attention in recent decades to alternative medicines and natural therapies has stimulated a new way of research interest in traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active compounds. Many plant extracts and plant products have been shown to possess significant antioxidant activity, which may be an important property of medicinal plants. The mulberry leaves have high nutritional and medical values, and they contain active ingredients, such as 1-deoxynojirimycin and resveratrol (Yagi et al., 1976; Zhao et al., 2005).

The 1-deoxynojirimycin (DNJ) was first isolated from its roots by Yagi *et al.* in 1976. DNJ is a glucose analogue with a secondary amine group instead of an oxygen atom in the pyranose ring of glucose. DNJ potently inhibits  $\alpha$ -glucosidase in the small intestine by binding to the active center of  $\alpha$ -glucosidase (Junge et al., 1996). More DNJ has also been found in the leaves and fruits of *Morus alba* L. (Asano et al., 1994; Asano et al., 2001). DNJ inhibits postprandial hyperglycemia (Junge et al., 1996), by inhibiting  $\alpha$ -glucosidase in the small intestine. There have been findings from animal studies supporting the hypothesis that mulberry leaves delay the onset of diabetes, as indicated by the fact that a rapid increase in the postprandial blood glucose level was inhibited (Hikino et al., 1985; Kong et al., 2008; Kimura et al., 1995).

Resveratrol (RSV; 3,5,4'-trihydroxystilbene) is a phenolic phytoalexin found in grapes, grape juice, red wines, peanuts, berries of *Vaccinium* species, including blueberries, bilberries, cranberries and *Morus alba*. Resveratrol has intracellular antioxidant activity and activates SIRT1, a NAD<sup>+</sup>-dependent histone deacetylase involved in mitochondrial biogenesis and the enhancement of peroxisome proliferator- $\gamma$ -activated receptor coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) and FOXO activity. The anti-diabetic, neuroprotective and anti-adipogenic actions of resveratrol may be mediated via SIRT1 activation (McAnulty et al., 2013).

The present study was designed to evaluate the antihyperglycemic, antiinflammation and antioxidant action of 1-deoxynojirimycin (DNJ) and resveratrol (RSV) on diabetic mice.

## MATERIAL AND METHODS

The experiments were carried out male mice, average body weight 25 – 26g, bred in the constant light conditions LD 12:12 and fed with standard diet with unlimited access to water. All the experiments were performed with the acceptance (No. 122/2010) of the Local Ethical Committee, Cracow, Poland.

The animals were divided into six groups. Control group (C) (I group) received an intraperitoneal (i.p.) injection of 0.1 mol/L sodium citrate buffer (pH 4.5).

Diabetes mellitus (DM) was induced in groups II, V, and VI and received a single i.p. injection of streptozotocin - STZ, 60 mgKg<sup>-1</sup> body weight, freshly dissolved in 0.1 M citrate buffer (pH 4.5). DM was verified by measuring blood glucose in tail nick blood samples. Mice with non fasting blood glucose levels of  $\geq 20$  mmol/L after 48 h of STZ injection or greater and symptoms of polyuria, polyphagia, and polydipsia were considered diabetic. Animals III experimental group was administered 1-deoxynojirimycin

(DNJ) in dose 0,75 mgKg<sup>-1</sup> body weight/day for 14 days. Mice IV experimental group was administered resveratrol (RSV) in dose 30 mgKg<sup>-1</sup> body weight/day for 14 days. Animals group V: diabetic group receiving DNJ in dose 0,75 mgKg<sup>-1</sup> body weight/day for 14 days. Group VI: diabetic group receiving RSV in dose 30 mgKg<sup>-1</sup> body weight/day for 14 days. Animals were orally fed by gastric intubation with 1-deoxynojirimycin and resveratrol.

Synthetic 1-Deoxynojirimycin hydrochloride, synonym: 1,5-Dideoxy-1,5-imino-D-sorbitol hydrochloride (DNJ), resveratrol (RSV), synonym: 3,4',5-Trihydroxy-*trans*-stilbene, 5-[(1E)-2-(4-Hydroxyphenyl)ethenyl]-1,3-benzenediol and streptozotocin (STZ) were purchased from Sigma-Aldrich Corp. St. Louis, MO, USA. STZ-induced diabetic (STZ-DM) mice were used as the severe insulin-deficient diabetic model. STZ is widely used as a strong inducer of diabetes in the experimental animals (Rerup, 1970). STZ selectively destroys the pancreatic cells that secrete insulin, inhibits syntheses and the release of insulin, and produces DM (Gilman et al., 1990). STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Ledoux et al. 1986).

Thirty minutes after the last injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery. All blood samples were processed immediately and frozen at minus 70 degrees centigrade until assay. Serum level of interleukin-6 (IL-6) was assessed as surrogate markers of inflammation. The livers were homogenized with a homogenizer (Ultra Turrax T25, Rose Scientific Ltd., Edmonton, Canada) in 10 volumes of a 50 mM sodium phosphate buffer (pH 7.4) at 4 °C. Homogenates were centrifuged (Beckman, U.S.A.) at 15000g for 10 min, and the supernatant obtained was used for the following antioxidant enzyme measurements. The supernatant were collected and stored at -70°C for further analysis. Levels of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activity were measured as surrogate markers of oxidative stress. Serum level of insulin and plasma glucose levels were assessed as surrogate markers of diabetes.

Total GPx (EC 1.11.1.9) activity in liver tissues was determined according to Paglia and Valentine's method (Paglia and Valentine, 1967). The enzyme solution was added to a mixture containing hydrogen peroxide and glutathione in 0.1 mM Tris buffer (pH 7.2) and the absorbance at 340 nm was measured. Activity was evaluated from a calibration curve, and the enzyme activity was defined as nmoles of NADPH oxidized per mg protein per min. Total SOD (EC 1.15.1.1) activity was determined by the inhibition of cytochrome c reduction, according to Flohe and Otting method (Flohe and Otting, 1984). The reduction of cytochrome c was mediated by superoxide anions generated by xanthine/xanthine oxidase system and monitored at 550 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of cytochrome c reduction by 50%. Total CAT activity was based on that of Aebi (Aebi, 1984). In brief, the reduction of 10 mM H<sub>2</sub>O<sub>2</sub> in 20 mM of phosphate buffer (pH 7.0) was monitored by measuring the absorbance at 240 nm. The activity was calculated using a molar absorption coefficient, and the enzyme activity was defined as nmoles of dissipating hydrogen peroxide per mg protein per min. The concentration of GSH in hepatic tissues was estimated by evaluating free-SH groups, using the 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) method as described by Sedlak and Lindsay (Sedlak and Lindsay, 1968).

The protein content of hepatic tissue extract was analyzed using the Bradford pro-

tein assay (Bradford, 1976) using the protein-dye kit (Bio-Rad, Richmond, CA, USA). A commercially available bovine serum albumin (Sigma Chemical, St Louis, MO, USA) was used as a standard solution. Changes in optical density were monitored at 595nm.

Plasma insulin was detected by an ELISA kit (Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden). The level IL-6 was measured by ELISA using cytoscreen immunoassay kits (Peprotech, Rocky Hill, NJ, USA).

The results were expressed as means  $\pm$  standard deviation. The statistical analysis of the results was carried out with Statistica program version 9. The distribution was tested using Shapiro-Wilk test. Differences between consecutive groups were analyzed using one-way ANOVA followed by post hoc analysis with Tukey test. Statistical significance was defined at  $P < 0.05$ .

## RESULT AND DISCUSSION

Under the diabetic condition, increase glucose levels are significant sources of free radicals and inducers of oxidative stress. The administration of 1-deoxynojirimycin at dose  $0,75 \text{ mgKg}^{-1}$  body weight/day and resveratrol at dose  $30 \text{ mgKg}^{-1}$  body weight/day to STZ-induced diabetic mice led to significant decreases in the levels of glucose versus DM group. The untreated diabetic animals showed significantly raised serum glucose levels and reduced serum insulin concentration as compared to the control group. The administration of DNJ or RSV however, significantly restored glucose and insulin levels in the diabetic mice to near control ( $P < 0.05$ ), (Table 1).

Table 1. Effect of oral administration of DNJ and RSV on serum glucose and insulin concentration in STZ- induced diabetic mice (mean  $\pm$  SD)

Groups	Glucose (mg/dl)	Insulin (ng/ml)
Control group (C)	$115.4 \pm 4.2^a$	$0.79 \pm 0.08^a$
Diabetes mellitus (DM)	$462.4 \pm 17.3$	$0.37 \pm 0.06$
1-deoxynojirimycin (DNJ)	$98.2 \pm 3.6^a$	$0.87 \pm 0.03^a$
Resveratrol (RSV)	$103.6 \pm 1.3^a$	$0.81 \pm 0.02^a$
Diabetes mellitus+1 deoxynojirimycin (DM+DNJ)	$303.7 \pm 11.5^a$	$0.69 \pm 0.04^a$
Diabetes mellitus+resveratrol (DM+RSV)	$329.83 \pm 9.51^a$	$0.51 \pm 0.02^b$

Significance:  $^aP < 0.001$ ;  $^bP < 0.01$  versus DM.

DNJ- $0,75 \text{ mgKg}^{-1}$  body weight/day for 14 days, RSV- $30 \text{ mgKg}^{-1}$  body weight/day for 14 days.

Since redox imbalance is causally linked to inflammatory processes, we evaluated the expression levels of some major factors implicated in inflammation in the diabetic mice model. Throughout exercise, in the DM group, IL-6 mean concentrations were quantitatively greater than in the control group. As shown in figure 1, mice in diabetic group showed significant increase in serum concentrations of IL-6 as compared with control group. Administration of RSV or DNJ once daily for 14 days a significant decrease in IL-6 level compared to the diabetic group ( $P < 0.05$ ), (Fig.1).

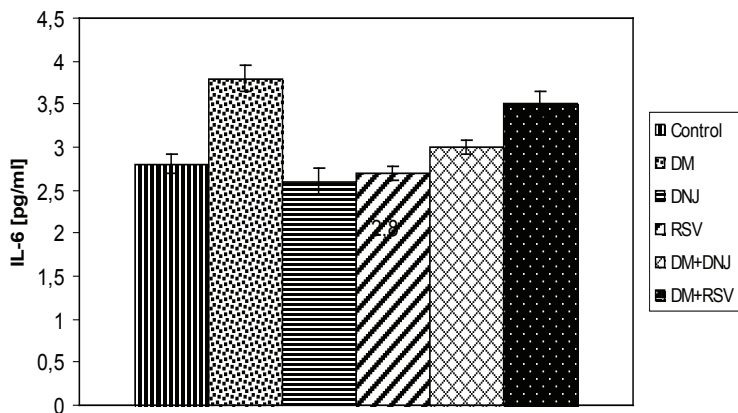


Fig. 1. Effect of 1-deoxyojirimycin and resveratrol on inflammatory cytokine IL-6 in STZ-induced diabetic mice. Values are given as means  $\pm$  SD of at least ten values.

We also found that the activities of enzymatic antioxidants (GPx, SOD and CAT) were significantly reduced in the liver tissue of the diabetic animals, but they were significantly improved following treatment with the DNJ and RSV for 14 days ( $P < 0.05$ ), (Fig. 2-4).

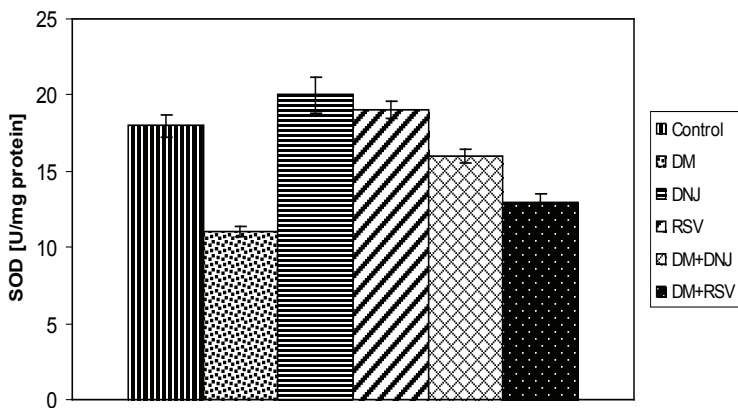


Fig. 2. Effect of 1-deoxyojirimycin and resveratrol on superoxide dismutase (SOD) activities in the liver tissue of normal and streptozotocin induced diabetic mice.

Values are given as means  $\pm$  SD of at least ten values.

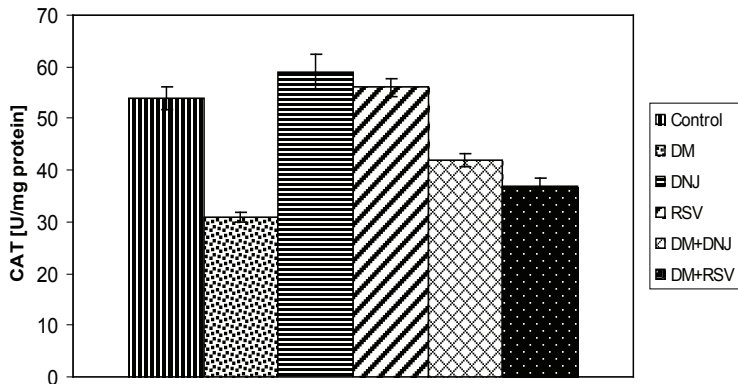


Fig. 3. Effect of 1-deoxyojirimycin and resveratrol on catalase (CAT) activities in the liver tissue of normal and streptozotocin induced diabetic mice. Values are given as means  $\pm$  SD of at least ten values.

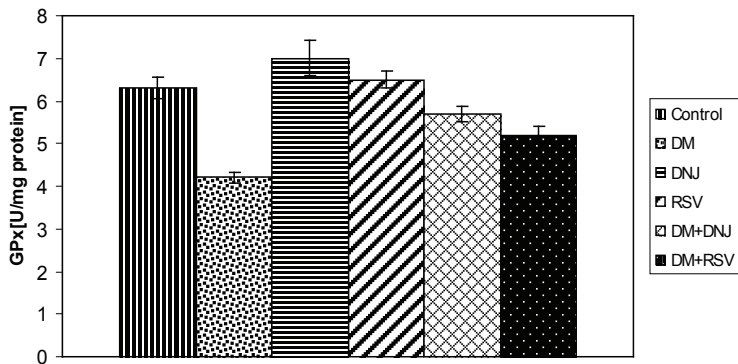


Fig. 4. Effect of 1-deoxyojirimycin and resveratrol on glutathione peroxidase (GPx) activities in the liver tissue of normal and streptozotocin induced diabetic mice. Values are given as means  $\pm$  SD of at least ten values.

DM resulted in significant decrease in the level of reduced glutathione (GSH) when compared to normal control. Administration of RSV or DNJ once daily for 14 days a significant increase in GSH level compared to the diabetic group ( $P < 0.05$ ), (Fig. 5).



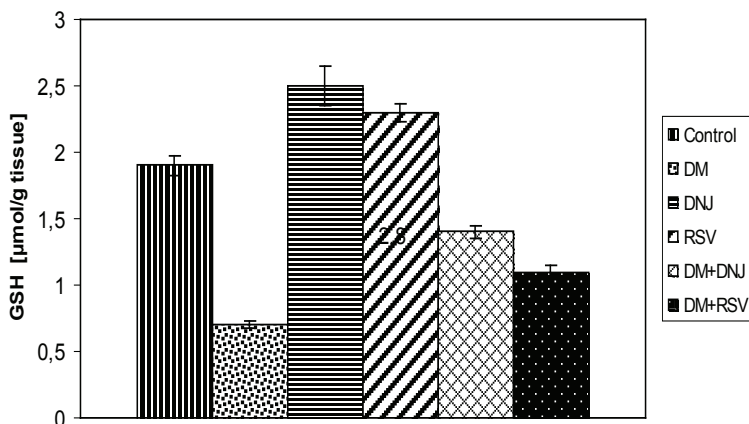


Fig. 5. Effect of 1-deoxynojirimycin and resveratrol on concentration of reduced glutathione (GSH) in the liver tissue of normal and streptozotocin induced diabetic mice. Values are given as means  $\pm$  SD of at least ten values.

The many studies indicate that hyperglycemia-induced diabetic complications are likely from oxidative dysfunction and inflammatory effect (Murugan and Pari, 2006; Dandona et al., 2004). In prevention and complementary therapy in early stages and of type 1 diabetes phytotherapy may be beneficial. For several plant materials hypoglycaemic effect had been found both in animal studies and in clinical trials (Greń and Formicki, 2012; Greń et al., 2013). Although some of them have not been tested clinically but have been used for a long time in traditional medicine. Among medicinal plants that may be efficient in insulin – resistance and in treatment and prevention of type 1 diabetes mellitus the most popular is *Morus alba L.* Among the biologically active ingredients contained in the morus, most important therapeutic attributed to 1-deoxynojirimycin (DNJ) and resveratrol (RSV) (Zdrojewicz Z, Belowska-Bień, 2005). Hyperglycemia increases the inflammatory markers tumor necrosis factor  $TNF-\alpha$ , interleukin IL-1 and IL-6 (Dhindsa et al., 2004; Pickup, 2004). Overproduced proinflammatory cytokines enhances inflammatory stress in diabetes and diabetic complications. A great deal of evidence indicates close ties between inflammatory and metabolic pathways (Pickup and Mattock, 2003; Wellen and Hotamisligil, 2005; Hotamisligil et al., 1996). Several studies suggested that  $TNF-\alpha$ , IL-6 and other inflammatory mediators may activate the intracellular pathways, such as the I-kappa, I-kappa-B, kinase-b, nuclear factor-kappa B and the protein c-Jun N-terminal kinase axes, amplify and aggravate low-grade inflammation, and these processes may become self-perpetuating through a positive feedback loop created by the proinflammatory cytokines, and lead to diabetes (Shoelson et al., 2006; Ishihara and Hirano, 2002). In diabetes, the suppressed proliferation potential of diabetic T lymphocytes with mitogen stimulated was achieved by a decreased expression of adenosine kinase (Sakowicz-Burkiewicz et al., 2006). The increased production of IL-6 and  $IFN-\gamma$  by STZ-induced diabetic rats was identified as autoimmune diabetes (Shoelson et al., 2006). These results our research support the fact that biologically active components of mulberry are a potent agent against diabetes-associated inflammatory injury via inhibiting inflammatory cytokines production. Both inflammation

and oxidative stress play a major role in the development of tissue insulin resistance (Dandona et al., 2004). Oxidative stress may be an important factor in the pathogenesis of different diabetic complications (Sakowicz-Burkiewicz et al., 2006).

In biological systems, antioxidants such as GSH, a major nonenzymatic antioxidant involved in the maintenance of the redox balance, ameliorate cellular oxidative damage. At cellular and molecular levels, redox imbalance causes the activation of redox-sensitive transcription factors that lead to inflammation (Chung et al., 2001). Therefore, enhanced oxidative stress due to uncontrolled ROS is a major factor in both acute and chronic inflammation and inflammatory-related diseases including diabetes (Lin et al., 2005). The decrease in markers of oxidative stress in diabetes was attenuated by 1-deoxynojirimycin and resveratrol. This suggests that DNJ and RSV would probably ameliorate diabetic oxidative stress. In diabetes mellitus, oxidative stress may be attributed to a combination of hyperglycemia-induced glycooxidation, sorbitol system activation, and reduced GSH synthesis. Reduced activities of GPx, CAT, and SOD in hepatic tissues of diabetic group mice were observed in our study. Treatment with DNJ and RSV reversed this change. According to the results, *Morus alba L.* may have an antioxidative effect against the pathological alterations caused by ROS.

## CONCLUSION

The pathogenesis of diabetes and diabetes complications is complex. 1-deoxynojirimycin and resveratrol has a beneficial effect in the STZ-induced diabetic animal model. The present investigation shows that DNJ or RSV possesses several treatment-oriented properties, including the control of hyperglycemia, antioxidant effects, and anti-inflammatory effects. Considering these observations, it appears that biologically active components of mulberry may be a useful adjunct supplement to delay the development of diabetes and diabetes complications. However, more work is necessary to further elucidate the role of 1-deoxynojirimycin and resveratrol, particularly looking at the underlying mechanism of treatment.

## REFERENCES

- AEBI, H.: Catalase in vitro. *Met. Enzym.*, 105:121–126, 1984.
- ASANO, N., TOMIOKA, E., KIZU, H., MATSUI, K.: Sugars with nitrogen in the ring isolated from the leaves of *Morus bombycis*. *Carbohydr. Res.*, 253:235–245, 1994.
- ASANO, N., YAMASHITA, T., YASUDA, K., IKEDA, K., KIZU, H., KAMEDA, Y., KATO, A., NASH, R.J., LEE, H.S., RYU, K.S.: Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba L.*) and silkworms (*Bombyx mori L.*). *J. Agric. Food Chem.*, 49:4208–4213, 2001.
- BRADFORD, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.*, 72(1-2):248–254, 1976.
- CERIELLO, A., TESTA, R.: Antioxidant anti-inflammatory treatment in type 2 diabetes. *Diab. Care.*, 32(2):S232–S236, 2009.
- CHUNG, H.Y., KIM, H.J., KIM, J.W., YU, B.P.: The inflammation hypothesis of aging: molecular modulation by calorie restriction. *Ann. NY. Acad. Sci.*, 928:327–335, 2001.

DANDONA, P., ALJADA, A., BANDYOPADHYAY, A.: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immun.*, 25(1)4–7, 2004.

DESHPANDE, M.C., VENKATESWARLU, V., BABU, R.K., TRIVEDI, R.K.: Design and evaluation of oral bioadhesive controlled release formulations of miglitol, intended for prolonged inhibition of intestinal alpha-glucosidases and enhancement of plasma glycogen like peptide-1 levels. *Int. J. Pharm.*, 380:16–24, 2009.

DHINDSA, S., TRIPATHY, D., MOHANTY, P.: Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor- $\kappa$ B in mononuclear cells. *Metabolism: Clin. Exp.*, 53(3)330–334, 2004.

FLOHE, L., OTTING, F.: Superoxide dismutase assays. *Met. Enzym.*, 105:93–104, 1984.

GILMAN, A.G., RALL, T.W., NIES, A.S., TAYER, P.: Goodman and Gilman's The Pharmacological Basis of Therapeutics (8th ed.). New York: Pergamon, pp. 1317–1322, 1463–1495, 1990.

GREŃ, A., FORMICKI, G.: Antioxidants in therapy and prevention of diabetes mellitus. In *Xenobiotics : soil, food and human health interactions*, Rzeszów, Wydawnictwo Uniwersytetu Rzeszowskiego, pp. 276-291, 2012.

GREŃ, A., FORMICKI, G., KAPUSTA, E., SZAROMA, W., MUCHACKA R., KOPAŃSKA, M., KRASKA, K.: Effect of some foodstuffs on the development in diabetes. *JMBFS*, 2:1205-1214, 2013.

HIKINO, H., MIZUNO, T., OSHIMA, Y., KONNO, C.: Isolation and hypoglycemic activity of moran A, a glycolprotein of *Morus alba* root barks. *Planta Med.*, 51:159–160, 1985.

HOTAMISLIGIL, G.S., PERALDI, P., BUDAVARI, A., ELLIS, R., WHITE, M.F., SPIEGELMAN, B.M.: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- $\alpha$ - and obesity-induced insulin resistance. *Science.*, 271(5249)665–668, 1996.

ISHIHARA, K., HIRANO, T.: IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytok. Grow. Fact. Rev.*, 13(4-5)357–368, 2002.

JAKUS, V.: The role of free radicals, oxidative stress antioxidant systems in diabetic vascular disease. *Bratisl. Lek. List.*, 101:541–51, 2000.

JUNGE, B., MATZKE, M., STOLTEFUSS, J.: *Chemistry and structure-activity relationships of glucosidase inhibitors*. In *Handbook of Experimental Pharmacology*, 119. Springer-Verlag; New York, pp. 411–482, 1996.

Kimura, M., Chen, F., Nakashima, N., Kimura, I., Asano, N., Koya, S.: Antihyperglycemic effects of N-containing sugars derived from mulberry leaves in streptozocin-induced diabetic mice. *J. Trad. Med.*, 12:214–219, 1995.

KING, G.L., LOEKEN, M.R.: Hyperglycemia-induced oxidative stress in diabetic complications. *Histochem. Cell Biol.*, 122:333–8, 2004.

KONG, W.H., OH, S.H., AHN, Y.R., KIM, K.W., KIM, J.H., SEO, S.W.: Antiobesity effects and improvement of insulin sensitivity by 1-deoxynojirimycin in animal models. *J. Agric. Food Chem.*, 56:2613–2619, 2008.

LEDOUX, S.P., WOODLEY, S.E., PATTON, N.J., WILSON, L.G.: Mechanisms of nitrosourea-induced beta-cell damage. Alterations in DNA. *Diabetes*, 35:866–872, 1986.

LIN, Y., BERG, A.H., IYENGAR, P.: The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J. Biol. Chem.*, 280(6)4617–4626, 2005.

MCANULTY, L.S., MILLER, L.E., HOSICK, P.A., UTTER, A.C., QUINDRY, J.C., MCANULTY, S.R.: Effect of resveratrol and quercetin supplementation on redox status and inflammation after exercise. *Appl. Physiol. Nutr. Metab.*, 38(7)760-765, 2013.

MURUGAN, P., PARI, L.: Antioxidant effect of tetrahydrocurcumin in streptozotocin-nicotinamide induced diabetic rats. *Life Sci.*, 79(18)1720–1728, 2006.

PAGLIA, D.E., VALENTINE, W.N.: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70(1)158–169, 1967.

PICKUP, J.C.: Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diab. Care.*, 27(3)813–823, 2004.

PICKUP, J.C., MATTOCK, M.B. Activation of the innate immune system as a predictor of cardiovascular mortality in type 2 diabetes mellitus. *Diab. Med.*, 20(9):723–726, 2003.

RERUP, C.C.: Drugs producing diabetes through damage of the insulin secreting cells. *Pharmacol. Rev.*, 22:485–518, 1970.

SAKOWICZ-BURKIEWICZ, M., KOCBUCH, K., GRDEN, M., SZUTOWICZ, A., PAWELCZYK, T.: Diabetes-induced decrease of adenosine kinase expression impairs the proliferation potential of diabetic rat T lymphocytes. *Immun.*, 118(3)402–412, 2006.

SEDLAK, J., LINDSAY, R.H.: Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.*, 25(C)192–205, 1968.

SHOELSON, S.E., LEE, J., GOLDFINE, A.B.: Inflammation and insulin resistance. *J. Clin. Invest.* 116:1793-1801, 2006.

WELLEN, K.E., HOTAMISLIGIL, G.S.: Inflammation, stress, and diabetes. *J. Clin. Invest.* 115(5)1111–1119, 2005.

YAGI, M., KOUNO, T., AOYAGI, Y., MURAI, H.: The structure of moranoline, a piperidine alkaloid from *Morus species*. *Nip. Nogeik. Kais.*, 50:571–572, 1976.

ZDROJEWICZ, Z., BELOWSKA-BIEŃ, K.: Resveratrol-its activity and clinical role. *Adv. Clin. Exp. Med.*, 14(5)1051-1056, 2005.

ZHAO, K., SU, Z.R., YANG, B.W., TAN, F., DENG, J.: Determination of resveratrol and polydatin in mulberry. *Food Sci.*, 31(14)241-244, 2005.

## ZAŠTITINI EFEKT 1-DEOXYNOJIRIMYCINA I RESVERATROLAPROTIV DIABETIČKOG OKSIDATIVNOG STRESA I INFLAMACIJE

AGNIESZKA GREŃ, GRZEGORZ FORMICKI, NORBERT LUKAČ,  
PETER MASSÁNYI, ROBERT STAWARZ

### Izvod

Dijabetes melitus (DM) je indukovana kod miševa grupe II, V, i VI. Kod životinje III eksperimentalne grupe je primenjen 1-deokinojirimicin (DNJ) u dozi 0,75 mg/kg<sup>-1</sup> telesne težine dnevno, tokom 14 dana. Kod miševi IV eksperimentalne grupe primenjen je resveratrol (RSV) u dozi 30 mg/kg<sup>-1</sup> telesne težine dnevno, toko 14 dana. Životinje grupa V: dijabetična grupa, primala je DNJ, a VI dijabetična grupa je primala RSV. Trideset minuta nakon poslednje injekcije, životinje su anestetizirani i dekapitovane. Se-

rumski nivo interleukin - 6 (IL - 6) je ocenjen kao surogat marker inflamacije. Nivoi redukovanog glutationa u jetre (GSH), aktivnost glutation peroksidaze (GPK), katalaze (CAT) i superoksid dismutaze ( SOD ) mereni su kao surogat marker oksidativnog stresa. Serumski nivo insulina u plazmi i nivoa glukoze su ocenjeni kao surogat marker dijabetesa. Administracija DNJ ili RSV značajno utiče na obnavljanje glukoze, insulina, IL-6, kao i nivo aktivnosti GSH i SOD, CAT, GPKS u dijabetičnih miševa, do nivoa vrlo sličnih onom u kontrolne grupe. Rezultati su pokazali da DNJ i RSV sa svojim antidijabetičnim, antizapaljenskim i antioksidantnim svojstvima, može biti potencijalni biljni lek u lečenju dijabetesa i problema jetre.

**Ključne reči:** *oksidativni stres, inflamacija, 1-deoxynojirimycin, resveratrol, diabetes.*

Received / *Primljen*: 10.02.2014.

Accepted / *Prihvaćen*: 25.02.2014.

## ZINC CONCENTRATION IN THE MILK SERUM OF COWS IN THE EARLY AND MID LACTATION\*

ZORANA KOVAČEVIĆ, DRAGICA STOJANOVIĆ, IVANA DAVIDOV,  
GORDANA ŽUGIĆ, MIODRAG RADINOVIĆ, MIHAJLO ERDELJAN<sup>1</sup>

**SUMMARY:** The cows in early lactation take less food than they need to produce milk, which is reflected in the change in the metabolic status of cows and can affect to the health of the cows significantly. *There are* lower zinc concentration in the blood in early lactation, which is related to increased number of somatic cells in milk and occurrence of inflammatory processes. *The primary function of zinc is as an antioxidant. It is the micronutrient which has been established as an essential component of the dairy cow diet for maintaining health.* Also, zinc plays an important role in the immune response. The total content of zinc in the body is affected by strong homeostatic regulation. *For human consumption the best source of zinc are foods of animal origin. The value of zinc concentration in the milk serum of cows in early lactation was  $24.78 \pm 13.4 \mu\text{mol/l}$ , while in mid lactation it was  $27.62 \pm 12.8 \mu\text{mol/l}$ .* The results of our study shows that zinc concentration in the milk serum of cows at different periods of lactation, in first and in sixth month of lactation is approximate and stable during lactation.

**Key words:** zinc, milk serum, lactation, cow.

### INTRODUCTION

The cows in early lactation take less food than they need to produce milk, which is reflected in the change in the metabolic status of cows and can affect to the health of

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Zorana Kovačević, Mr Pharm, Teaching Assistant, Dragica Stojanović, DVM, PhD, Associate professor, Ivana Davidov, DVM, PhD, Assistant professor, Miodrag Radinović, DVM, PhD, Assistant professor, and Mihajlo Erdeljan, DVM, Teaching Assistant. University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia. Gordana Žugić, PhD, Agencija za lekove i medicinska sredstva Srbije, Vojvode Stepe 458, 11000 Belgrade, Serbia. Corresponding author: Zorana Kovačević, e-mail: zoranakovacevic@yahoo.co.uk, phone.: +381/21-485-3524.

\*This paper is the part of project TR31062, supported by Ministry of Science and Education, Serbia.

the cows significantly (Cincović et al., 2011; Cincović et al., 2012). There are lower zinc concentration in the blood in early lactation, which is related to increased number of somatic cells in milk and occurrence of inflammatory processes (Davidov et al., 2013).

The primary function of zinc is as an antioxidant. It is the micronutrient which has been established as an essential component of the dairy cow diet for maintaining health (Andrieu, 2008). Zinc is an essential component of many enzymes involved in the synthesis of DNA and RNA. He enters in the composition of the enzyme that prevents free radical activity (Prasad et al., 2004; Gressley, 2009). Also, zinc plays an important role in the immune response (Weiss and Spears, 2006).

The total content of zinc in the body is affected by strong homeostatic regulation. The amount of zinc excreted in the feces is proportional to food intake and the amount of zinc in the body. Homeostatic control mechanisms are complex and the main ways of the changes are in the extent of absorption of the meal, excretion in urine, feces and milk, as well as the deposit of non-harmful forms of trace element which can be mobilized in the case of a defect. (Baker and Hammerman, 1995). Approximately 20-30% of the food ingested zinc is absorbed, mainly in the duodenum and proximal jejunum. Absorption of zinc in both processes: passive diffusion and active transport (Lee et al., 1989, Cope et al., 2009). The absorption of zinc is affected by numerous factors such as the solubility of zinc in the intestinal tract, species and categories of animals, the composition of food and the most important content of zinc in the diet (Wiking et al., 2008; Cope et al., 2009; Rabbiee et al., 2010). After intestinal absorption, zinc enters the portal circulation and distributes by blood to tissues and organs bounded to albumin (Gordon et al., 1981). Content of zinc in blood is 0,5 % of the total amount of zinc in the body. There is 75-88% zinc in erythrocytes, 12-22% in plasma and in leukocytes and platelets around 3% (Kolarski, 1995). For human consumption the best source of zinc are foods of animal origin. Most zinc is in seafood and in red meat. Poultry, eggs, cheeses, milk, yogurt, nuts and whole grain cereals are good sources of zinc. Amount of zinc in cow milk is 0.3-0.4mg/100g (Wiking et al., 2008).

Adequate concentrations of zinc in the food, and consequently in the blood, have positive impact on proper immune response while zinc deficiency leads to improper functioning of the immune system and irregular keratinisation (Hutcheson, 1989; Reddy and Frey, 1990).

The content of zinc in the milk of dairy cows did not show high variability, and its average value was within the recommended limit for zinc content in consumer milk (Hosnedlova et al., 2005).

The aim of this study was to determinate the zinc concentration in milk serum in dairy cows in the early and mid period of lactation.

## MATERIAL AND METHODS

The study was performed on thirty Holstein cows approximate same body weight, ages 3 to 5 years and in first to third lactation, and they giving approximately the same amount of milk. All cows were stabling with dry straw for bedding and with *ad libidum* access to potable water, and feed by total mixed ration. The total mixed ration contained maize silage, grass silage, cracked wheat, soybean meal, rapeseed meal, sugar beet and hay.

The milk samples were taken in two productive periods, in the postpartum period

(in the first lactating month) and in the mid lactation (in the sixth lactating month). The same sampling procedure was used each time.

Milk from all four quarters was taken before morning milking. When quarter milk samples were taken the teat ends were disinfected and milk samples were then stored at room temperature for 48 hours due to allocation of milk serum. Featured milk serum of cows in the first and sixth month of lactation were centrifuged at 1200 rpm for 15 minutes to completely set aside serum, and then frozen at  $-20^{\circ}\text{C}$  for further analysis. When we have collected all the samples of milk, milk serum was further analyzed by atomic absorption spectrometry (AAS) on a Perkin Elmer Elan 6100 ICPMS, Massachusetts, USA.

For statistical analysis we used test of correlation by Statgraphic Centurion (USA). The first step was to determinate a descriptive statistical parameters. The difference in the concentration of zinc in the milk serum in the first and sixth months of lactation was investigated by Wilcoxon test, while the correlation between the concentration of zinc in the first and sixth month is determined by the Spearman correlation test with post-hoc analysis of variance.

## RESULTS AND DISCUSSION

The value of zinc concentration in the milk serum of cows in early lactation was  $24.78 \pm 13.4 \mu\text{mol/l}$ , while in mid lactation it was  $27.62 \pm 12.8 \mu\text{mol/l}$ . These results are shown in figure 1. and in table 1.

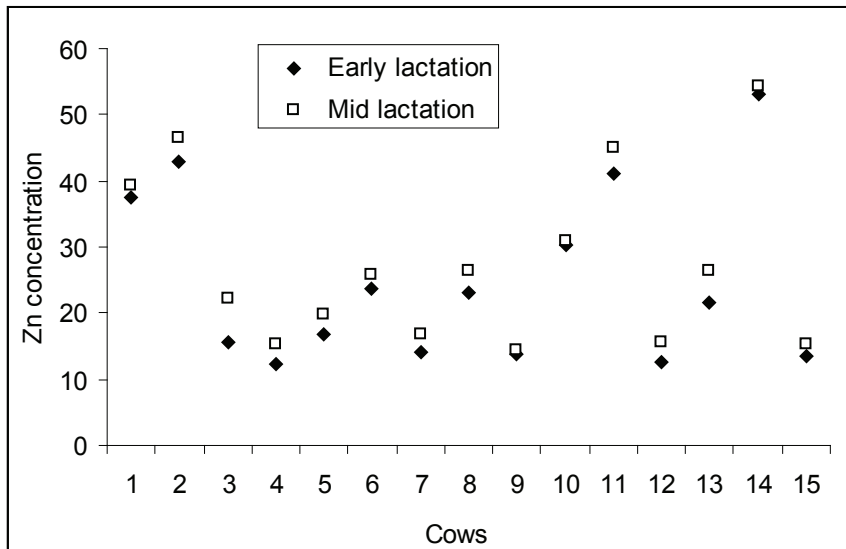


Figure 1. Presentation of the concentration of zinc in cows during two periods of lactation



Table 1. Descriptive statistics for the value of zinc in the milk serum of cows

	Early lactation	Mid lactation
Count	15	15
Average	24,78 <sup>a</sup>	27,62 <sup>a</sup>
Standard deviation	13,1458	12,9856
Coeff. of variation	53,05%	47,015%
Minimum	12,4	14,3
Maximum	53,1	54,4
Range	40,7	40,1
Std. Skewness	1,49566	1,3767
Std. Kurtosis	-0,216956	-0,328336

<sup>a</sup> Values with the same superscripts are not significantly different ( $p > 0.05$ ).

The difference in the concentration of zinc in the milk serum in the first and in the sixth month of lactation did not show statistically significant ( $p > 0,05$ ). These results are shown in figure 2. and in figure 3.

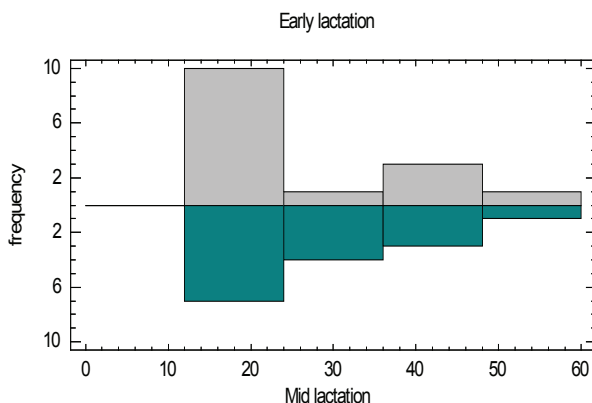


Figure 2. Frequency distribution of zinc concentration in the milk serum in early and mid lactation

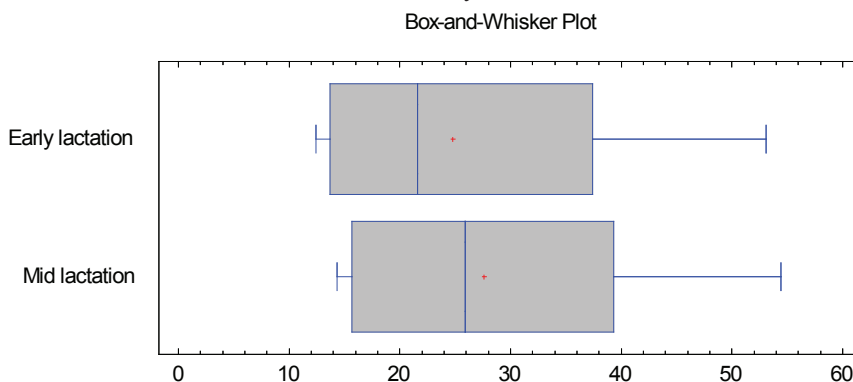


Figure 3. The average concentration (line) median (dot) and standard deviation of zinc concentration in the early and mid lactation

There is significant positive correlation between the value of zinc in the first and in the sixth month of lactation ( $r = 0.99$ ;  $r^2 = 98,54\%$ ; standard error of estimation = 1.62). Using regression analysis there was a formula that describes the relationship between concentration of zinc in the milk serum in the first and in the sixth month of lactation and it is:

$Zn \text{ mid lactacion} = 3.32086 + 0.989595 \times Zn \text{ early lactacion}$ . Analysis of variance indicates a high statistical significance this regressim model ( $p < 0,01$ ) and it can be seen in figure 4. and in table 2.

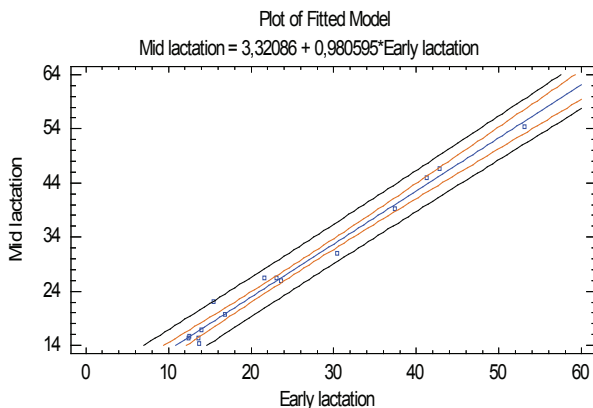


Figure 4. Linear regression and correlation of zinc values in the milk serum between the two groups

Table 2. Analysis of variance of the linear model to investigate the relationship between the concentration of zinc in the early and mid lactation

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	2326,38	1	2326,38	880,05	0,00001
Residual	34,365	13	2,64346		
Total (Corr.)	2360,74	14			

Since there is a significant positive correlation between the value of zinc in the two periods of lactation and that the difference in the concentration of zinc in milk serum is not statistically significant it could be concluded that the value of the zinc in milk serum is stable during time which is shown in the results of the other authors like Hosnedlova et al. (2005.) where the content of zinc in the milk of dairy cows did not show high variability, and its average value was within the recommended limit for zinc content in consumer milk. Since there is a lack of available results in literature about zinc concentration during the lactation period it could be concluded that concentration of zinc is stable during the lactation.

## CONCLUSION

Zinc concentration in the milk serum of cows in early lactation was  $24.78 \pm 13.4$   $\mu\text{mol/l}$ , while in mid lactation it was  $27.62 \pm 12.8$   $\mu\text{mol/l}$  and this value of zinc concentration in the milk serum of cows at different periods of lactation, in first and in sixth month, is approximate and stable during lactation.

## REFERENCES

- ANDIEU, S.: Is the role for organic trace element supplements in transition cow health? *Vet. J.*, 176:77-83, 2008.
- BAKER, D.H., HAMMERMAN, C.B.: Zinc availability. In *Bioavailability for nutrients for animals*. C.B. Hammerman, D.H. Baker, A.J. Lewis Eds. Academic Press, San Diego, USA, 367-398, 1995.
- CINCOVIĆ, M.R., BELIĆ, B., VIDOVIĆ, B., KRČMAR, L.J.: Reference values and frequency distribution of metabolic parameters in cows during lactation and in pregnancy. *Contemporary agriculture*, 60(1-2)175-182, 2011.
- CINCOVIĆ, M.R., BELIĆ, B., RADOJIČIĆ, B., TOHOLJ, B., STANČIĆ, I., STEVANČEVIĆ, M.: Periparturient metabolic profile, blood picture and body condition score in healthy cows and cows with associated periparturient disease. *Contemporary agriculture*, 61(3-4)205-220, 2012.
- COPE, C.M., MACKENZIE, A.M., WILDE, D. AND SINCLAIR, L.A.: Effects of level and form of dietary zinc on dairy cow performance and health. *J. Dairy Sci.*, 92:2128-2135, 2009.
- DAVIDOV, I., RADINOVIĆ, M., ERDELJAN, M., CINCOVIĆ, M.R., STANČIĆ, I., BELIĆ, B.: Relations between blood Zinc concentrations and udder health in dairy cows. *Rev. Vet. Med.*, 164(4)183-190, 2013.
- GRESSLY, T.F.: Zinc, copper, manganese and selenium in dairy cattle rations. *Proceedings of the 7<sup>th</sup> Annual Mid-Atlantic Nutrition Conference*, pp.65-71, 2009.
- GORDON, F.E., GORDON, C.R., PASSAL, B.D.: Zinc metabolism-Basic, clinical and behavioral aspects. *J. Pediatr.*, 99:341-349, 1981.
- HOSNEDLOVA, B., TRAVNICEK, J., CHRASTNY, V.: Zinc and copper concretion in milk of dairy cows in the south Bohemia region. *XII<sup>th</sup> International Congress, ISAH 2005*, Warsaw, Poland, Vol I, pp 256.
- HUTCHESON, D.P.: Nutritional factors affect immune response in cattle. *Feedstuffs*, 61:16-24, 1989.
- KOLARSKI, D.: *Osnovi ishrane domaćih životinja*. Naučna knjiga. Beograd, 1995.
- LEE, H.H., PRASAD, S.A., BREWER, J.G., OWZANG, C.: Zinc absorption in human small intestine. *Am. J. Physiol.*, 256:687-691, 1989.
- PRASAD, A.S., BAO, B., BECK, Jr. F.W., KUCUK, O. SARKAR, F.H.: Antioxidant effects of zinc in humans. *Free Radic. Biolo. and Med.*, 37:1182-1190, 2004.
- RABIEE, A.R., LEAN, I.J., STEVENSON, M.A. AND SOCHA, M.T.: Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: A meta-analysis. *J. Dairy Sci.*, 93:4239-4251, 2010.
- REDDY, P.G., FREY, R.A.: Nutritional modulation of immunity in domestic food animals. *Adv. Vet. Sci. Comp. Med.*, 35:255-281, 1990.
- WEISS, W.P., SPEARS J.W.: Vitamin and trace mineral effect on immune function of ruminants. In: Sejrnsen K., Hvelplund T., Nielsen M.O. (Eds.), *Ruminant Physiology*. Wageningen Academic Publishers, Utrecht, The Netherlands, pp. 473-496, 2006.
- WIKING, L., LARSEN, T. AND SEHESTED, J.: Transfer of dietary zinc and fat to milk-evaluation of milk fat quality, milk fat precursors and mastitis indicators. *J. Dairy Sci.*, 91:1544-1551, 2008.

## KONCENTRACIJA CINKA U MLEČNOM SERUMU KRAVA U RANOJ I SREDNJOJ LAKTACIJI

ZORANA KOVAČEVIĆ, DRAGICA STOJANOVIĆ, IVANA DAVIDOV,  
GORDANA ŽUGIĆ, MIODRAG RADINOVIĆ, MIHAJLO ERDELJAN

### Izvod

Kod krava u ranoj laktaciji uzimaju manje hrane nego što im je potrebno za proizvodnju mleka, što se ogleda u nastanku promena u metaboličkom statusu krava i može značajno uticati na zdravlje krava. U ranoj laktaciji postoje snižene koncentracije cinka u krvi, što je u vezi sa povećanim brojem somatskih ćelija u mleku i nastankom upalnih procesa. Primarna funkcija cinka je antioksidantna. To je mikronutritient za koji je utvrđeno da je bitan sastojak u ishrani mlrčnih krava za održavanje zdravlja. Takođe, cink ima značajnu ulogu i u imunološkom odgovoru organizma. Ukupan sadržaj cinka u organizmu je pod uticajem jake homeostatske regulacije. Za ishranu ljudi, najbolji izvor cinka su namirnice animalnog porekla. Koncentracija cinka u mlečnom serumu krava u ranoj laktaciji je iznosila  $24.78 \pm 13.4 \mu\text{mol/l}$ , dok je u srednjoj laktaciji iznosila  $27.62 \pm 12.8 \mu\text{mol/l}$ . Rezultati našeg istraživanja ukazuju da je koncentracija cinka u mlečnom serumu krava u različitim periodima laktacije ima približne vrednosti i tokom perioda laktacije je stabilna.

**Ključne reči:** cink, mlečni serum, laktacija, krava.

Received / *Primljen*: 22.11.2013.

Accepted / *Prihvaćen*: 21.02.2014.

## SCREENING TEST FOR DETECTION OF COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) SENSITIVITY TO ABAMECTIN\*

SLAVICA VUKOVIĆ, DUŠANKA INĐIĆ,  
SONJA GVOZDENAC, SNEŽANA TANASKOVIĆ<sup>1</sup>

*SUMMARY: Screening test was carried out to assess the sensitivity to 19 filed populations of Colorado potato beetle (*Leptinotarsa decemlineata* Say.) to abamectin during 2008 (Futog, Čenej, Vilovo, Kovilj and Čurug), 2009 (Stepanovićevo, Gložane, Žabalj, Sirig, Šajkaš, Ljutovo, Šimuže, Bačko Gradište, Novo Miloševo i Kikinda) and 2012 (Zmajevo, Despotovo, Vilovo and Čurug). The test provided a quick assessment of sensitivity of overwintered adults to abamectin (commercial insecticide Abastate) applied at label rate (0.75 l/ha), two-fold (1.5 l/ha) and five-fold higher rates (3.75 l/ha), by soaking method (5 sec). Mortality was assessed after 72 h of exposition. Results were corrected for the mortality in the control and presented as efficacy (%) after the treatment. According to IRAC (method 011), with slight corrections, the sensitivity of Colorado potato beetle was classified by scale 1-5 (1 - highly sensitive population ( $E = 100\%$ ); 2 - sensitive ( $100 > E \geq 95\%$ ); 3 - slightly resistant ( $95 > E \geq 90\%$ ); 4 - resistant ( $90 > E \geq 50\%$ ); 5 - highly resistant ( $E < 50\%$ ). From five tested populations in 2008, four were highly sensitive ( $E = 100\%$ ) to abamectin at label rate and one was sensitive ( $E = 97.5\%$ ). In 2009, out of ten tested populations, three were slightly resistant ( $E = 92.6-94.6\%$ ), five were resistant ( $E = 64.6-88.7\%$ ) and two were highly resistant ( $E = 22.5-48.3\%$ ) to abamectin at label rate. Out of four tested populations in 2012, one was highly sensitive ( $E = 100\%$ ), one was sensitive ( $E = 95.8\%$ ) and two were slightly resistant ( $E = 90.6-94.2\%$ ). The survival of adults in treatments with higher rates of abamectin, that the label one, prove that there is a part of population which has endured changes in susceptibility or selection to resistance.*

**Key words:** screening test, Colorado potato beetle, sensitivity, abamectin.

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Slavica Vuković, PhD, assistant professor, Dušanka Inđić, PhD, full professor, Sonja Gvozdenc, MSc, research associate, University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, Novi Sad, Snežana Tanasković, PhD, assistant professor, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia.

Corresponding author: Slavica Vuković, e-mail: vukovic@polj.uns.ac.rs, Phone: +381214853333.

\* The research was conducted within the project III 46008, funded by the Ministry of Education and Science of the Republic of Serbia.

## INTRODUCTION

Pesticide use in agriculture has, aside from well known advantages, caused some negative effects in the course of time. Such are changes in sensitivity of harmful insects, or better known, their resistance to insecticides. The most frequently mentioned example for the resistance occurrence is Colorado potato beetle (CPB), which represents the most harmful species in potato production.

Considering the facts about the share of potato in human nutrition, amounts and diversity of pesticides (insecticides, fungicides, herbicides) used for protection of this crop even in storage process, and the quality of this feed stuff, the risk of pesticide residues is of great importance (Indić et al., 2006a). To ensure the expected yield in Serbia potato crops are treated with insecticides two to four times during vegetation (Zabel et al., 2000). According to several authors, CPB resistance to carbamates and organophosphates, as well as to pyrethroids (Indić, 1997; Perić et al. 1997) was registered in a number of populations in Serbia (Indić, 1997; Perić et al., 1997; Stanković et al., 2004; Indić et al, 2006; Indić et al., 2009; Marčić and Perić, 2009; Indić et al., 2012). Literature data indicate that CPB resistance was noted for 42 insecticides, belonging to different chemical groups (organophosphates, carbamates, pyrethroids), which justifies the fact of CPB ranking among 10 species that most rapidly develop resistance (Mota-Sanchez et al., 2006; Whalon et al., 2008). In practice, the problem of resistance in our region is resolved in accordance with principles of integrated pest management using different measures (manual collection, rarely bioinsecticides and growth regulators) which reduces the selection pressure of insecticides to insects. However, farmers that have large areas under potato production, mostly use chemical measures. Thus, within the strategy of delay or slowing down the resistance process it is recommended to use mixtures of insecticides with different modes of action. Although, other measures are familiar, such as predators and parasitoids, plant extracts, joined cropping (Copping and Menn, 2000; Gokce et al., 2006), the selection of potato varieties to CPB resistance, or spatial distance of areas under potato production, which directly or indirectly reduce selection pressure of insecticides, they are not common in practice.

The aim of the study was the creation of simple and rapid test for detection of sensitivity levels of CPB to insecticides and confirmation of the resistance, as well as providing simplified presentation of the results. The method is based on the assessment of sensitivity of overwintered adults to insecticides. It allows a quick formation (24-72 h) of information network related to production regions and on these bases further establishment of strategy for rational use of insecticides in CPB control. However, the insight into the part of population which survived the insecticides applied at label and higher rates, allow us to make statement that a certain part of population has endured changes in sensitivity.

## MATERIAL AND METHODS

The site selection. For monitoring of CPB sensitivity to insecticides, based on the advice of experts from Agricultural extension service, 19 sites on the territory of Serbia during 2008 (Futog, Čenej, Vilovo, Kovilj and Črug), 2009 (Stepanovićevo, Gložane, Žabalj, Sirig, Šajkaš, Ljutovo, Šimuže, Bačko Gradište, Novo Miloševo and Kikinda) and 2012 (Zmajevo, Despotovo, Vilovo and Črug) were selected and marked with GPS

coordinates.

Applied insecticide. Insecticide Abastate (a.i. abamectin) was applied at 0.75 l/ha – label rate; 1.5 l/ha – two-fold label rate and 3.75 l/ha – five-fold label rate.

Test insect. This bioassay included overwintered adults of CPB field populations, which were not in direct contact with insecticides prior to testing. Insects were kept in laboratory conditions, without additional feeding, at temperature of  $23 \pm 2$  °C and normal photoperiod (16/8h).

Toxicological experiment. Bioassay was based on the assumption that CPB populations have reduced sensitivity to insecticides and on evaluation of response of overwintered adults to insecticide applied at label rate (the rate determined in field experiments during the registration process and found to cause 100% mortality) and higher rates. Insecticides were applied by insects soaking for 5 sec. The experiment was set up in four replicates with 30 adults per replication (sex ratio 1:1). The sex was determined according to Tribelj and Korol (2001). Assessment of insecticide efficacy consisted of counting the number of dead (with no signs of vitality), paralyzed (uncoordinated movements and inability to move) and alive insects (normal mobility and vitality). The effect was determined 24, 48 and 72 h after insecticide application. Results were corrected for mortality in the control (Schneider Orelli, 1947) and expressed as the efficacy (E%) achieved only after 72 h. Sensitivity was evaluated on the scale 1-5 (1 - highly sensitive populations /E = 100%; 2 - sensitive /100> E ≥ 95%; 3 - slightly resistant /95> E ≥ 90%; 4 - resistant /90> E ≥ 50%; 5 - highly resistant /E <50%/), which was created as a slight modification of IRAC method No. 011 (Anonymous, 2009) that refers to pollen beetles (*Meligethes* spp.).

## RESULTS AND DISCUSSION

In our conditions abamectin is used less frequently for the control of the Colorado potato beetle, compared to insecticides from organophosphates, carbamates and synthetic pyrethroids chemical groups.

Abamectin is an analog of a metabolite produced by fungus *Streptomyces avermitilis*, which is according to IRAC - classified into the group avermectins, and exhibits insecticidal and acaricidal activities. It has contact and digestive action. Since it is agonist of  $\gamma$  agonist - aminobutyric acid, the mechanism of action is based on the interruption of neurotransmission. Toxicity is manifested primarily in cease of insect feeding and afterwards in paralysis of irreversible character (Janjic and Elezović, 2008). Stumpf and Nauen (2002) mention the possibility of different biochemical and pharmacokinetic mechanisms being responsible for the emergence of resistance to abamectin in target organisms. In resistant Colorado potato beetle races to abamectin, the enhanced enzyme activity is registered (carboxylesterase and monooxygenase), as well as the increased metabolism and excretion of this compound. In our conditions, abamectin is relatively rarely used for the control of Colorado potato beetle, compared to insecticides belonging to the group of organophosphates, carbamates and synthetic pyrethroids.

By applying the above described screening test, in our conditions, in 2007, a monitoring of CPB sensitivity towards several insecticides from different chemical groups (organophosphates, carbamates, pyrethroids, phenyl pyrazoles, abamectin) was conducted and the results are presented in several papers (Indić et al., 2009; 2012). The mentioned bioassay is based on the assumption that the populations of Colorado potato beetle have

reduced sensitivity to the insecticide. This is based on measuring the response of overwintered adults to insecticides applied at label and higher rates, but can also be used to estimate the sensitivity of the population towards newly introduced substances. The analysis of the efficacy of insecticide applied at label rate in this test, tended to provide the conditions close to the once in the field, because it is expected to have high efficiency. Otherwise it is suspected that a change in sensitivity has occurred. The application of higher rates (two- and five-fold) of the insecticide at was used to confirm that the individuals from the same population can survive even higher quantities of insecticide i.e. the evidence that the change in sensitivity has already occurred. Mentioned indicates which part of population is sensitive to label rate of insecticide, or which tolerates it, or exhibits reduced sensitivity, or that individuals have developed an appropriate mechanism of resistance. Knowing that there is no correlation between the increase in the amount of insecticides and increase mortality in the population, namely that there is a high percentage of survival of insects regardless on the rate of insecticide, and that the population has become resistant, requires: unconditional exclusion of such insecticide from the production, compliance of application strategies, regular recording or monitoring and mapping of such populations. Based on the efficacy of insecticides obtained in screening test (after 72 h exposure of insects) and in some degree modified scale for the classification of susceptibility to insecticides, a classification of CPB susceptibility to abamectin was carried out. This test can be performed in the modest experimental conditions, without special requirements for specific equipment, handling procedures are very simple and the results are clear and very applicative because they do not require complicated statistical analysis, except for precise classification of the scale 1-5. The overwintered adults were chosen as test insects in evaluation of sensitivity to insecticides for various reasons. Firstly, it is known that they were not in direct contact with insecticides, also, they are easily maintained for a long period prior to inclusion in the test, no need to be fed during the tests, and the effects of insecticides (mortality, paralysis, vitality) are easily observable and measurable.

Based on tests performed in 2008 the adults of CPB from the site Futog, Vilvo, Kobilj and Čurug were highly sensitive to abamectin, while from Čenej were sensitive since 2.5 % of adults survived the treatment with abamectin applied at label rate (Table 1).

The results of the sensitivity of CPB in 2009 are presented in Table 2. Three CPB populations (Gložane, Sirig and Kikinda) were slightly resistant to abamectin applied at label rate, i.e. in mentioned populations 5.4 to 7.4 % of adults survived the treatment, and the increase of the application rate caused efficiency of 100%. Five CPB populations (Stepanovićevo, Žabalj, Šajkaš, Šimuže and Novo Miloševo) were resistant ( $90 > E \geq 50$  %), and survival rate ranged from 11.3 to 35.4 %. These populations exhibited different sensitivity to the increase of insecticide rate. Population from Stepanovićevo, even at five-fold higher rates can be classified as slightly resistant, meaning that the population has extremely heterogeneous susceptibility to abamectin. In other populations the increase of the application rate caused the increase in sensitivity. Populations from Ljutovo and B. Gradište were highly resistant to abamectin. The heterogeneity in sensitivity of CPB populations was noted. In the population from B. Gradište, after 72 hours, 77.5 % of adults survived abamectin applied at label rate, and from Ljutovo population 51.7 %. In these two populations, abamectin did not express 100 % efficacy even after the application of five-fold higher rates.

In 2012 the tests were repeated at sites Vilovo and Čurug. Compared to the results from the 2008 the population from Vilovo, in 2012 had survival rate 9.4 %, thus can



be classified as slightly resistant, while the increase of the application rate caused the efficiency of 100 %. The level of sensitivity of the population from Čurug remained at the same level as in 2008 i.e. highly sensitive. The population from Despotovo was sensitive, with the survival rate 4.4% and the one from Zmajevu was slightly resistant, since 5.8 % of adults survived the treatment, while at higher rates than the label one, it exhibited high sensitivity (Table 3).

Similar results were obtained by Marčić and Perić (2009), when testing the efficacy of abamectin under field conditions (2004 and 2005) for the suppression of the first generation of CPB larvae. In both years, abamectin expressed high efficacy (94.8-100 %) when applied in combination with adjuvant, although the sensitivity differs depending on developmental stage and age of larvae (Indić, 1994). Test results of Mohamed (2010) in laboratory conditions show that abamectin caused high mortality (71-88.9 %) of CPB larvae ( $L_1$  -  $L_3$ ) after three days, and 100 % of mortality after seven days. The mortality of  $L_4$  larvae was 48.9 % after three days, and 100 % after seven days. The same author assessed the adulticidal effect of abamectin when applied at label rate, 50 % and 25 % lower quantities. At all applied rates abamectin achieved high mortality of adults (51.1-71.1 %) after three and after seven days (75.6-95.6 %), depending on the applied rate of insecticide.

Table 1. Sensitivity (scale 1-5) of overwintered CPB adults to **abamectin in 2008**

Sites	kg; l/ha	efficacy (%) of abamectin				
		1	2	3	4	5
Futog	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Čenej	0.75	-	97.5 (2.5)	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Vilovo	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Kovilj	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Čurug	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-

1 - highly sensitive population ( $E = 100\%$ ); 2 - sensitive ( $100 > E \geq 95\%$ );

3 - slightly resistant ( $95 > E \geq 90\%$ ); 4 - resistant ( $90 > E \geq 50\%$ ); 5 - highly resistant ( $E < 50\%$ )

Table 2. Sensitivity (scale 1-5) of overwintered CPB adults to abamectin in 2009

Sites	kg; l/ha	efficacy (%) of abamectin				
		1	2	3	4	5

Stepanovićevo	0.75	-	-	-	73.9 (26.1)	-
	1.5	-	-	-	-	37.6 (62.4)
	3.75	-	-	92.9 (7.1)	-	-
Gložane	0.75	-	-	94.6 (5.4)	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Žabalj	0.75	-	-	-	69.1 (30.9)	-
	1.5	-	-	90.8 (9.2)	-	-
	3.75	100	-	-	-	-
Sirig	0.75	-	-	92.6 (7.4)	-	-
	1.5	-	98.9 (1.1)	-	-	-
	3.75	100	-	-	-	-
Šajkaš	0.75	-	-	-	88.7 (11.3)	-
	1.5	-	-	95.6 (4.4)	-	-
	3.75	100	-	-	-	-
Ljutovo	0.75	-	-	-	-	48.3 (51.7)
	1.5	-	-	-	83.3 (16.7)	-
	3.75	-	99.2 (0.8)	-	-	-
Šimuže	0.75	-	-	-	87.2 (12.8)	-
	1.5	-	97.2 (2.8)	-	-	-
	3.75	100	-	-	-	-
Bačko Gradište	0.75	-	-	-	-	22.5 (77.5)
	1.5	-	-	-	-	25.1 (74.9)
	3.75	-	95.6 (4.4)	-	-	-
Novo Miloševo	0.75	-	-	-	64.6 (35.4)	-
	1.5	-	-	-	84.5 (15.5)	-
	3.75	-	99.1 (0.9)	-	-	-
Kikinda	0.75	-	-	93.6 (6.4)	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-

1 - highly sensitive population (E= 100%); 2 - sensitive(100 > E ≥95%);

3 - slightly resistant (95>E ≥90%); 4 - resistant (90>E ≥50%); 5 - highly resistant (E < 50%)

Table 3. Sensitivity (scale 1-5) of overwintered CPB adults to abamectin in 2012

Sites	kg; l/ha	efficacy (%) of abamectin				
		1	2	3	4	5
Zmajev	0.75	-	-		-	-
	1.5	100	-	94.2 (5.8)	-	-
	3.75	100	-		-	-
Despotovo	0.75	-	95.6 (4.4)	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Vilovo	0.75	-	-	90.6 (9.4)	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Čurug	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-

1 - highly sensitive population (E= 100%); 2 - sensitive(100 > E ≥95%);

3 - slightly resistant (95>E ≥90%); 4 - resistant (90>E ≥50%); 5 - highly resistant (E < 50%)

## CONCLUSION

Based on the results of the screening test of sensitivity of 19 populations of CPB, in Vojvodina, to abamectin in 2008, 2009 and 2012 the following conclusions can be drawn:

- In 2008, all CPB populations (Futog, Vilovo, Kovilj Čurug ) were highly sensitive to abamectin applied at label rate, apart from Čenej population which was sensitive because the survival rate was 2.5 %;
- In 2009, three CPB populations (Gložan, Sirig, Kikinda) were slightly resistant, five (Stepanovićevo, Žabalj, Šajkaš, Šimuže, Novo Miloševo) were resistant and two (Ljutovo, B. Gradište) were highly resistant to abamectin. In all populations the manifested survival rate was ranging from 11.3 to 77.5 %;
- In 2012, the population from Čurug was highly sensitive, from Despotovo sensitive and from Zmajev and Vilovo) were slightly resistant to abamectin, since 4.4-9.4 % of CPB adults survived abamectin applied at label rate;
- The application of this screening test provides quick and reliable measuring of which part of CPB population has endured the changes in sensitivity to tested insecticides and provide data of great importance for the extensive agricultural practices as well as for sophisticated (at the molecular level) research.

## REFERENCES

- ANONIMUS: IRAC Susceptibility Test Methods Series, Version 3, Method No. 011, 2009.
- COPPING, L.G., AND MENN, J.J.: Biopesticides a review of their action, applications and efficacy. Pest Management Science, 56:651-676, 2000.
- GOKCE, A., ISAACS, R. AND WHALON, M.E.: Behavioural response of Colorado

potato beetle (*Leptinotarsa decemlineata*) larvae to selected plant extracts. Pest Management Science, 62:1052–1057, 2006.

INĐIĆ D.: Efekti zajedničkog delovanja insekticida na krompirovu zlaticu *Leptinotarsa decemlineata* Say. Doktorska disertacija, Univerzitet u Beogradu, Poljoprivredni fakultet, 1-107, 1994.

INĐIĆ, D.: Effect of joint action of insecticides on colorado potato beetle (*Leptinotarsa decemlineata* Say). Review of Research Work at the Faculty of Agriculture, Belgrade, 42(1) 7-22, 1997.

INĐIĆ, D., KLOKOČAR ŠMIT, Z., VUKOVIĆ, S.: Pesticidi visokog rizika i njihova zamena u zaštiti krompira. Studija, Poljoprivredni fakultet i Pokrajinski sekretarijat za nauku i tehnološki razvoj APV, Novi Sad, 1-39, 2006a.

INĐIĆ, D., VUKOVIĆ, S., KLOKOŠAR-ŠMIT, Z.: Rapid detection of Colorado potato beetle (*Leptinotarsa decemlineata* Say.) sensitivity to insecticides. XVII Czech and Slovak Plant Protection, 2006, Prag, Proceedings, 459-464.

INĐIĆ, D., VUKOVIĆ, S., GRAHOVAC, M.: Osetljivost krompirove zlatice (*Leptinotarsa decemlineata* Say.) prema insekticidima i uspostavljanje monitoringa rezistentnosti. XXIV Seminar zaštite bilja Vojvodine, Biljni lekar, 16-26, Novi Sad, 2009.

INĐIĆ, D., VUKOVIĆ, S., TANASKOVIĆ, S., GRAHOVAC, M., KERESI, T., GVOZDENAC, S., SAVČIĆ-PETRIĆ, S.: Screening Test for Detection of *Leptinotarsa decemlineata* (Say) Sensitivity to Insecticides. Pesticidi i fitomedicina, 27 (1) 59-67, 2012.

JANJIĆ, V., ELEZOVIĆ, I.: Pesticidi u poljoprivredi i šumarstvu u Srbiji, 2008. Šesnaesto, izmenjeno i dopunjeno izdanje, Društvo za zaštitu bilja, Beograd, 2008.

MARČIĆ, D., PERIĆ, P.: Field evaluation of natural and syntetic insecticides against *Leptinotarsa decemlineata* Say. IV Balkan Symposium on vegetables and Potatoes, Plovdiv, Bulgaria, 2009, Acta Horticulture, 2 (830) 391-396.

MOTA-SANCHEZ, D., HOLLINGWORTH, R.M., GRAFIUS, E.J., MOYER, D.D.: Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Pest Management Science, 62:30-37, 2006.

MOHAMED, O.: Biological efficacy of some biorational and conventional insecticides in the control of different stages of the colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Plant Protection Scienci, 46 (3)123-134, 2010.

PERIĆ, I., MILOŠEVSKI, N., KLJAJIĆ, P.: Insecticide susceptibility of the Colorado potato beetle in the vicinity of Belgrade, Yugoslavia. Acta Horticulturae, 462: 983-990, 1997.

SCHNEIDER-ORELLI, O.: Entomoloisches Praktikum. Aufl. Aarau, 1947.

STANKOVIĆ, S., ZABEL A., KOSTIĆ, M., MANOJLOVIĆ, B., RAJKOVIĆ, S.: Colorado potato beetle (*Leptinotarsa decemlineata* Say) resistance to organophosphates and carbamates in Serbia. Journal of Pest Science, 77(1) 11-15, 2004.

STUMPF, N., NAUEN, R.: Biochemical markers linked to abamectin resistance in *Tetranychus urticae*. Pesticide Biochemistry&Physiology, 72, 111-121, 2002.

ТРИБЕЛЬ, С.О. И КОРОЛЬ, Т.С.: Колорадський жук. Захист рослин, Інститут захисту послин, Київ, 1-31, 2001.

WHALON, M.E., HOLLINGWORTH, R.M., MOTA-SANCHEZ, D.: The MSU database of pesticide resistance, 2008, <http://www.pesticideresistance.org>.

ZABEL, A., RAJKOVIĆ, S., MANOJLOVIĆ, B., STANKOVIĆ, S., VELJKOVIĆ, I.:

New pesticides in potato protection against the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] and late blight [*Phytophthora infestans* (Mont. de Bary)] on potato. 2nd Balkan Symposium on Vegetables and Potatoes, Thessaloniki, Greece, 2000, Proceeding, Acta Horticulturae, 579: 491-500.

## PRIMENA SCREENING TESTA PRI DETEKCIJI OSETLJIVOSTI KROMPIROVE ZLATICE (*LEPTINOTARSA DECEMLINEATA* SAY) NA ABAMEKTIN

SLAVICA VUKOVIĆ, DUŠANKA INĐIĆ,  
SONJA GVOZDENAC, SNEŽANA TANASKOVIĆ

### Izvod

Screening testom je ispitana osetljivost 19 poljskih populacija krompirove zlatice (*Leptinotarsa decemlineata* Say.) prema abamektinu tokom 2008. (Futog, Čenej, Vilovo, Kovilj i Čurug), 2009. (Stepanovićevo, Gložane, Žabalj, Sirig, Šajkaš, Ljutovo, Šimuže, Bačko Gradište, Novo Miloševo i Kikinda) i 2012. godine (Zmajevno, Despotovo, Vilovo i Čurug). Pomenuti test je omogućio brzu procenu osetljivosti populacija prezimelih imaga krompirove zlatice prema abamektinu (preparat Abastate) koji je primenjen u preporučenoj (0.75 l/ha), dva (1.5 l/ha) i pet (3.75 l/ha) puta većim količinama od preporučene, metodom potapanja imaga u trajanju 5 sec. Smrtnost insekata je određena posle 72 h ekspozicije. Rezultati su korigovani za smrtnost u kontroli i prikazani preko efikasnosti. Prema IRAC (metoda br. 011), uz neznatnu korekciju, izvršena je procena osetljivosti krompirove zlatice prema skali 1-5 (1 - visoko osetljiva populacija ( $E=100\%$ ); 2 - osetljiva ( $100 > E \geq 95\%$ ); 3 - blago rezistentna ( $95 > E \geq 90\%$ ); 4 - rezistentna ( $90 > E \geq 50\%$ ); 5 - visoko rezistentna ( $E < 50\%$ )). Od pet ispitanih populacija krompirove zlatice u 2008. godini, prema količini za praktičnu primenu abamektina četiri su visoko osetljive ( $E=100\%$ ) i jedna osetljiva ( $E=97.5\%$ ). U 2009. godini od 10 ispitanih populacija, tri su blago rezistentne ( $E=92.6-94.6\%$ ), pet je rezistentno ( $E=64.6-88.7\%$ ) i dve su visoko rezistentne ( $E=22.5-48.3\%$ ) na količinu za praktičnu primenu abamektina. Od četiri ispitane populacije u 2012. godini jedna populacija je visoko osetljiva ( $E=100\%$ ), jedna osetljiva ( $E=95.8\%$ ) i dve su blago rezistentne ( $E=90.6-94.2\%$ ). Preživljavanje insekata primenom viših količina od onih za praktičnu primenu, dokaz je više da postoji deo populacije kod kojih je došlo do promene u osetljivosti ili selekcije na rezistentnost.

**Ključne reči:** screening test, krompirova zlatica, osetljivost, abamektin.

Received / *Primljen*: 12.02.2014.

Accepted / *Prihvaćen*: 24.02.2014.

## VISITORS' SATISFACTION: THE CASE OF THE "ŠARGAN – MOKRA GORA" NATURE PARK

ZORAN A. RISTIĆ, IMRE NAĐ,  
MILOSAVA MATEJEVIĆ, MILUTIN KOVAČEVIĆ<sup>1</sup>

*SUMMARY: The "Šargan – Mokra Gora" Nature Park is a touristic destination with exquisite nature features. Although tourism industry is developing, it succeeded in preserving authenticity so Nature Park is very attractive to domestic and foreign visitors. The aim of this paper is to investigate visitor satisfaction with the tourist offer of the "Šargan– Mokra Gora" Nature Park. The results showed that visitors are generally satisfied with the elements identify in the destination. Analysis of structure and preferences of the visitors could enables more adequate positioning of "Šargan– Mokra Gora" on the touristic market. At the same time, it is necessary to provide framework for development of quality, contemporary and innovative tourist offer that would enhance competitiveness of Šargan- Mokra Gora, and therefore its tourist offer.*

**Key words:** Šargan- Mokra Gora, Nature Park, visitors' satisfaction, touristic destination.

### INTRODUCTION

Šargan-Mokra Gora is specific touristic destination, since besides profiling touristic industry it succeeded in preserving authenticity, which gives it special importance and makes it very attractive for domestic and foreign tourists. By Law on environmental protection (*Official gazette of the Republic of Serbia no. 81/2008*) for this area was given a Nature Park status, which is a nature area of high national importance – category one (*Official gazette of the Republic of Serbia no. 66/91 and 83/92*). In the Nature Park, specific ways of tourist movements have developed. Most often are outing touristic movements (Marković et al., 2014). Moreover, this Nature Park is extremely attractive

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Zoran A. Ristić, PhD, associate professor, Imre Nađ, PhD, associate professor, Milosava Matejević, MSc, assistant, Milutin Kovačević, MSc, assistant, University of Novi Sad, Faculty of Sciences, 21000 Novi Sad, Serbia.

Corresponding author: Zoran A. Ristić, e-mail: zoran.ristic@dgt.uns.ac.rs, phone: +381 21 485-28-45.

destination for eco-, country-, fishing-, hunting-, bicycle- and manifestation tourism (Obradović and Simić, 2004; Štetić, 2002). This destination is especially attractive due to “Drvengrad” at Mečavnik, created by Emir Kusturica, and “Šarganska osmica” railroad with trains and railroad objects (Obradović, 2006; Bešlić, 1987).

The aim of this paper is to research visitors satisfaction by tourist offer in “Šargan – Mokra Gora” Nature Park. The starting idea of this paper is that the “Šargan – Mokra Gora” Nature Park has a range of possibilities for satisfying its visitors. It is important to determinate visitors’ satisfaction, in order to improve tourist offer of Sargan

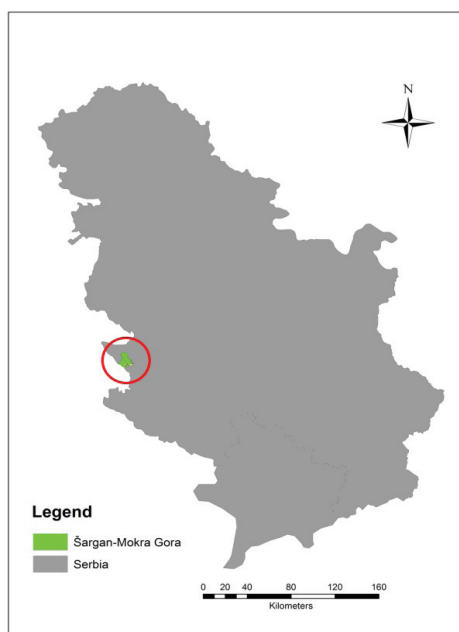


Fig. 1. Location of Nature Park „Šargan-Mokra Gora“ in Serbia

## MATERIALS AND METHODS

Results of investigation were obtained by surveying visitors in “Šargan-Mokra Gora” Nature Park. Written materials regarding this area, as space plans, master plans and other documents were also used. Based on the literature review the questionnaire was formed. Total of 29 elements were identified in order to measure visitors satisfaction with the tourist offer in the natural park. The survey was conducted in the summer during 2010. Investigation was carried out on the localities of “Bele vode”, “Jatare” and “Drvengrad” at Mečavnik, with representative sample of 50 respondents.

For measurement purposes, 29 elements were identified and used to represent overall tourist offer in the destination such as attractiveness of the climate, amicability of the local population, amicability of tourism staff, helpfulness, foreign language knowledge of touristic workers, road network and accessibility of the destination, parking space, available information about destination, information at the destination, touristic signalization at destination, manifestations, souvenirs, organization of a destina-

tion, promenades and marked trails, crowdedness, scenery, environmental preservation, cleanliness, children's objects, lodging objects, restaurant objects, culture facilities, entertainment facilities, active vacation facilities, eco-tourism potentials, hunting tourism potentials, outing offer, local gastronomy, price/quality ratio and general evaluation of Mokra Gora offer as the destination. Subjects graded these elements by five-point Likert scale, with grades ranging from 1 (extremely unsatisfied) to 5 (extremely satisfied).

Methods used in this paper were questionnaires, direct (personal) contact with Nature Park management, local population, personal insights, photographing etc. Those were classic "field research" with the aim to obtain as much relevant data as possible "at the spot". Besides fieldwork, data were gathered using different written sources (laws, strategies, acts, programs, scientific papers, books, magazines) and electronic sources (Internet browsing, multimedia presentations etc).

## RESULTS

The socio-demographic data of respondents are shown in Table 1.

Table 1. Respondents structure  
*Tabela 1. Struktura ispitanika*

Total number of respondents/ <i>Ukupan broj ispitanika</i>	50
<b>Gender/<i>Pol</i></b>	
female/ <i>žene</i>	54%
male/ <i>muškarci</i>	46%
<b>Age/<i>Godine</i></b>	
16-25	26%
26-35	36%
36-45	20%
46-55	8%
56-65	6%
65 and more/ <i>i više</i>	4%
<b>Education level/<i>Obrazovanje</i></b>	
Primary school/ <i>Osnovna škola</i>	6%
High school/ <i>Srednja škola</i>	44%
College/ <i>Viša škola</i>	18%
University/ <i>Fakultet</i>	28%
Master/doctoral degree / <i>Magistar, Doktor nauka</i>	4%
<b>Daily spending/<i>Dnevna potrošnja</i></b>	
less than 30 €/ <i>manje od</i>	68%
31-50€	28%
more than 51€/ <i>više od</i>	4%

At the question from which country they come, most of subjects said Serbia (44 respondents), and from those, from Belgrade were 46%, from Vojvodina 31%, and from



other parts of Serbia 23%. Seven subjects were from abroad (USA, Italy, Slovenia, Australia and Montenegro). Survey has encompassed more women than men. If we compare their answers, we note that most of women came for the first time (32%) and that they plan to come again (44%). In male population, most were here between two and five times (28%) and 12% does not know whether they will come again. Due to age, a large percent of subjects were between 16 and 25 years of age (26%). This is a young category, people who are near the end of schooling or recently graduated, and therefore they are financially dependent and have no family. This is confirmed by the fact that 70% of young subjects said that they are spending less than 30 Euros during sojourn at the destination. In most cases, they come with friends or a partner, and reason to come was fun, relaxation and recreation, cultural facilities and new experiences.

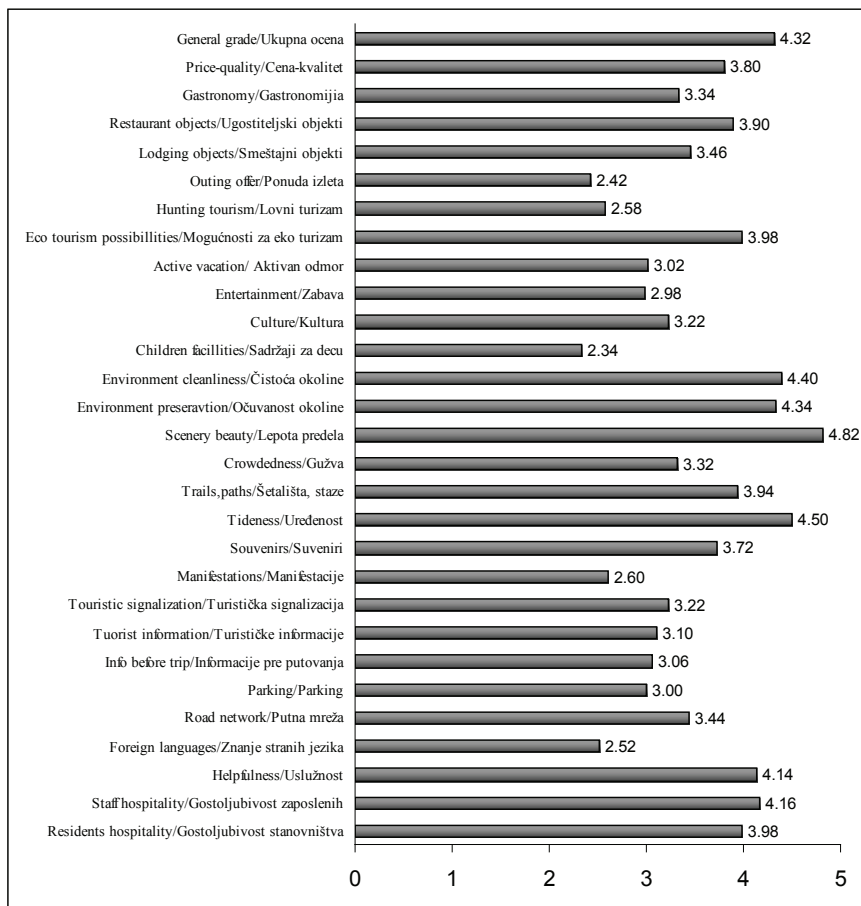
The next age group, and the most numerous one, was subjects between 26 and 35 years of age. According to statistics, these persons are financially independent and often have started a family. The most often travel with family or friends. In comparison to previous group, here more subjects spend more between 30 and 50 Euros (60%), while others spend less than 30 Euros. In most cases, they came because of nature beauty, relaxation and recreation. In analyzing subjects older than 35, it is notable that they are mostly first-time visitors to “Šargan-Mokra Gora” (63%). In most cases, they come with a spouse or with a whole family (children). They spend between 30 and 50 Euros (42%), while some spend more than 50 Euros (10%). From those respondents, 73% would come again to this destination. Reasons to visit these parts are well-preserved nature, climate and local gastronomy.

By analyzing answers in this survey, it was concluded that most subjects (68%) spend less than 30 Euros, and 28% spends between 30 and 50 Euros. Only 4% of subjects spend more than 50 Euros, and those were foreign tourists. As we said above, age group 36-45 years spends most, and after them those from 26-35 years. In addition, more women spend between 30 and 50 Euros (24%), while man said they spend less than 30 Euros at this destination (36%).

Education level of subjects did not have any relevance in this analysis. Share of every category is leveled, and only difference was regarding hunting activities, as it was indicated in previous research (Marković et al., 2011). All subjects with masters or doctoral degree were interested to come to hunt at this destination and they believe that it is possible to combine hunting and sustainable game management. Most subjects with high-school degree would not come to hunt at this destination (76%). At the question whether they would like to come again to “Šargan-Mokra Gora”, most subjects answered affirmatively (74%); 22% said they might come again, while 4% does not want to come here again. The arithmetic means of all answers to survey is shown in Graph 1. Results of investigation have shown that subjects consider geographical and touristic position of “Šargan – Mokra Gora” as very favorable destination. Relatively good infrastructure (3.44) nevertheless should be renewed and maintained. Tourist signalization is graded by 3.22 but further communication with subjects, especially foreign ones, revealed that certain info boards are written in Cyrillic letters only, which is a problem for strangers. Most subjects arrived by a car, and parking lots (3) are located at a small area, which is a downside, having in mind that most visitors come by private cars and are daily excursionists. Nature beauty (4.82), preservation (4.34) and cleanliness (4.4) were graded quite high. Nature beauty is also one of main reasons that tourist come to these parts. Other positively graded elements were amicability of touristic staff and of

local population. Helpfulness is also high (4.14), but knowledge of foreign languages in staff received rather low grade.

Poor transportation connections (city busses) and low cooperation with travel agencies led to conclusion that very small number of tourists arrives through travel agencies. Busses operate several times a day, mostly to Višegrad and Sarajevo, and in transition they drop off tourists who use this type of transportation. Since there are no concrete activities and no outing in offer, outing activities offer within the destination itself has unfavorable tendency. There are a number of localities that can be used, because this Nature Park has natural and cultural characteristics promising development of different types of tourism (eco, sustainable, country, manifestation etc).



Graph. 1. Elements evaluation offered by respondents

Graf. 1. Ocena elemenata ponude od strane ispitanika

Possibilities for development of ecotourism were positively graded by subjects (3.48), while for hunting tourism grades were somewhat lower (2.58), as well as for active vacation possibilities (3.02).

Considerable cultural wealth may be additional, but also the main motive bringing tourists. Still, visitor satisfaction by presence and programs of manifestation at this

destination was graded poorly (2.6). Children facilities almost don't exist (2.34). Since most of survey subjects were middle-age pairs, they probably come with their children. Additional activities for them would most certainly complement and brought tourist offer of the destination to higher-level.

High grades are given to restaurant objects (3.9) and boarding objects (3.46). This destination got higher grades mostly because of relatively high number of various category objects, from plain village cottages to luxurious hotels and apartments. In addition, they are all unique: for instance, at Mečavnik there are no two identically decorated rooms.

Regarding price/quality ratio, average grade is 3.8. Most visitors are fascinated by natural beauties, but it is far from enough. For longer stay of tourists, it is necessary to form more ample offer with various attractive activities, and new facilities that will attract visitors and bring them back on this destination another time.

## DISCUSSION AND CONCLUSIONS

The results showed that visitors are generally satisfied with the elements identify in the destination. However, for longer stay of tourists, it is necessary to form more ample offer with various attractive activities, and new facilities that will attract visitors and bring them back on this destination another time. Regarding this matter, development of the "Šargan- Mokra Gora" Nature Park in a long-term, must be guided with the principles of sustainability and ecotourism – not only as perspective branch in tourism in this region, but also due to general values and tendencies to organize general economy recovery of the region, without consequences to environment, i.e. sustainable development based on ecotourism and complementary activities (agriculture, transport, commerce) (Ristić, 2006).

Need to redirect towards sustainable development, which may be identified to justified, coordinated, scientifically based and rationally based and attainable activities that would not interfere basic ecological relations in an area which should bear a mark of ecological quality and keep it for the sake of future generations, understands creating not only most favorable conditions for fulfillment of present and future needs of tourists, but also the needs of local population. At the same time, redirection from classic and always coveted mass tourism to ecotourism must not be organized by rejecting present systems but by gradual changes, improvements and refinements for better future.

Touristic potentials of this destination are not used enough. With improvements of transportation infrastructure, opening airport at Ponikve, including cattle-breeding huts to tourist offer, "Šargan- Mokra Gora" may become interesting for foreign visitors too. Increase of number of tourists will bring more jobs for local population too.

As a tourist destination, it must be strategically defined entirety, with characteristic elements. It is necessary to create distinct image of this destination which must fulfill demands of present world of globalization, but at the same time to be recognized by specific offers, shaped according to investigations and consideration of general and specific trends ad the tourist market (Armenski et al., 2009).

The knowledge of the needs and wishes of tourists is an essential input for a tourist destination to make marketing-related decision (Djeri et al., 2007). Modern tourists do not wish to use only the basic offer of a destination; they want individual or organized experience outside strict borders of predestined touristic localities (Djeri et al., 2007).

For that reason, there is even more need to emphasize need to exploit potential tourist offer of specific types of tourism within the Nature Park, which will create competitiveness of the destination itself, certainly with respect to principles of sustainable and eco tourism, since this area has not only local but also global importance, so the preservation of nature and of biodiversity must be the first priority.

## REFERENCES

- ARMENSKI, T., ZAKIĆ, L., DRAGIN, A.: Percepcija inostranih turista o imidžu Srbije. Glasnik srpskog geografskog društva, 89(1)39-63, 2009.
- BEŠLIĆ, M.: Mokra Gora, Antropogeografska i etnološka istraživanja. Narodni muzej, Užice, 1987.
- DJERI, L., PLAVŠA, J., ČEROVIĆ, S.: Analysis of Potencial Tourists' Behavior in the Process of Deciding Upon a Tourist Destination Based on a Survey Conducted in Bačka Region. Internat. Sci. Journal Geographica Pannonica, 11(1)70-76, 2007.
- MARKOVIĆ, V., DAVIDOVIĆ, N., ĐURĐEV, B., DRAGIN, A.: Influence of age and educational level on the bahaviour of hunters in Vojvodina Province (Serbia). Turizam, 15(4)159-168, 2011.
- OBRADOVIĆ, D.: Šargan i Mokra Gora – Predeo izuzetnih odlika. Zemlja i ljudi, 56:51-56, 2006.
- OBRADOVIĆ, D. SIMIĆ, M.: Turističke vrednosti Šargana i Mokre Gore. Zaštita prirode, 56(1)93-109, 2004.
- RISTIĆ, Z.: Lovstvo. Zavod za udžbenike i nastavna sredstva, Beograd, 2006.
- OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA no. 66/91. Law on environmental protection, National assembly of the Republic of Serbia, Belgrade, 1991.
- OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA no. 83/92. Law on environmental protection, National assembly of the Republic of Serbia, Belgrade 1992.
- OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA no. 81/2008. Law on environmental protection, National assembly of the Republic of Serbia, Belgrade, 2008.
- ŠTETIĆ, S.: Nove tendencije svetskih turističkih kretanja. Turizam, 6:7-10, 2002.
- MARKOVIĆ, V., STANKOV, U., KLAUČO, M., JOVANOVIĆ, T., RISTIĆ, Z.: Human impacts on the land cover trough landscape metrics: Nature Park “Šargan-Mokra Gora” (Serbia). Rocznika Ochrona Srodowiska, 16:2014 issn 1506-218X (in press).

## ZNAČAJ ZADOVOLJSTVA POSETILACA PARKA PRIRODE „ŠARGAN - MOKRA GORA”

ZORAN A. RISTIĆ, IMRE NAĐ, MILOSAVA MATEJEVIĆ, MILUTIN  
KOVAČEVIĆ

### Izvod

Park prirode “Šargan - Mokra Gora” je turistička destinacija izuzetnih prirodnih vrednosti koja je i pored razvoja turističke delatnosti uspešla da sačuva potpunu autentičnost zbog koje je izuzetno atraktivna za domaće i inostrane posetioce . Cilj ovog

rada je istraživanje turističke tražnje i zadovoljstva posetilaca turističkom ponudom parka prirode «Šargan - Mokra Gora». Rezultati su pokazali da su turisti generalno zadovoljni elementima destinacije. Analiza strukture i preferencija turističke tražnje omogućava adekvatno pozicioniranje ove destinacije na turističkom tržištu. Istovremeno, potrebno je stvoriti okvir za razvoj kvalitetne, savremene i inovativne turističke ponude koja će povećati konkurentnost, a samim tim i turističku ponudu.

**Ključne reči:** *Šargan- Mokra Gora, park prirode, zadovoljstvo posetilaca, turistička destinacija.*

Received / *Primljen*: 03.10.2014

Accepted / *Prihvaćen*: 21.03.2014.

## EFFECT OF PGPR ON THE GERMINATION AND GROWTH OF ENGLISH RYEGRASS AND MICROBIOLOGICAL ACTIVITY IN ITS RHIZOSPHERE\*

DRAGANA STAMENOV, SIMONIDA ĐURIĆ, TIMEA HAJNAL JAFARI<sup>1</sup>

*SUMMARY: The objective of this study was to examine the effect of plant growth promoting bacteria *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. on germination and growth of English ryegrass and microbiological activity in its rhizosphere. One hundred seeds of perennial ryegrass inoculated with the appropriate bacteria were placed on filter paper in the dark at 22 C° until germination. Five and fourteen days after, hypocotyl and root length of seedlings were measured. The experiment was conducted in 10 l volume vegetation pots, with the following variants: 1. *Pseudomonas* sp. P12, 2. *Bacillus* sp. B1, 3. *Streptomyces* sp. A3, 4. control - no inoculation. Thirty and sixty days after sowing, length of stem and roots of plants (cm) were determined. Thirty days after sowing, the number of microorganisms was determined, using the dilution method. Application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1 had a negative effect on ryegrass seed germination, while the use of *Streptomyces* sp. A3 gave positive results. On average, the application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1 had the greatest effect on the stem and root length of English ryegrass. The number of the investigated groups of microorganisms increased in all variants in comparison to the control. The best effect was achieved with *Bacillus* sp. B1.*

**Key words:** microorganisms, germination, yield, English ryegrass. `

### INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are originally defined as root-colonizing bacteria that can be found in the rhizosphere, at root surfaces and in association

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Dragana Stamenov, PhD, research associate, Simonida Đurić, PhD, assistant professor, Timea Hajnal Jafari, PhD, researcher, University of Novi Sad, Faculty of Agriculture, Department for Field and Vegetable crops, Trg D. Obradovića 8, 21000 Novi Sad, Serbia.

Corresponding author: Dragana Stamenov, e-mail: draganastamenov@yahoo.com

\* This research is part of an ongoing project TR-31027 financed by the Ministry of Science, Republic of Serbia.

with roots, enhancing the growth of the plant, either directly, and/or indirectly. Treatment with PGPR has increased the germination percentage, seedling vigor, plant stand, root and shoot growth, total biomass of plants, seed weight, early flowering, increased grain, fodder, fruit yields etc. (Ramamoorthy et al., 2001).

On the other hand, after being introduced into the soil, PGPR may affect the indigenous microbial population. Likewise, the indigenous microorganisms can affect the inoculants. What kind of effect will be expressed depends on the conditions in soil, plant species, adaptation ability of the introduced microorganisms etc. (Egamberdiyeva, 2007). Some groups of microorganisms may be stimulated, some may be inhibited or there may be no effect of the introduced microorganisms on the structure of the indigenous population (Dobbelaere et al., 2003). Artursson et al. (2006) concluded that the use of PGPR in plant production results in the increase in the number and enzymatic activity of microorganisms, microbial biomass, microbial diversity in the rhizosphere and improved production characteristics of soil.

Different bacteria that have been reported as PGPR belong to the following genera: *Pseudomonas*, *Bacillus*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Streptomyces*, *Trichoderma*, *Variovovax*, *Xanthomonas* and *Phyllobacterium* (Berg, 2009). Most popular bacteria studied and exploited as biocontrol agents include the species of fluorescent *Pseudomonas*, *Bacillus* and actinomycetes *Streptomyces* sp. (Ade-semoye et al., 2008).

Therefore, the objective of this study was to examine the effect of plant growth promoting bacteria *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. on the germination and growth of English ryegrass and microbiological activity in its rhizosphere.

## MATERIAL AND METHODS

PGPR *Pseudomonas* sp. P12, *Bacillus* sp. B1, *Streptomyces* sp. A3 (from the collection of the Faculty of Agriculture, Novi Sad) were used as inoculants. English ryegrass (*Lolium perenne* L. Calibra) was taken from the collection of Institute of Forage Crops, Kruševac, Serbia.

The effect of PGPR on seed germination of English ryegrass was evaluated in controlled conditions. Before inoculation, the seed sterilization was performed by 70% ethanol and 0.1% HgCl<sub>2</sub> solution and rinsing with sterile water. After that, one hundred seeds of perennial ryegrass inoculated with the appropriate bacteria were placed on filter paper in the dark at 22 C° until germination. Five and fourteen days after, hypocotyl and root length of seedlings were measured.

The variants of the experiment were the following: 1. *Pseudomonas* sp. P12, 2. *Bacillus* sp. B1, 3. *Streptomyces* sp. A3, 4. control - no inoculation. The experiment was conducted in 10 l volume vegetation pots. The pots were filled with soil having optimum characteristics for plant production. Before sowing, 50 ml of the inocula was introduced into the pots. The number of cells in 1 ml of the inoculum was 5x10<sup>8</sup>CFU/ml. Thirty and sixty days after sowing, length of stem and roots of plants (cm) were determined.

Thirty days after sowing, the number of microorganisms was determined, using the dilution method (Trolldenier 1996). Appropriate nutrient media were used (Hi Media Laboratories Pvt. Limited, Mumbai, India): nutrient agar for the total number of bacteria, synthetic agar for actinomycetes, potato dextrose agar for fungi and medium

with mannitol for azotobacter.

The data were statistically processed using STATISTICA 12 software (Hamburg, Germany). The significance of the difference between the applied treatments was tested using Fisher's LSD test.

## RESULTS AND DISCUSSION

In controlled conditions, the applied inoculants had different effects on the seed germination (Table 1).

Five days after inoculation, the highest percentage of germination (96%) was found in the variant in which *Streptomyces* sp. A3 was applied, while in the variant with *Pseudomonas* sp. P12, germination percentage was the lowest (11%). On hypocotyl and root length, the greatest impact had the application of *Streptomyces* sp. A3. Application of *Bacillus* sp. B1 and *Pseudomonas* sp. P12, compared to the control, had a negative effect on the hypocotyl and root length of seedling (Table 1).

Table 1. The percentage of seed germination, root and hypocotyl length

Variants	Seed germination (%)		Root length (mm)		Hypocotyl length (mm)	
	5days	14days	5days	14days	5days	14days
<i>Pseudomonas</i> sp. P12	11,0	89,0	1,0	6,0	13,0	90,0
<i>Bacillus</i> sp. B1	18,0	90,0	3,0	4,0	14,0	70,0
<i>Streptomyces</i> sp. A3	96,0	97,0	45,0	55,0	34,0	88,0
control	94,0	96,0	43,0	47,5	30,0	83,0

After 14 days, in the variant where *Streptomyces* sp. A3 was applied, the highest percentage of germination was determined. High percentage of seed germination was determined in other variants as well. *Streptomyces* sp. A3 had the highest effect on the hypocotyl and root length of the seedling. Variants in which *Bacillus* sp. B1 and *Pseudomonas* sp. P12 were added negatively affected the root length. On the other hand, the application of *Pseudomonas* sp. P12 had a good influence on the hypocotyl length.

Negative effect of *Bacillus* and *Pseudomonas* on the seed germination of perennial ryegrass could be due to their ability to produce hydrogen cyanide gas, which in large quantities inhibits the germination and growth of plant roots (Stamenov, 2014). These results suggest that investigated isolates should be applied after plants emergence, but not during the sowing. This is in accordance with the studies of Heydari et al. (2008), who determined the inhibitory effect of the hydrogen cyanide producing *Pseudomonas* on germination of wheat and rye. Alstrom and Burns (1989) also studied the hydrogen cyanide producing *Pseudomonas* which inhibited the development of the beans. In contrast to these studies, Niranjan et al. (2004) reported that the application of *P. fluorescens* had a positive effect on germination of millet grains. Moreover, Shaukat et al. (2006) reported that the introduction of *Azospirillum*, *Azotobacter* and *Pseudomonas* in the rhizosphere of sunflower and wheat had a positive effect on the germination and the length of the seedlings. However, these isolates were not determined as a hydrogen cyanide producing bacteria.

After thirty days, all the applied treatments had a positive effect on the stem and root length of the plant, but the highest effect was achieved by using *Pseudomonas* sp.



P12 and *Bacillus* sp. B1 (Table 2). In these variants, increase of the stem and root length was statistically significant.

Table 2. The effect of inoculation on the stem and root length (cm)

Measured	Plant	control	<i>Pseudomonas</i> sp. P12	<i>Bacillus</i> sp. B1	<i>Streptomyces</i> sp. A3
30 days	Stem	10,5 <sup>b</sup>	19,0 <sup>a</sup>	18,5 <sup>a</sup>	11,5 <sup>b</sup>
	Root	3,0 <sup>b</sup>	5,0 <sup>a</sup>	4,5 <sup>ab</sup>	3,75 <sup>ab</sup>
60 days	Stem	10,75 <sup>a</sup>	12,0 <sup>a</sup>	11,75 <sup>a</sup>	11,5 <sup>a</sup>
	Root	2,5 <sup>a</sup>	4,0 <sup>a</sup>	4,0 <sup>a</sup>	3,5 <sup>a</sup>
Average	Stem	10,62	15,5	15,12	11,5
Average	Root	2,75	4,5	4,25	3,62

\*The different superscripts indicate significant difference at P<0.05 according to Fisher's test.

After sixty days, all applied treatments acted positively on the stem and root length of plants, but these changes were not statistically significant. On average, the stem and root length was the largest in the variant with *Pseudomonas* sp. P12 and lowest in the control.

Positive effect of inoculation on the grass yield was found in the research of Dragomir et al. (2007), who examined the effects of *Azospirillum* and *Beijerinckia* inoculation on the dry matter of cocksfoot (*Dactylis glomerata* L). Rennie et al. (1983) found that introduction of *Bacillus* sp. positively influenced the yield of grass, which is in accordance with the results of our study. El-Tarabily (2008) reported that several *Streptomyces* species have the ability to improve plant growth by increasing seed germination and root elongation, which is the case with our *Streptomyces* species. Similarly, Biswas et al. (1994) proved that the introduction of *Azotobacter* and *Azospirillum* positively affected the growth of annual and perennial grasses.

Microbiological processes can additionally be stimulated by introducing biofertilizers. These microorganisms reproduce in soil and with their enzymatic activity raise and maintain the appropriate level of organic matter in soil (Hajnal-Jafari, 2010). In this study, the quantity of systematic and physiological groups of microorganisms in the rhizosphere of ryegrass depended on the applied inoculants. The number of investigated microbial groups in relation to the control was increased in all of the variants (Table 3).

Table 3. The effect of inoculation on the number of microorganisms in the rhizosphere of English ryegrass

Variants	The number of microorganisms in 1 g of absolutely dry soil (CFU/g)			
	Total nu. (10 <sup>6</sup> )	Fungi (10 <sup>4</sup> )	Actinomycetes (10 <sup>5</sup> )	Azotobacter (10 <sup>2</sup> )
<i>Pseudomonas</i> sp. P12	37.16 <sup>a</sup>	11.87 <sup>b</sup>	6.69 <sup>cb</sup>	118.93 <sup>b</sup>
<i>Bacillus</i> sp. B1	30.84 <sup>d</sup>	27.6 <sup>a</sup>	8.29 <sup>ab</sup>	130.06 <sup>a</sup>
<i>Streptomyces</i> sp. A3	33.45 <sup>b</sup>	3.67 <sup>c</sup>	4.88 <sup>dc</sup>	55.07 <sup>c</sup>
Control	20.54 <sup>c</sup>	3.55 <sup>c</sup>	3.93 <sup>d</sup>	37.52 <sup>c</sup>

\*The different superscripts indicate significant difference at P<0.05 according to Fisher's test

Application of *Pseudomonas* sp. P12 led to the increase of the total number of microorganisms, while isolate *Bacillus* sp. B1 affected positively the number of fungi, actinomycetes and *Azotobacter*. Application of *Streptomyces* sp. isolates A3 increased the number of actinomycetes. On average, the best effect on the microbial abundance had the application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1. Applied inoculants affected the number of *Azotobacter* notably while the lowest effect was found on the total number of microorganisms. Similarly, Nannipieri et al. (2003) demonstrated the positive effect of *Pseudomonas* sp. on the total number of bacteria and enzymatic activity in the soil. The research of Stamenov et al. (2012) demonstrated a positive effect of *Pseudomonas fluorescens* and *Bacillus* sp. on the total number of microorganisms, fungi, *Azotobacter* in the rhizosphere of perennial ryegrass.

Microorganisms have not yet found significant practical use in the production of forage grasses, even though more qualitative yield has been sought in livestock production. The results of this study demonstrate that positive results in the production of perennial ryegrass can be achieved by the use of microorganisms. Therefore, it is very important to carry out further studies of the relationship between applied microorganisms and ryegrass under field conditions in order to optimize the method and time of application of inoculants.

## CONCLUSION

Application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1 had a negative effect on ryegrass seed germination, while the use of *Streptomyces* sp. A3 gave positive results. On average, the application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1 had the greatest effect on the stem and root length of English ryegrass. The number of the investigated groups of microorganisms increased in all variants in comparison to the control. The best effect was achieved with *Bacillus* sp. B1. The results of our research confirmed that using specific species of microorganisms has positive effects in the production of English ryegrass which justifies the use of biofertilizers in the production of forage crops.

## REFERENCES

- ADESEMOYE, A.O., OBINI, M., UGOJI, E.O.: Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. *Braz. J. Microbiol.*, 39:423-426, 2008.
- ALSTROM, S., BURNS, R.G.: Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. *Biology and Fertility of Soil*, 7:232-238, 1989.
- BERG, G.: Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.*, 84:11-48, 2009.
- BISWAS, B.C., TEWATIA, R.C., PRASAD, N., DAS, S.: Biofertilizers in Indian Agriculture. Fertilizer Association of India, New Delhi, India, 1-43, 1994.
- EL-TARABILY, K.A.: Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. *Plant Soil*, 308:161-174, 2008.
- DOBBELAERE, S., VANDERLEYDEN, J., OKON, Y.: Plant growth-promoting ef-

- fects of diazotrophs in the rizosphere, *Crit.Rev.Plant Sci.*, 22:107-149, 2003.
- DRAGOMIR, C., MOISUC, A.: Bacterial inoculation effect upon yield capacity in alfalfa and orchard grass, *Lucrări Științifice, Facultatea de Agricultură USAMVB Timișoara*, 38:275-278, 2007.
- EGAMBERDIYEVA, D.: The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils, *Appl. Soil Ecol.*, 36:184-189, 2007.
- HAJNAL-JAFARI, T.: Uticaj inokulacije na prinos i mikrobiološku aktivnost u zemljištu pod usevom kukuruza. Doktorska disertacija. Poljoprivredni fakultet, Novi Sad, 2010.
- HEYDARI, S., MOGHADAM, P.R., ARAB, S.M.: Hydrogen cyanide production ability by *Pseudomonas Fluorescence* bacteria and their inhibition potential on weed. Proceedings of the Competition for Resources in a Changing World: New Drive for Rural Development, October 7-9, Hohenheim, Germany, 2008.
- NANNIPIERI, P., ASCHER, J., CECCHERINI, M.T., LANDI, L., PIETRAMELLARA, G., RENELLA, G.: Microbial diversity and soil functions, *Eur. J.Soil Sci.*, 54:665-670, 2003.
- NIRANJAN-RAJ, S., N.P. SHETTY, SHETTY, H.S.: Seed-bio-priming with *Pseudomonas fluorescens* isolates, enhances growth of pearl millet plants and induce resistance against downy mildew. *Int. J. Pest Manage.*, 50:41-48, 2004.
- RAMAMOORTHY, V., VISWANATHAN, R., RAGHUCHANDER, T., PRAKASAM, V., SAMIYAPPAN, R.: Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protec.* 20:1-11, 2001.
- RENNIE, R. J., RENNIE, D. A.: Techniques for quantifying N<sub>2</sub> fixation in association with nonlegumes under field and green-house conditions. *Can. J. Microbiol.*, 29:1022-1035, 1983.
- SELDIN, L., VAN ELSAS, J. D., PENIDO, E.G. C.: *Bacillus azotofans* sp. nov., a nifogen species from Brazilian Soils and grass roots. *Int. J. Syst. Bact.*, 34:451-456, 1984.
- SHAUKAT, K., AFFRASAYAB, S., HASNAIN, S.: Growth responses of *Triticum aestivum* to plant growth promoting rhizobacteria used as a biofertilizer. *Res. J. Microbiol.*, 1:330-338, 2006.
- STAMENOV, D., JARAK, M., ĐURIĆ, S., MILOŠEV, D., HAJNAL JAFARI, T.: The use of plant growth promoting Rhizobacteria in the production of English Ryegrass, *Plant, Soil and Environment*, 58(10) 477-480, 2012.
- STAMENOV, D.: Karakterizacija mikroorganizama promotora rasta i njihovo preživljavanje u rizosferi engleskog ljujla, Doktorska disertacija, Poljoprivredni fakultet, Novi Sad, 2014.
- TROLLDENIER G.: Plate Count Technique. In *Methods in Soil Biology*. Ed.Franz Schinner, Ellen Kandeler, Richard Ohlinger, Rosa Margesin. Springer-Verlag Berlin Heildeberg., 20-26, 1996.

# UTICAJ PRIMENE PGPR NA KLIJAVOST I RAST ENGLESKOG LJULJA I NA MIKROBIOLOŠKU AKTIVNOST U RIZOSFERI BILJKE

DRAGANA STAMENOV, SIMONIDA ĐURIĆ, TIMEA HAJNAL JAFARI

## Izvod

Cilj istraživanja je bio da se ispita efekat primene mikroorganizama promotora rasta *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. na klijavost i rast engleskog ljulja, kao i na mikrobiološku aktivnost u rizosferi biljke. Seme engleskog ljulja inokulisano određenom bakterijom, stavljeno je na filter papir na 22 °C na naklijavanje. Petog i četrnaestog dana, merena je dužina stabaoaceta i korenka klice, kao i procenat klijavosti. Varijante ogleđa su bile sledeće: 1. *Pseudomonas* sp. P12, 2. *Bacillus* sp. B1, 3. *Streptomyces* sp. A3, 4. Kontrola- bez inokulacije. Ogleđ je postavljen u posudama zapremine 10l. Trideset i šezdeset dana nakon setve, merena je dužina nadzemnog dela i korena biljke (cm). Trideset dana nakon setve, metodom agarnih ploča određivana je brojnost pojedinih grupa mikroorganizama. Primena *Pseudomonas* sp. P12 i *Bacillus* sp. B1 imala je negativan efekat na klijavost semena, dok je primena *Streptomyces* sp. A3 dala pozitivne rezultate. U proseku, primena *Pseudomonas* sp. P12 i *Bacillus* sp. B1 imala je najbolji efekat na dužinu nadzemnog dela i korena biljke. Brojnost ispitivanih grupa mikroorganizama u odnosu na kontrolu povećala se u svim varijantama. Najbolji efekat postignut je primenom *Bacillus* sp. B1.

**Ključne reči:** mikroorganizmi, klijavost, prinos, Engleski ljulj.

Received / *Primljen*: 17.03.2014

Accepted / *Prihvaćen*: 14.04.2014.

## THE IMPORTANCE OF LEGISLATION TO IMPROVING THE REPRODUCTIVE HEALTH OF PIGS

NENAD STOJANAC, OGNJEN STEVANČEVIĆ, MARKO R. CINCOVIĆ<sup>1</sup>

*SUMMARY: The aim of this study was to investigate the boars, sows and gilts for the presence of infectious pathogens that cause deterioration of health and reproduction of pigs. This research was conducted in accordance with the Ordinance on establishing a program of measures for animal health protection and it included breeding boars, sows and gilts, which had an abortion. Tested boars and breeding sows which had an abortion were seronegative to *Brucella* spp. and *Listeria* spp., while on two farms seropositive results for *Leptospira* spp. were found. Seroprevalence of Aujeszky's disease and PRRS in boars was 29.21% and 41.57%. All boars were seronegative for tuberculosis, and no pathogens of *Brucella* spp., *Leptospira* spp., and *Listeria* spp. were found in aborted fetuses and parts of placenta of sows and gilts. The results of this study point out the importance of veterinary legislation in health care and reproduction of pigs, which is reflected in the regular monitoring of breeding animals, prevention of disease occurrence and spreading and control of infectious diseases in pigs.*

**Key words:** veterinary legislation, abortion, reproductive health, pigs.

### INTRODUCTION

Reproductive health is one of the primary factors for successful pigs breeding. Infectious factors are the leading cause that reduce reproductive efficiency and profitability of pig production (Stojanac et al., 2013). The health status of boars used for artificial insemination (AI) in the intensive pig production is the basis for successful reproduction (Britt et al., 1999; Stančić et al., 2012). In Serbia, according to the Ordinance on establishing a program of measures for animal health protection for the 2013 (Službeni glasnik, 91/13, poglavlje IX), provides for a compulsory testing of breeding boars twice

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Nenad Stojanac, PhD, Research Assistant, Ognjen Stevančević, DVM, Assistant, Marko R. Cincović, PhD, Assistant Professor, University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad, Serbia.

Corresponding author: Nenad Stojanac, e-mail: stojanac.n@gmail.com; phone: +381 21 485-3515.

a year on: brucellosis, tuberculosis, Aujeszky's disease (AD), leptospirosis and porcine respiratory and porcine respiratory and reproductive syndrome (PRRS).

The pregnancy is the longest period in the sows reproductive cycle (114 days), normally ending with birth. Each premature birth represents an abortion and leads to reproductive efficiency reduction. In addition, abortion of infectious etiology represents a potential source of infection for other animals and humans (Pozzi and Alborali, 2012). For this reason, the Ordinance on establishing a program of measures for animal health protection for 2013 (Službeni glasnik, 91/13, poglavlje VIII), provides for a compulsory reporting of every abortion of sows and gilts to the veterinary organization. Also, there is an obligation to investigate abortions in sows and gilts for the presence of brucellosis, leptospirosis and listeriosis, which can cause infections in humans.

The objectives of this study were to examine five pig farms in Vojvodina during the 2013, for the following: (1) breeding boars for brucellosis, tuberculosis, AD, leptospirosis and PRRS, (2) sows and gilts that have had an abortion for the presence of brucellosis, leptospirosis and listeriosis.

## MATERIALS AND METHODS

*Trial Farms:* The study was conducted on five commercial farrow-to-finish pig farms which are located in AP Vojvodina, with the capacity over 1,000 sows, with a closed production cycle. The study included 89 breeding boars, used for AI of sows on the investigated farms. In the course of research 53 abortions in sows and gilts were recorded on the studied farms. All studied farms implement a system all in/all out at all stages of production.

*Experimental design:* Blood samples were collected from breeding boars using the method of plexus brachiocephalicus puncture and were sent to the laboratory of an authorized institution. The testing was conducted for the presence of antibodies specific for *Brucella* spp., *Leptospira* spp., *Aujeszky's disease* (AD), and *porcine respiratory and reproductive syndrome* (PRRS). At the same time with taking the blood samples from boars avian and bovine tuberculin were administered intracutaneously (i.c.) in the left and right ear. Reading the results was performed on two occasions, 48 and 72 hours after administration of the tuberculin. Based on the reaction obtained, the results of tuberculin test may be: negative (point of administration of tuberculin is not swollen or the swelling is the size of a pea and is not tempered), suspicious (swelling is slightly tempered, approximately 2 cm in diameter and is not surrounded by a red zone; these pigs should have tuberculin re-administered after 60 days) and positive (swelling is pasty, tempered, somewhat more sensitive, 2-5 cm in size and surrounded by a red zone, the middle of the swelling is always tempered and often covered with crust. Pigs which reacted positively to bovine or avian tuberculin, are considered positive in tuberculosis).

*Blood samples* were taken from sows and gilts which had an abortion by puncture of plexus brachiocephalicus, and aborted fetuses and parts of the placenta were taken, in the presence of a veterinary inspector, and sent to the lab of an authorized institution. Aborted fetuses and placenta were tested for presence of *Brucella* spp., *Leptospira* spp., and *Listeria* spp., while blood samples of sows and gilts were tested for presence of antibodies specific for *Brucella* spp., *Leptospira* spp., and *Listeria* spp.

*Data analysis:* Data were entered into an Excel spreadsheet (Microsoft Excel 2010) and imported into Stata (Stata 8 Intercooled for Windows 9x) in which data were

analyzed. Descriptive analysis was done in MiniTab version 14 (MiniTabR14b) and Excel (Microsoft Excel 2010).

## RESULTS

All tested boars were in good shape with no clinical signs of diseases. A total of 89 boars was tested for the presence of antibodies specific for *Brucella* spp., *Leptospira* spp., AD and PRRS (Table 1).

Table 1. The presence of antibodies specific for *Brucella* spp., *Leptospira* spp., AD and PRRS in breeding boars

Farm	No. boars tested	<i>Brucella</i> spp.	<i>Leptospira</i> spp.	AD	PRRS
I	15	0	0	11	7
II	17	0	2	8	6
III	12	0	1	0	0
IV	22	0	0	7	14
V	23	0	0	0	10
Total	89	0	3	26	37

All the tested breeding boars after i.c. administration of tuberculin had a normal appetite, behavior and libido in the next 72 hours. All 89 breeding boars tested were negative for tuberculosis.

Aborted fetuses and parts of placenta in all 53 tested abortions of sows and gilts were negative for presence of *Brucella* spp., *Leptospira* spp., and *Listeria* spp. The presence of antibodies specific for *Brucella* spp., *Leptospira* spp., and *Listeria* spp. is shown in Table 2.

Table 2. Presence of antibodies specific for *Brucella* spp., *Leptospira* spp., and *Listeria* spp. in the blood of sows and gilts which had an abortion

Farm	No. blood samples tested from sows and gilts	<i>Brucella</i> spp.	<i>Leptospira</i> spp.	<i>Listeria</i> spp.
I	7	0	0	0
II	3	0	0	0
III	14	0	1	0
IV	12	0	1	0
V	17	0	0	0
Total	53	0	2	0

## DISCUSSION

In this study, all tested animals, breeding boars, sows and gilts which had an abortion were seronegative for the presence of *Brucella* spp. Also, *Brucella* spp. was not isolated from aborted fetuses and parts of placenta. Regular monitoring, which is prescribed by the Ordinance on establishing a program of measures for animal health protection for 2013 (Službeni glasnik, 91/13), provides a basis for preventing the disease occurrence and spreading and control of zoonotic infections, which can cause reproductive disorders in humans (Xavier et al., 2010). The results of this study are similar to the findings of other authors who report very low seroprevalence of *Brucella* spp. (Hernández et al., 2013).

World Organisation for Animal Health (OIE) in its report states that climate changes lead to the emergence of the disease in animals, and six infectious diseases are described as particularly important for Europe (Dufour et al., 2008). *Leptospira* spp. a bacteria of zoonotic potential is among these six pathogens. This research found three seropositive breeding boars on two farms (II and III) and two seropositive breeding sows that have had an abortion on two farms (III and IV). *Leptospira* spp. was not isolated from the aborted fetuses and placenta, while occurrence of leptospirosis was correlated with the appearance of high humidity, or greater precipitation (Boqvist et al., 2012).

All of the breeding sows tested were seronegative to *Listeria* spp. *Listeria* spp. was not isolated from the aborted fetuses and sows, which represents a lower value of the prevalence of *Listeria* spp. compared to the findings of other authors (Autio et al., 2004).

Twelve months before the research, vaccination of pigs against the Aujeszky's disease was not carried out. Of the 89 tested breeding boars 29.21% were seropositive, which is a higher seroprevalence compared to the findings of other authors (Tummaruk and Tantilertcharoen, 2012). High seroprevalence in our study is certainly a consequence of vaccination of breeding boars in a preceding period, but it is also undoubtedly an indication that pathogen of Aujeszky's disease is present endemically on the tested farms.

Thirty-seven (41.57%) of the tested boars were seropositive for PRRS, i.e. on 4/5 (80%) of the examined farms a presence of PRRS was found. Other researchers around the world report similar results (Kim et al., 2002; Tummaruk and Tantilertcharoen, 2012).

Tuberculosis of pigs is rare compared to other swine diseases, but its zoonotic potential still presents a risk to human health. In our study all tested breeding boars were negative.

## CONCLUSION

The results of this study point to the importance of veterinary legislation in health care and reproduction of pigs, which is reflected in the regular monitoring of breeding animals. Serological and bacteriological findings obtained suggest that abortions in sows and gilts are not of infectious etiology, while complete diagnostics of abortions requires further research.



## REFERENCES

- AUTIO, T., MARKKULA, A., HELLSTROM, S., NISKANEN, T., LUNDEN, J., KORKEALA, H.: Prevalence and Genetic Diversity of *Listeria Monocytogenes* in the Tonsils of Pig. *J. Food Protect.*, 67(4)805-808, 2004.
- BOQVIST, S., ELIASSON-SELLING, L., BERGSTRÖM, K., MAGNUSSON, U.: The association between rainfall and seropositivity to *Leptospira* in outdoor reared pigs. *Vet. J.*, 193:135-139, 2012.
- BRITT, J.H., ALMOND, G.W., FLOWERS, W.L.: Diseases of the Reproductive System. In: *Diseases of Swine* (Straw BE, D'Allaire S, Mengeling WL, Taylor DJ, eds.). 8th ed., Iowa State University Press, Ames, Iowa, pp. 898-911, 1999.
- DUFOUR, B., MOUTOU, F., HATTENBERGER, A.M., RODHAIN, F.: Global change: Impact, management, risk approach and health measures – The case of Europe. *Revue Scientifique et Technique de Office International des Epizooties*, 27:529–550, 2008.
- HERNÁNDEZ, M., GÓMEZ-LAGUNA, J., TARRADAS, C., LUQUE, I., GARCÍA-VALVERDE, R., REGUILLO, L., ASTORGA, R.J.: A serological Survey of *Brucella* spp., *Salmonella* spp., *Toxoplasma gondii* and *Trichinella* spp. in Iberian Fattening Pigs Reared in Free-Range Systems. *Transbound Emerg. Dis.*, 2013.
- KIM, S.M., HAN, T.U., KANG, S.Y., SHIN, K.S., KIM, C.J., KIM, J.T., KIM, H.S.: Seroprevalence of Antibody to Porcine Reproductive and Respiratory Syndrome Virus in Diagnostic Submissions. *J. Vet. Sci.*, 3(3)159-161, 2002.
- POZZI, P.S. and ALBORALI, G.L.: Reproductive diseases in sows (*Sus scrofa domestica*): A Review. *Isr. J. Vet. Med.*, 67(1) 24-33, 2012.
- PRAVILNIKU O UTVRĐIVANJU PROGRAMA MERA ZDRAVSTVENE ZAŠTITE ŽIVOTINJA ZA 2013. GODINU. Službeni glasnik RS, br. 91/13, 2013.
- STANČIĆ, I., RADOVIĆ, I., DRAGIN, S., ERDELJAN, M., APIĆ, I.: Veterinary and zootechnical situation in artificial insemination at swine farm units in Vojvodina (Serbia). *Contemporary Agriculture*, 61(1-2)54-60, 2012.
- STOJANAC, N., STEVANČEVIĆ, O., POTKONJAK, A., KOVAČEVIĆ, Z., TOHOLJ, B., LJUBOJEVIĆ, D., KRAGIĆ, S.: Seroprevalence of infectious causes of abortion in sows. *Contemporary Agriculture*, 62(3-4) 206-211, 2013.
- TUMMARUK, P. and TANTILERTCHAROEN, R.: Seroprevalence of porcine reproductive and respiratory syndrome, Aujeszky's disease, and porcine parvovirus in replacement gilts in Thailand. *Trop. Anim. Health Prod.*, 44:983–989, 2012.
- XAVIER, M.N., PAIXÃO, T.A., DEN HARTIGH, A.B., TSOLIS, R.M., SANTOS, R.L.: Pathogenesis of *Brucella* spp. *The Open Veterinary Science Journal*, 4:109-118, 2010.

# ZNAČAJ ZAKONSKIH PROPISA ZA UNAPREĐENJE REPRODUKTIVNOG ZDRAVLJA SVINJA

NENAD STOJANAC, OGNJEN STEVANČEVIĆ, MARKO R. CINCOVIĆ<sup>1</sup>

## Izvod

Cilj ovog rada je bio da se ispitaju priplodne svinje (nerastovi, krmače i nazimice) na prisustvo infektivnih uzročnika, koji dovode do narušavanja zdravstvenog stanja i reprodukcije svinja. Ovo istraživanje je urađeno u skladu sa Pravilniku o utvrđivanju programa mera zdravstvene zaštite životinja i ispitani su priplodni nerastovi i krmače i nazimice koje su pobacile. Ispitani nerastovi i plotkinje koje su pobacile su bili seronegativni na *Brucella* spp i *Listeria* spp., dok su na po dve farme utvrđeni seropozitivni nalazi za *Leptospira* spp. Seroprevalenca bolesti Aujeckog i PRRS kod nerastova je iznosila 29,21% i 41,57%. Svi nerastovi su bili negativni na tuberkulozu, a iz pobačenih plodova i delova placente krmača i nazimica nisu ustanovljeni uzročnici *Brucella* spp., *Leptospira* spp. i *Listeria* spp. Rezultati ovog istraživanja ukazuju na značaj veterinarskih zakonskih propisa u zdravstvenoj zaštiti i reprodukciji svinja, koji se ogleda u redovnom monitoringu priplodnih životinja, sprečavanju nastanka, širenja i kontrole infektivnih bolesti svinja.

**Ključne reči:** veterinarski zakonski propisi, pobačaj, reproduktivno zdravlje, svinje.

Received / *Primljen*: 14.04.2014.

Accepted / *Prihvaćen*: 28.04.2014.

## CONCENTRATION OF BLOOD HSP70 AND ITS RELATION WITH LIPIDE MOBILISATION AND KETOGENESIS IN DAIRY COWS DURING PERIPARTURIENT PERIOD\*

MARKO R. CINCOVIĆ, BRANISLAVA BELIĆ<sup>1</sup>

*SUMMARY: Heat shock proteins (HSP) are a family of stress-responsive proteins, which has important role in cell protection from different stressors. Cows suffer from metabolic stress during early lactation, which is characterized with lipolysis increased and ketogenesis. The aim of this study is to measure HSP70 concentration in blood during periparturient period, and examine relationship between HSP70 concentration, lipolysis and ketogenesis. The experiment included 20 Holstein-Friesian cows. Blood samples were taken by venepunction of v.coccigea at week before calving and 1, 2, 4 and 8 weeks after calving. Concentration of HSP70 in blood is significantly higher in early lactation in comparison with week before calving. Also, higher concentration of NEFA and BHB were found in first and second weeks after lactation. HSP70 are in positive correlation with NEFA and BHB. Partially, correlations in first and second week after calving were stronger (when there is higher lipid mobilization and ketogenesis) then overall correlation, but it loses in fourth and eight weeks after calving. We can conclude that increases HSP70 concentration in first two weeks after calving is depend of level of lipid mobilization and ketogenesis. Metabolic adaptation as lipid mobilisation and ketogenesis upregulates HSP70 in dairy cows during early lactation.*

**Keys words:** cows, HSP70, lipolysis, ketogenesis.

### INTRODUCTION

Heat shock proteins (HSP) are a family of stress-responsive proteins that modulate cell function and contribute to protein homeostasis (Asea, 2008). HSP show some important functions: protection against apoptotic stimuli, assistance in de novo folding

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Marko R. Cincović; PhD, Assist. prof.; Branislava Belić, PhD, Assoc.prof.; Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Trg D.Obradovica 8, Novi Sad.

Corresponding author: Marko R. Cincović, mcincovic@gmail.com, phone: +381214853516

\* This research was a part of project TR31062 funded by the Serbian Ministry of Science.

of polypeptides, and prevention of protein misfolding and aggregation (Atalay et al. 2009; Mayer and Bukau 2005). Intracellular role of HSP is well-known. Certain results show an increase in the blood concentration of HSP70 in the various actions of stress and disease (De Maio, 2011). HSP are primarily released into blood during tissue injury, causing cell lysis or necrosis (Kimura et al. 2004; Lancaster and Febbraio 2005), but other results demonstrated increase concentration of HSP70 by exocytotically release in response to stressors which are not definitive reasons for cell death and necrosis (Fleshner and Johnson 2005; Johnson and Fleshner 2006).

Cows suffer from metabolic stress during early lactation, which is characterized with negative energy balance, lipolysis, ketogenesis, and dominant inflammation response (Sordillo et al., 2009; Cincović et al. 2012; Đoković et al., 2014). To date, only a limited number of studies investigated HSP70 concentration in dairy cows during periparturient period and its relation with metabolic stress. Kristensen et al. (2004) showed that Hsp72 is present in plasma from female dairy cattle and that the age and stage of lactation affect the concentration level. Results obtained from Catalani et al. (2010) provided the first evidence on dairy cows that both cellular and circulating Hsp72 are subjected to changes during the periparturient period.

The aim of this study is to measure HSP70 concentration in blood during periparturient period, and examine relationship between HSP70 concentration, lipolysis and ketogenesis.

## MATERIAL AND METHODS

*Animals:* The experiment included 20 Holstein-Friesian cows. Cows were kept in equal environmental and technology condition.

*Laboratory analysis:* Blood samples were taken by venepuncture of v.coccigea at week before calving and 1, 2, 4 and 8 weeks after calving. Blood samples were immediately transported to the laboratory. HSP70 concentration was measured in blood serum using a ELISA method (Cusabio, Ch) at Rayto ELISA reader. Concentration of NEFA and BHB was measured by standard biochemical kits (Randox, UK) using a Rayto spectrophotometer.

*Statistics model:* Difference in blood HSP70 concentration was analysed using a ANOVA model with post-hoc LSD test. Relationship between NEFA, BHB and HSP70 was examined by testing of Pearson's correlation coefficient. Relationship between HSP70 concentrations at different week of observation was analysed Pearson's correlation coefficient.

## RESULTS AND DISCUSSION

Concentration of HSP70 in blood was from 2.55 ng/ml in week before calving to 4.11 ng/ml in second week after calving. Concentration of HSP70 was significantly lower in week before calving in comparison to postpartal weeks, but difference in concentration of HSP70 at different week postcalvig was not observed. Results are present in Table 1. Correlation between HSP70 in different week was positive. Results are present in Table 2. Our results are in line with results obtained from Kristensen et al. (2004) and Catalani et al. (2010). Kristensen et al. (2004) were not observed statistically significant

difference between HSP concentrations in early and mid lactation, but concentration in early lactation showed tendency to be higher. Molvarec et al. (2007) reported that serum concentration of HSP72 is lower in pregnant than in non-pregnant women.

Concentration of NEFA was higher in first week after calving. Statistically higher concentration of BHB was observed in first and second weeks after calving. Results are present in Table 3. Positive correlation was observed between HSP70, NEFA and BHB through all weeks. Results are present in Table 4. Partially, correlations in first and second week after calving were stronger than overall correlation, but it loses in fourth and eight weeks after calving. We can conclude that increases HSP70 concentration in first two weeks after calving is depend of level of lipid mobilization and ketogenesis. In early lactation cows are in negative energy balance, with metabolic stress and inflammation. Eitam et al. (2009) reported that a prolonged low-energy diet promoted Hsp response with a significant increase of Hsp90 but unchanged levels of Hsp70 mRNA in white blood cells, and a lower expression of Hsp70 in milk somatic cells. In human with diabetes was founded increased concentration of HSP70. Same authors showed inverse correlation between fasting glucose concentration and HSP70 (Nakhjavani et al., 2010). Liver lipidosis and insulin resistance are typical findings during early lactation. Increased HSP70 concentration could be protective response to this process. Namely, intracellular HSP72 alleviated insulin resistance and reduced fat accumulation in the hepatocytes (Morino et al. 2008). Lipid mobilisation and ketogenesis upregulated HSP70 in dairy cows during early lactation.

Table 1: Concentration of HSP70 in blood during periparturient period

	-1	1	2	4	8
HSP70	2.55±0.6 <sup>a</sup>	3.77±0.5 <sup>b</sup>	4.11±0.4 <sup>b</sup>	3.8±0.4 <sup>b</sup>	3.9±0.5 <sup>b</sup>

<sup>a,b,c</sup> Values with different superscripts, within the row, significant differ (p < 0.05).

Table 2: Correlation between HSP70 concentrations in different weeks

	1	2	4	8
-1	0.49*	0.45*	NS	NS
1	/	0.56**	NS	NS
2	/	/	0.46*	0.45*
4	/	/	/	NS

\*p<0.05; \*\*p<0.01

Table 3: Concentrations of NEFA and BHB during periparturient period

	-1	1	2	4	8
NEFA	0.35±0.09 <sup>a</sup>	0.68±0.12 <sup>b</sup>	0.49±0.15 <sup>a</sup>	0.41±0.1 <sup>a</sup>	0.29±0.07 <sup>a</sup>
BHB	0.51±0.12 <sup>a</sup>	0.73±0.15 <sup>b</sup>	0.91±0.2 <sup>b</sup>	0.43±0.1 <sup>a</sup>	0.47±0.08 <sup>a</sup>

<sup>a,b,c</sup> Values with different superscripts, within the row, significant differ (p < 0.05).

Table 4: Correlation of HSP70 with NEFA and BHB during all time of observation and partially for each week

	All time	-1	1	2	4	8
Hsp70:NEFA	0.44*	0.33	0.62**	0.51*	0.25	0.19
Hsp70:BHB	0.5*	0.37	0.51*	0.57**	0.3	0.1

\*p<0.05; \*\*p<0.01

## CONCLUSION

Concentration of HSP70 in blood is significantly higher in early lactation in comparison with week before calving. Also, higher concentration of NEFA and BHB were found in first and second weeks after lactation. HSP70 are in positive correlation with NEFA and BHB. Partially, correlations in first and second week after calving were stronger (when there is higher lipid mobilization and ketogenesis) then overall correlation, but it loses in fourth and eight weeks after calving. We can conclude that increases HSP70 concentration in first two weeks after calving is depend of level of lipid mobilization and ketogenesis. Metabolic adaptation as lipid mobilisation and ketogenesis upregulates HSP70 in dairy cows during early lactation.

## REFERENCES

- ASEA, A.: Hsp70: a chaperokine. *Novartis Found Symp.* 291:173–179. doi: 10.1002/9780470754 030.ch13., 2008
- ATALAY, M., OKSALA, N., LAPPALAINEN, J., LAAKSONEN, D.E., SEN, C.K., ROY, S.: Heat shock proteins in diabetes and wound healing. *Curr Protein Pept Sci.*, 10:85–95, 2009.
- CATALANI, E., AMADORI, M., VITALI, A., BERNABUCCI, U., NARDONE, A., LACETERA, N.: The Hsp72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. *Cell Stress & Chaperones*, 15(6)781-790, 2010.
- CINCOVIĆ, M.R., BELIĆ, B., RADOJIČIĆ, B., HRISTOV, S., ĐOKOVIĆ, R.: Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. *Acta veterinaria (Beograd)*, 62(4)429-444, 2012.
- DE MAIO, A.: Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: A form of communication during injury, infection, and cell damage. *Cell Stress and Chaperones*, 16:235–249, 2011.
- DJOKOVIĆ R., CINCOVIĆ, M., KURCUBIC, V., PETROVIĆ, M., LALOVIĆ, M., JAŠOVIĆ, B., STANIMIROVIĆ, Z.: Endocrine and Metabolic Status of Dairy Cows during Transition Period. *Thai J Vet Med.* 44(1)59-66, 2014.
- EITAM, H., BROSH, A., ORLOV, A., IZHAKI, S.A.: Caloric stress alters fat characteristics and HSP70 expression in somatic cells of lactating beef cows. *Cell Stress Chaperones*, 14:173–82, 2009.
- FLESHNER, M., JOHNSON, J.D.: Exogenous extra-cellular heat shock protein 72: releasing signal(s) and function. *Int. J. Hyperthermia*, 21:457–471, 2005.

JOHNSON, J.D, FLESHNER, M.: Releasing signals, secretory pathways, and immune function of endogenous extracellular heat shock protein 72. *J. Leukoc. Biol.*, 79:425–434, 2006.

KIMURA, F., ITOH, H., AMBIRU, S., SHIMIZU, H., TOGAWA, A., YOSHIDOME, H., OHTSUKA, M., SHIMAMURA, F., KATI, A., NUKUI, Y., MIYAZAKI, M.: Circulating heat-shock protein 70 is associated with postoperative infection and organ dysfunction after liver resection. *Am. J. Surg.*, 187:777–784, 2004.

KRISTENSEN, T.N., LØVENDAHL, P., BERG, P., LOESCHCKE, V.: Hsp72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. *Cell Stress & Chaperones*, 9(2):143–149, 2004.

LANCESTER, G., FEBBRAIO, M.: Mechanisms of stress-induced cellular HSP72 release: implications for exercise-induced increases in extracellular HSP72. *Exerc. Immunol. Rev.*, 11:46–52, 2005.

MAYER, M.P., BUKAU, B.: Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol. Life. Sci.*, 62:670–684, 2005.

MOLVAREC, A., RIGÓ, J. Jr., NAGY, B., WALENTIN, S., SZALAY, J., FUST, G., KARADI, I., PROHASZKA, Z.: Serum heat shock protein 70 levels are decreased in normal human pregnancy. *J. Reprod. Immunol.*, 74:163–169, 2007.

MORINO, S., KONDO, T., SASAKI, K., ADACHI, H., SUICO, M.A., SEKIMOTO, E., MATSUDA, T., SHUTO, T., ARAKI, E., KAI, H.: Mild electrical stimulation with heat shock ameliorates insulin resistance via enhanced insulin signalling. *PLoS ONE* 3:e4068. doi:10.1371/journal.pone.0004068, 2008.

NAKHJAVANI, M., MORTEZA, A., KHAJEALI, L., ESTEGHAMATI, A., KHALILZADEH, O., ASGARANI, F., OUTERIO, T.F.: Increased serum HSP70 levels are associated with the duration of diabetes. *Cell Stress Chaperones*, 15(6):959–964, 2010.

SORDILLO, L.M., CONTRERAS, G.A., AITKEN, S.L.: Metabolic factors affecting the inflammatory response of periparturient dairy cows. *Animal Health Research Reviews*, 10(1):53–63, 2009.

## **KONCENTRACIJA HSP70 U KRVI I NJEGOVA VEZA SA LIPIDNOM MOBILIZACIJOM I KETOGENEZOM KOD KRAVA U PERIPARTALNOM PERIODU**

MARKO R. CINCOVIĆ, BRANISLAVA BELIĆ

### **Izvod**

Proteini toplotnog udara (heat shock proteins, HSP, eng.) predstavlja familiju proteina čija koncentracija raste prilikom delovanja različitih stresora na organizam jedinke. Tokom rane laktacije krave pate od metaboličkog stresa koji se karakteriše povećanom lipidnom mobilizacijom i ketogenezom. Cilj ovog rada je da se ispita veza između koncentracije HSP70 sa neesterifikovanim masnim kiselinama (NEFA) i beta-hidroksibutiratom (BHB) kod krava u peripartalnom periodu. U ogled je uključeno 20 krava Holštajn-frizijske rase. Krv je uzorkovana punkcijom v.coccigea u nedelji pre teljenja, zatim u prvoj, drugoj, četvrtoj i osmoj nedelji posle teljenja. Koncentracija HSP70

je bila značajno viša u nedeljama posle teljenja u odnosu na nedelju pre teljenja. Viša koncentracija NEFA i BHB nađena je u prvoj i drugoj nedelji posle teljenja u odnosu na ostale periodu. Koncentracija HSP70 pozitivno korelira sa vrenostima NEFA i BHB. Parcijalna korelacija pokazuje da su veze jače u prvoj i drugoj nedelji posle teljenja, što je period kada su lipidna mobilizacija i ketogeneza naizraženije. Koncentracija HSP70 u prve dve nedelje posle teljenja je zavisna od nivoa lipidne mobilizacije i ketogeneze. Metabolički stres koji se karakteriče lipidnom mobilizacijom i ketogenezom povećava koncentraciju HSP70 u krvi tokom rane laktacije.

**Ključne reči:** krave, HSP70, lipoliza, ketogeneza.

Received / *Primljen*: 15.04.2014.

Accepted / *Prihvaćen*: 28.04.2014.



## INSULIN RESISTANCE IN COWS DURING DRY PERIOD AND EARLY LACTATION\*

MARKO R. CINCOVIĆ, BRANISLAVA BELIĆ, RADOJICA ĐOKOVIĆ, BOJAN TOHOLJ, TALIJA HRISTOVSKA, BILJANA DELIĆ, MAJA DOŠENOVIĆ<sup>1</sup>

*SUMMARY: Insulin resistance is pathophysiological term defined as change in insulin responsiveness (insulin response to glucose) or insulin sensitivity (tissue response to insulin) or both. The aim of this study was to investigate insulin resistance in dairy cows during dry period and early lactation, and determine influence of insulin, glucose and NEFA to RQUICKI index of insulin resistance. Cows in early lactation showed lower concentration of glucose and insulin and higher concentration of NEFA compared to cows in dry period. Insulin:glucose index are 2.5 in dry period and 2.05 in early lactation. RQUICKI index was lower during early lactation. Determination of RQUICKI value using a concentration of glucose, insulin and NEFA was correct in 78.9% in dry period and 84.5% in early lactation. During dry period insulin and glucose showed significant influence to RQUICKI value (negative correlation), but there was found absence of NEFA influences. However, NEFA only showed significant influences to RQUICKI in early lactation (negative correlation). Insulin and glucose determined RQUICKI in 56% from 78.9% of whole model, but in early lactation NEFA determined 32% from 84.5%. Higher glucose concentration means higher insulin response in dry period with consequently poor insulin binding and glucose transport. In this condition it is need higher concentration of insulin to neutralize same concentration of glucose. Therefore, found higher insulin:glucose ration was found in dry period in relation to early lactation. Insulin resistance is more intense in early lactation compared to dry period. Based on metabolic change insulin resistance in dry period is consequence of decreased insulin sensitivity of peripheral tissue with compensatory increased insulin:glucose ration. During early lactation there is decrease insulin and glucose concentration with lower insulin response to glucose. Absence of insulin increases lipid mobilization which determined insulin resistance level.*

**Keys words:** *insulin resistance, lactation, cows.*

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Marko R. Cincović; PhD, Assistant prof.; Branislava Belić, PhD, Asoc.prof.; Radojica Đoković, PhD, Full prof., Bojan Toholj, Assistant prof.; Taliya Hristovska, DVM, Teching Assistant; Biljana Delić, MSc, Teching Assistant, Maja Došenović, DVM; Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Trg D.Obradovica 8, Novi Sad

Corresponding author: Marko R. Cincović, mcincovic@gmail.com, phone: +381214853516

\* This research was a part of project TR31062 funded by the Serbian Ministry of Science.

## INTRODUCTION

Insulin resistance is pathophysiological term defined as change in insulin responsiveness (insulin response to glucose) or insulin sensitivity (tissue response to insulin) or both. Insulin resistance could be at a few levels: prereceptor (problems in insulin-receptor interaction), receptor (decreased receptor number) and postreceptor level (intracellular problems, change in GLUT receptor) (Hayirli, 2006). During early lactation cows showed many endocrine and metabolic change prior to negative energy balance, partus and lactation. These changes included lower insulin concentration, lower glucose concentration and higher NEFA concentration in early lactation compared to dry cows (Wathes et al., 2007; Cincović et al., 2011; Cincović et al., 2012, Đoković et al., 2014).

Insulin resistance could be measured by intravenous glucose tolerance test, hyperinsulinemic euglycemic clamp (gold standards). These methods demand some equipment and they are not appropriate for everyday work. Because of that for everyday praxis were formed formulas for calculation of insulin resistance level (Hayirli, 2006). The most useful formula is RQUICKI formula (Holtenius and Holetenius, 2007; Balogh et al., 2008; Keresztes et al., 2009) which included basal level of insulin, glucose and NEFA. RQUICKI showed good correlation with metabolic adaptation during insulin resistance (Haarstrich, 2011; Cincovic, 2013).

In periparturient period exists the quite number of factor which be able to leads cows to insulin resistance. In dry period positive energy balance, obesity or change in gestation hormone activity with consequently higher insulin and glucose concentration could be introduction to insulin resistance. Hiperinsulinemia is in association with inability of insulin to suppress glucose output from liver and to increase glucose oxidation and lipomobilisation in peripheral tissue. In early lactation cows suffer from negative energy balance and glucose is direct to mammary gland without insulin control. Peripheral tissue of cows has to use fatty acid for energy purpose and cows come to lipid mobilisation and ketogenesis with higher NEFA in blood. Lower glucose concentration means decrease in insulin response. Lower insulin concentration increased lipolitic effects of other hormones with increase NEFA concentration. Elevated NEFA concentration causes inhibition of insulin-stimulated glucose uptake by peripheral tissues, decreases the number of GLUT 4, and disturbs intracellular insulin signalling pathways in the liver and peripheral tissues (Hayirli, 2006, Bjerre-Harpøth et al., 2012).

The aim of this study was to investigate insulin resistance in dairy cows during dry period and early lactation, and determine influence of insulin, glucose and NEFA to RQUICKI value of insulin resistance.

## MATERIAL AND METHODS

*Animals:* In experiment were included 40 cows of Holstein Friesian breeding (20 in early lactation and 20 in dry period). Cows were kept in same condition of feeding and in same technology environment. They had similar marks of body condition (3.5-4 before calving and 3.2-3.5 after calving). The diet was suited to the energy requirement of late pregnancy and early lactation cows.

*Blood sampling:* Blood was taken from *v.jugularis* in 5-10 days before calving and 5-10 days after calving. Vacutainers with heparin were used, and after collection blood samples were pack in fridge. Blood samples were analyst immediately after sample

collection.

*Laboratory analysis:* Concentration of glucose and insulin was measured by colorimetric methods using a standard kit (Randox, UK). Concentration of insulin was measured by ELISA kit (Cusabio, Ch). All measurement were done using a Rayto spectrophotometer.

RQUICKI: Revised quantitative sensitivity insulin check index (RQUICKI) was calculated using a standard formula  $RQUICKI = 1 / [\log(\text{glucose mg/dL}) + \log(\text{insulin } \mu\text{U/mL}) + \log(\text{NEFA mmol/l})]$ .

*Statistic model:* Difference in insulin, glucose and NEFA concentration and RQUICKI value between dry and early lactation cows was determined using a t-test. Influence of insulin, glucose and NEFA to RQUICKI index was analysed by regression analysis according to formula:  $RQUICKI = a + b(\text{insulin}) + b(\text{glucose}) + b(\text{NEFA})$ . Power of linear model was calculated (analysis of variance, F statistics) for dry period and early lactation separately. Regression parameter beta was analysed for influence of insulin, glucose and NEFA to RQUICKI value separately for dry period and early lactation. Partial coefficient of correlation and determination was calculated to determine influence of glucose, insulin and NEFA on RQUICKI in dry period and early lactation.

## RESULTS

Cows in early lactation showed lower concentration of glucose and insulin and higher concentration of NEFA compared to cows in dry period. RQUICKI index was lower during early lactation. These results are present in Table 1. Determination of RQUICKI value using a concentration of glucose, insulin and NEFA was correct in 78.9% in dry period and 84.5% in early lactation. Linear model are significant both in dry period and early lactation. Insulin:glucose index are 2.5 in dry period and 2.05 in early lactation. Results are present in Table 2.

During dry period insulin and glucose showed significant influence to RQUICKI value (negative correlation), but there was found absence of NEFA influences. However, NEFA only showed significant influences to RQUICKI in early lactation (negative correlation). These results are present in table 3. Insulin and glucose determined RQUICKI in 56% from 78.9% of whole model, but in early lactation NEFA determined 32% from 84.5%.

Table 1: Concentration of insulin, glucose, NEFA and value of RQUICKI in dry period and early lactation

	Dry period	Early lactation	p
Insulin ( $\mu\text{U/l}$ )	7.89 $\pm$ 2.1	5.11 $\pm$ 1.9	<0.05
Glucose (mmol/l)	3.1 $\pm$ 0.55	2.5 $\pm$ 0.32	<0.01
NEFA (mmol/l)	0.2 $\pm$ 0.12	0.75 $\pm$ 0.25	<0.001
RQUICKI	0.5 $\pm$ 0.04	0.44 $\pm$ 0.05	<0.05
Insulin:glucose	2.51 $\pm$ 0.15	2.05 $\pm$ 0.13	<0.01

Table 2: Prediction of RQUICKI value according to linear model

	Dry period	Early lactation
Power of model	10.95 (p<0.01)	12.16 (p<0.01)
Correct determination of RQUICKI by model	78.9%	84.5%

Tabela 3: Testing of b parameters in regression model for determination RQUICKI

	Regression parameter	Value of <i>b</i> parameter	SD	Partial correlation coefficient	Partial determination coefficient	p
Dry period	Constant	-0.71	1.05	/	/	NS
	Glucose	-0.28	0.08	-0.46	21%	<0.05
	Insulin	-0.34	0.09	-0.59	35%	<0.05
	NEFA	0.03	0.015	0.33	11%	NS
Early lactation	Constant	-0.84	1.18	/	/	NS
	Glucose	0.021	0.021	0.25	6%	NS
	Insulin	0.063	0.054	0.41	17%	NS
	NEFA	-0.32	0.096	-0.56	32%	<0.05

## DISCUSSION

Insulin concentration is depend from period of lactation with significantly lower concentration in early lactation. This finding is confirmed by many authors. Wathes et al. (2007) were made linear model of insulin concentration during periparturient period which was characterized with lower insulin concentration in early lactation. During negative energy balance insulin concentration is lower then 12 mIU/L (0,5 ng/ml), but in cows with positive energy balance concentration is between 6 and 50 mIU/L (0,25-2,1 ng/ml) (Kunz et al., 1985). Our results are in relation with previous results.

Glucose concentration is lower in early lactation compared to dry period. Ruminant has lower glucose concentration then monogastric animal, between 2.2-3.3 mmol/l. Average glucose concentration 10-15 days before calving was 2,7 mmol/L, at 5-10 day after calving 2,4 mmol/l. New results showed lower glucose concentration in same period, because of higher production and metabolic load (Jovanović et al., 1987; Cincović et al., 2011; Đoković et al., 2014). NEFA concentration was higher in early lactation (Kovačević i sar., 2002; Krnić i sar., 2006; Cincović i sar., 2011). It is depend from level of lipid mobilisation. NEFA concentration higher then 0.5 mmol/L increases risk for periparturient disease and poor metabolic adaptation (Hachenberg et al., 2007; Kessel et al., 2008; Ospina et al., 2010; Cincović et al., 2012).

In dairy cows RQUICKI index is the best indicator of insulin resistance. Value of RQUICKI was between 0.38 and 0.65 (Holtenius and Holtenius, 2007; Balogh et al., 2008; Keresztes et al., 2009; Haarstrich, 2011; Gross et al., 2011). Our results are in relation with that.

Level of lipolysis is in positive correlation with insulin resistance in cows during

early lactation. Lipolysis becomes cows to similar metabolic changes as it exist during insulin resistance (hypoglycemia, ketogenesis, fatty liver) (Hayirli, 2006; Cincović, 2013). During lipolysis many adipokins, oxidative stress and proinflammatory cytokines stimulate insulin resistance phenomenon (Li et al., 2013). Metabolic syndrome in human and cows showed similar characteristic in terms of lipid metabolism (Lopatnicu i Ronzoni, 2013). Latest results of Prodanović et al. (2013) showed a negative correlation between the values of NEFA and insulin response after an intravenous glucose tolerance test.

In dry period cows are in positive energy balance (Grummer and Rastani, 2003). At this condition higher glucose concentration means higher insulin response with consequently poor insulin binding and glucose transport. In this condition it is need higher concentration of insulin to neutralize same concentration of glucose. Therefore, in dry period was found higher insulin:glucose concentration ( $2.51 \pm 0.15$ ) in relation to early lactation ( $2.05 \pm 0.13$ ).

Insulin and glucose are important factor to determine RQUICKI in dry period. Insulin and glucose concentration are in negative correlation with RQUICKI index, that's way we can conclude that increase insulin and glucose concentration in dry period are represent of insulin resistance. Adipose tissue very quickly response to external stimuli and was founded that intravenous application of glucose stimulates key lipogenic enzymes in relation to dose of sugar (Carra i sar., 2013). This could be the reason why the glucose and insulin are the most important determining factors of insulin resistance in the dry period. During early lactation NEFA only significantly influences RQUICKI value. NEFA are in negative relation with RQUICKI. We can conclude that higher lipid mobilization is important sign of insulin resistance in early lactation. These finding are in relation with general model of insulin resistance.

## CONCLUSION

Insulin resistance is more intense in early lactation compared to dry period. Insulin and glucose are important factor to determine the level of insulin resistance in dry period. Increase insulin and glucose concentration in dry period means insulin resistance. During early lactation NEFA only significantly influences level of insulin resistance, and higher lipid mobilization means higher insulin resistance in cows. Based on metabolic change insulin resistance in dry period is consequence of decreased insulin sensitivity of peripheral tissue with compensatory increased insulin:glucose ration. During early lactation there is decrease insulin and glucose concentration with lower insulin response to glucose. Absence of insulin increases lipid mobilization which determined insulin resistance level.

## REFERENCES

BALOGH, O., SZEPES, O., KOVACS, K., KLUCSAR, M., REICZIGEL, J., ALCAZAR J.A., KERESZTES, M., FEBEL, H., BARTYIK, J., FEKETE, S.G., FESUS, L., HUSZENICZA G.: Interrelationship of growth hormone AluI polymorphysm, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. *Veterinarni Medicina*, 53(11)604-616, 2008.

BJERRE-HARPØTH, V., FRIGGENS, N.C., THORUP, V.M., LARSEN, T., DAMGAARD, B.M., INGVARSTEN, K.L., MOYES, K.M.: Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. *J. Dairy Sci.*, 95:2362–2380, 2012.

CARRA, M., AL-TRAD, B., PENNER, G.B., WITTEK, T., GABEL, G., FÜR, M., ASCHENBACH, J.R.: Intravenous infusions of glucose stimulate key lipogenic enzymes in adipose tissue of dairy cows in a dose-dependent manner. *J. Dairy Sci.*, 96(7)4299-4309, 2013.

CINCOVIĆ, M.R., BELIĆ, B., RADOJIČIĆ, B., HRISTOV, S., ĐOKOVIĆ, R.: Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. *Acta veterinaria (Beograd)*, 62(4)429-444, 2012.

CINCOVIĆ, M.R., BELIĆ, B., VIDOVIĆ B., KRČMAR, L.J.: Reference values and frequency distribution of metabolic parameters in cows during lactation and in pregnancy. *Contemporary agriculture*, 60(1-2)175-182, 2011.

CINCOVIĆ M.R.: Upotreba indikatora insulinske rezistencije u proceni metaboličkog statusa krava u ranoj laktaciji. Specijalistički rad. Fakultet veterinarske medicine, Beograd, 2013.

DJOKOVIĆ R. CINCOVIĆ, M., KURCUBIC, V., PETROVIĆ, M., LALOVIĆ, M., JAŠOVIĆ, B., STANIMIROVIĆ, Z.: Endocrine and Metabolic Status of Dairy Cows during Transition Period. *Thai J. Vet. Med.*, 44(1)59-66, 2014.

GROSS, J., VAN DORLAND, H.A., BRUCKMAIER, R.M., SCHWARTZ, F.J.: Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. *J. Dairy Sci.*, 94:1820-1830, 2011.

GRUMMER, R.R., RASTANI, R.R.: When should lactating dairy cows reach positive balance? *Prof. Anim. Scientist* 19:197-203, 2003.

HAARSTRICH, D.: Evaluation of surrogate indices of insulin sensitivity by means of hyperinsulinemic-euglycemic glucose clamps in dairy cows; u: Insulinsensitivität und Insulinresponse nach einer Langzeit-Supplementation von konjugierten Linolsäuren bei laktierenden Milchkühen. Inaugural disertation. Tierärztliche Hochschule Hannover, 2011.

HACHENBERG, S., WEINKAUF, C., HISS S., SAUERWEIN, H.: Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the periparturient period. *J. Anim. Sci.*, 85:1923-1932, 2007.

HAYIRLI, A.: The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. *Vet. Res. Commun.*, 30:749–774, 2006.

HOLTENIUS, P., HOLTENIUS, K.: A model to estimate insulin sensitivity in dairy cows. *Acta Veterinaria Scandinavica*, 49:29-31, 2007.

JOVANOVIĆ, M. J, STAMATOVIĆ, S, ŠAMANC, H. et al.: Prilog proučavanju metaboličkog profila krava u laktaciji. *Vet. Glasnik*, 41(6)449-454, 1987.

KERESZTES M, FAIGL, V., KULCSÁR, M., BALOGH, O., FÖLDY, J., FÉBEL, H., CHILLARD, Y., HUSZENICZA, G.: Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. *Domest Anim Endocrinol.*, 37(4)250-261, 2009.

KESSEL, S., STROEHL, M., MEYER, H.H.D., HISS, S., SAUERWEIN, H., SCHWARZ, F.J., BRUCKMAIER, R.M.: Individual variability in physiological adap-

tation to metabolic stress during early lactation in dairy cows kept under equal conditions. *J. Anim. Sci.*, 86:2903-2912, 2008.

KOVAČEVIĆ, M., ŠAMANC, H., DAMJANOVIĆ, Z., KIŠARČIĆ, S.: Koncentracija triglicerida i NEFA u krvi krava u visokom graviditetu i posle teljenja. *Savremena poljoprivreda*, 51(3-4)111-113, 2002.

KRNIĆ, J., PAŠIĆ-JUHAS, E., KANAČKI, Z., HODŽIĆ, A., HAMAMDŽIĆ, M.: Uticaj nepovoljnih uslova ishrane na karakteristike metaboličkog profila krava. *Savremena poljoprivreda*, 55(3-4)136-142, 2006.

KUNZ, P.L., BLUM, J.W., HART, I.C., BICKEL, H., LANDIS J.: Effects of different energy intakes before and after calving on food intake, performance and blood hormones and metabolites in dairy cows. *Animal Production*, 40(2)219-231, 1985.

LI, Y., DING, L., HASSAN, W., ABDELKADER, D., SHANG, J.: Adipokines and Hepatic Insulin Resistance. *Journal of Diabetes Research*, Article ID 170532, 8 pages, 2013.

LOPATNICU, E.-R., RONZONI, A.: Metabolic syndrome in humans and in cows: a review. *Lucrări Științifice Medicină Veterinară*, XLVI(4)100-109, 2013.

OSPINA, P.A., NYDAM, D.V., STOKOL, T., OVERTON, T.R.: Evaluation of nonesterified fatty acids and  $\beta$ -hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *J. Dairy Sci.*, 93(20)546-554, 2010.

PRODANOVIĆ, R., KIROVSKI, D., VUJANAC, I., ĐURIĆ, M., KORIĆANAC, G., VRANJEŠ-ĐURIĆ, S., IGNJATOVIĆ, M., ŠAMANC, H.: Insulin responses to acute glucose infusion in Buša and Holstein-friesian cattle breed during the periparturient period: comparative study. *Acta veterinaria*, 63(4)373-384, 2013.

WATHES, D.C., CHENG, Z., BOURNE, N., TAYLOR, V.J., COFFEY, M.P., BROTH-ERSTONE, S.: Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. *Domestic Animal Endocrinology*, 33(2)203-225, 2007.

## **INSULINSKA REZISTENCIJA KOD KRAVA U PERIODU ZASUŠENJA I RANOJ LAKTACIJI**

MARKO R. CINCOVIĆ, BRANISLAVA BELIĆ, RADOJICA ĐOKOVIĆ, BOJAN TOHOLJ, TALIJAH HRISTOVSKA, BILJANA DELIĆ, MAJA DOŠENOVIĆ

### **Izvod**

Insulinska rezistencija je patofiziološki termin koji označava umanjen odgovor insulina na stimuluse i/ili povećanu rezistenciju perifernog tkiva na insulin. Cilj ovog rada je da se ispituju karakteristike insulinske rezistencije kod krava u periodu zasušanja i u ranoj laktaciji, kao i da se odredi uticaj glukoze, insulina i NEFA na vrednost RQUICKI indeksa insulinske rezistencije. Kod krava u ranoj laktaciji postoji značajno niža koncentracija insulina i glukoze, viša koncentracija NEFA. Odnos insulin:glukoza bio je 2.5 u zasušenom periodu, a 2.05 tokom rane laktacije. Vrednost RQUICKI indeksa je značajno niža, što ukazuje na više izraženu insulinsku rezistenciju u ranoj lakta-

ciji. RQUICKI indeks može se pravilno odrediti upotrebom vrednosti insulina, glukoze i NEFA u 78.9% slučajeva u periodu zasušenja i u 84.5% slučajeva tokom rane laktacije. Tokom zasušenja insulin i glukoza imaju značajan uticaj na vrednost RQUICKI. U ranoj laktaciji samo NEFA značajno determiniše RQUICKI indeks. Povećane vrednosti insulina i glukoze u periodu zasušenja, odnosno povećanje vrednosti NEFA u ranoj laktaciji znače pad vrednosti RQUICKI, odnosno izraženiju insulinsku rezistenciju. Na osnovu metaboličkih izmena možemo zaključiti da smanjena osetljivost tkiva na insulin kod krava u periodu zasušenja dovodi do porasta koncentracije insulina i glukoze u krvi, što objašnjava njihov uticaj na nastanak insulinske rezistencije. Smanjena koncentracija glukoze i insulina u ranoj laktaciji ima za posledicu povećanu mobilizaciju lipida kada raste koncentracija NEFA, koji determiniše nivo insulinske rezistencije u ovom periodu.

**Gljučne reči:** insulinska rezistencija, laktacija, krave.

Received / *Primljen*: 08.04.2014.

Accepted / *Prihvaćen*: 28.04.2014.



## SERUM ACTIVITIES OF AST, ALT, GGT AND LDH IN CLINICALLY HEALTHY DAIRY COWS DURING TRANSITIONAL PERIOD AND MID LACTATION\*

RADOJICA ĐOKOVIĆ, VLADIMIR KURČUBIĆ, ZORAN ILIĆ,  
MARKO R. CINCOVIĆ, MILUN PETROVIĆ, BILJANA DELIĆ<sup>1</sup>

*SUMMARY: This study examined the activities of aspartate-aminotransferase (AST), alanine- aminotransferase (ALT), gamma-glutamyltransferase (GGT) and lactate-dehydrogenase (LDH) in the blood serum of 45 dairy Simmental cows divided into three groups according to production period. The first group (n = 15) consisted of late pregnant dairy cows, the second group (n = 15) cows in the early lactation, and the third group (n = 15) cow in mid lactation. The significant higher activity (P<0.05) of AST was determined in the early lactation period than in dry period, while enzyme activity in the mid lactation period was higher (P>0.05) than in the dry period. ALT activity showed a lower (P>0.05) serum activities in early lactation cows than in the late pregnant and mid lactation cows. The higher serum activities of GGT (P>0.05) and LDH (P<0.05) were determined in early lactation cows than in the late pregnant and mid lactation cows. Research results showed possibility of mild degree of hepatic lesions, probably due to fat infiltration in early lactation cows. Serum AST enzyme activities were significant correlated (P<0.05) with ALT, GGT and LDH activities and may be most sensitive indicato in determining the functional liver state in dairy cows during transition period and mid lactation.*

**Keys words:** dairy cows, serum enzymes activities, transition period, mid lactation.

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Radojica Đoković, PhD, prof.; Vladimir Kurčubić, PhD, prof.; Zoran Ilić, PhD; Marko R. Cincović, PhD, assistant prof.; Milun Petrović, PhD, prof.; Biljada Delić, MSc, Teach.Asist.; Department of Animal Science, Faculty of Agronomy-Čačak, University of Kragujevac, Cara Dušana 34, Department of Veterinary medicine Faculty of Agriculture, University of Novi Sad, Trg D.Obradovica 8, Novi Sad. Department of Animal Science, Faculty of Agronomy, University of Priština, Kopaonička bb. Lešak.

Corresponding author: Radojica Djokovic, djokovici@ptt.rs, phone: +381214853516.

\* This research was a part of project TR31001 and TR31062 funded by the Serbian Ministry of Science.

## INTRODUCTION

The priority for intensive milk production is prevention of metabolic diseases and other disorders. Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. The diseases listed in this include: the fat liver syndrome, ketosis, laminitis, mastitis, milk fever, retained placenta, metritis and infertility. The metabolic profile, a series of specific blood analytical tests is routinely used to reveal metabolic problems in dairy cattle. It involves collecting blood samples from eight to twelve cows at 4 time periods relative to calving (dry, early lactation, peak lactation and mid lactation) and measuring selected blood metabolites (Gross et al., 2001; Oetzel, 2004; Stengarde et al., 2008). A part of that test is determining enzyme activities in the serum: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT),  $\gamma$ -glutamyl-transferase (GGT) and lactate-dehydrogenase (LDH). The activities of enzymes are highly important blood parameters used in evaluating the degree of hepatocyte damage. The fatty liver infiltration and the hepatocyte degeneration in dairy cows involve cell membrane damage and hepatocyte destruction coupled to the release of cytoplasm enzymes (AST, ALT, GGT, LDH) and marked increases in the circulating activities (Pechova et al., 1997; Lubojacka et al., 2005; Stojević et al., 2005). Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature (*Kauppinen, 1984; Meyer and Harvey, 1998*). The AST activity is found to be the most sensitive indicator in diagnosing fatty liver in cows (Pechova et al., 1997; Lubojacka et al., 2005). GGT is a microsomal and membrane-bound enzyme found mostly in the liver, kidneys and small intestines. The increase in the activity of this enzyme results from damages of the cellular structure of hepatocytes (Kupczynski et al., 2002; Lubojacka et al., 2005). According to Tainurier et al. (1984) the activity of AST and GGT enzymes shows occasional irregular, small changes during pregnancy and early lactation, while the activity of ALT decreases significantly in the seventh and eighth month of pregnancy and at the beginning of lactation. LDH is not an organ-specific enzyme, being found at high concentrations in the muscles, heart, kidneys and the liver. It is released into the blood in cases of acute cell damage of the above organs. The blood activity of LDH is correlated with the degree of fatty infiltration of hepatocytes (Pechova et al., 1997).

The aim of this study was to determine the activity of AST, ALT, GGT and LDH and their relations in the blood plasma in Simmental dairy cows during transitional period and the mid lactation, in order to identify the pathological processes and changes in the metabolic status.

## MATERIAL AND METHODS

This experiment was carried out at Simmental dairy herd with several metabolic and reproductive disorders. The cows were mid-yielding with a preceding lactation about 6,500 l. Three groups of clinically healthy cows were chosen from herd. One group consisted late pregnant cows (n=15) in period from 25 to 1 (13±9) days to calving, a second group included early post-partum cows (n=15) in the first month of lactation (16 ± 9 days) and third group (n = 15) in mid lactation (115±29 days). The experimental cows were kept in tie-stall barns. The diet and the housing facilities were adapted to

research purposes. The diet suited the energy necessary for cows in late pregnancy, early and mid lactation.

The blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Serum AST, ALT, GGT and LDH were measured in the biochemical laboratory by different colorimetric techniques using a spectrophotometer (Cobas Mira plus) and the corresponding commercial kits.

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA).

## RESULTS

The mean values of AST, ALT, GGT and LDH activities in the blood plasma of dairy cows are shown for each period in Table 1. In this study highest serum activities of AST, GGT and LDH were determined in group of early lactation cows than in late pregnant and mid lactation cows, and showed significant variations ( $P < 0.05$ ) for AST and LDH activities. The lowest serum ALT activities were determined in early lactation cows, without significant differences ( $P > 0.05$ ) compare to other two groups of cows.

Correlation coefficients for the serum enzyme activities in dairy cows during transition period and mid lactation are shown in Table 2.

Table 1. Mean values ( $\bar{x} \pm \text{SD}$ ) of AST, ALT, GGT and LDH activities in dairy cows during transition period and mid lactation (average  $\pm$  SD)

	Late pregnancy	Early lactation	Mid lactation
AST (U/l)	26.45 $\pm$ 8.97 <sup>a</sup>	33.55 $\pm$ 9.35 <sup>b</sup>	32.61 $\pm$ 8.90 <sup>b</sup>
ALT (U/l)	72.47 $\pm$ 24.16 <sup>a</sup>	66.87 $\pm$ 12.96 <sup>a</sup>	96.38 $\pm$ 80.46 <sup>a</sup>
GGT (U/l)	20.61 $\pm$ 4.16 <sup>a</sup>	25.51 $\pm$ 4.91 <sup>a</sup>	23.03 $\pm$ 9.94 <sup>a</sup>
LDH (U/l)	715.69 $\pm$ 160.72 <sup>a</sup>	1058.15 $\pm$ 205.45 <sup>b</sup>	670.59 $\pm$ 134.95 <sup>a</sup>

<sup>a,b,c</sup> Values with different superscripts, within the row, significant differ ( $p < 0.05$ ).

Table 2. Correlation coefficients for the serum enzyme activities calculated for all cows in the present study.

	ALT	GGT	LDH
AST	0.33*	0.32*	0.43*
ALT	-	0.07	0.28
GGT	-	-	0.18

\*Significant correlations ( $P < 0.05$ ).

## DISCUSSION

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to predict such disorders and eventual subclinical diseases it is necessary to determine physiological ranges of biochemical

parameters in a clinically healthy herd. (Stojević et al., 2005).

The serum AST activity is considered as the most sensitive indicator for diagnosing fatty liver in this species (Pechova et al., 1997; Kupczynski et al., 2002; Lubojacka et al., 2005). AST is located in the cytoplasm and mitochondria of different tissues and organs, and the highest activities are detected in heart and skeletal musculature, as well as in liver in cows (Lubojacka et al., 2005). Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage. In the present study, the serum AST activities were significantly higher ( $P < 0.05$ ) in early lactating cows than in late pregnant cows, corroborating that the development of fatty infiltration in liver has led to cell disruption and release of the intracellular enzymes into the blood flow. ALT activity in cows differs during certain production periods. The lowest ALT activity was measured during early lactation, while activity increased ( $P > 0.05$ ) in the mid lactation cows. In the dry period enzyme activity decreased, but it was not statistically higher ( $P > 0.05$ ) than in the early lactation cows. Similar result was obtained by Kauppinen (1984). The author considers that the role of ALT in predicting liver damage in ketosis is not significant. Tainturier et al. (1984) in their study presented information that ALT activity decreased in the seventh and eighth months of pregnancy and that it remained stable until the end of pregnancy, and in the first month of lactation. Our results confirm this only partially because in the period of mid lactation we measured the highest concentration of ALT.

GGT is a microsomal and membrane-bound enzyme (Lubojacka et al., 2005). The increase in this enzyme activity results from disruption of the cellular structure of hepatocytes (Kupczynski et al., 2002, Lubojacka et al., 2005). GGT activity also depended on the observed period. Higher values were measured in early lactation compared to late pregnancy and mid lactation period, without statistically differences ( $P > 0.05$ ). El-Ghoul et al. (2000) found that GGT activity in late pregnancy is much lower than in the first week after calving, and 6 weeks after delivery the activity increased. LDH is a not specific organ enzyme, found at high concentrations in muscles, heart, kidneys and liver (Pechova et al., 1997; Lubojacka et al., 2005). It is released into the blood flow in cases of acute cell damage of the above organs (Lubojacka et al., 2005). In this study, LDH activities was significant higher ( $P < 0.05$ ) in early lactation cows than in late pregnant and mid lactation cows. This result suggested that early lactation cows had metabolic disturbances, and mild degree of hepatic lesions, probably due to fat infiltration

In the present study, the serum AST, ALT, GGT and LDH activities measured in late pregnant in early and mid lactating cows were included inside the physiological limits (AST: 78-132 U/l, ALT: 7-35 U/l, GGT: 10-25 U/l and LDH: 692-1445 U/l), (Stojić, 1996), but they were higher after calving, expect ALT activities, corroborating that the development of fatty infiltration in liver has led to cell disruption and release of the intracellular enzymes into the blood flow. Moreover, according to Pechova et al. (1997), the blood activities of liver enzymes are correlated with the degree of fatty infiltration in the organ. In agreement, only the serum AST enzyme activities were significant correlated ( $P < 0.05$ ) with ALT, GGT and LDH activities and may be most sensitive indicator in determining the functional liver state in dairy cows during transition period and mid lactation.

## CONCLUSION

Biochemical examination of blood serum showed higher activities of AST, GGT, LDH in early lactation cows such as lower concentrations of ALT compared to the groups of late pregnant and mid lactation cows. The serum AST enzyme activities were significant correlated with ALT, GGT and LDH activities and may be most sensitive indicator in determining the functional liver state in dairy cows during transition period and mid lactation. This result suggested that early lactation cows had metabolic disturbances, and mild degree of hepatic lesions, probably due to fat infiltration.

## REFERENCES

- EL-GHOUL, W., HOFMANN, W., Y. KHAMIS, Y., A. HASSANEIN A.: Beziehungen zwischen Klauenerkrankungen und peripartalen Zeitraum bei Milchrindern. *Prakt. Tierarzt*, 82:862-868, 2000.
- GROSS, J., VAN DORLAND, H.A., BRUCKMAIER, R.M., SCHWARZ, F.J.: Performance and metabolic profile of dairy cows during a lactation and deliberately induced negative energy balance with subsequent realimentation. *J. Dairy Sci.*, 94:1820-1830, 2001.
- KAUPPINEN, K.: ALAT, AP, ASAT, GGT, OCT activities and urea and total bilirubin concentrations in plasma of normal and ketotic dairy cows. *Zbl. Vet. Med. A.*, 31:567-576, 1984.
- KUPCZYNSKI, R., CHUDOBA-DROZDOWSK, B.: Values of selected biochemical parameters of cows blood during their drying-off and the beginning of lactation. *Electr. J. Polish Agric. Univ.*, 55:225-231, 2002.
- LUBOJACKA, V., PECHOVA, A., DVORAK, R., DRASTICH, P., KUMMER, V., POUL, J.: Liver steatosis following supplementation with fat in dairy cows diets. *Acta Vet. Brno*, 74:217-224, 2005.
- OZEL, G.R.: Monitoring and testing dairy herds for metabolic diseases. *Vet. Clin. North Am. Food Anim. Pract.*, 20:651-674, 2004.
- PECHOVA, A., LLEK, J., HALOUZKA, R.: Diagnosis and control of the development of hepatic lipidosis in dairy cows in the peri-parturient period. *Acta Vet. Brno*, 66:235-243, 1997.
- MEYER, D.J., HARVEY J.W.: Evaluation of hepatobiliary system and skeletal muscle and lipid disorders. In: *Veterinary Laboratory Medicine. Interpretation and Diagnosis.* (Meyer, D.J., Harvey, J.W., Eds.) 2nd ed., W.B. Saunders company Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. pp. 157-187, 1998.
- STOJEVIĆ, Z., PIRSLJIN, J., MILINKOVIC-TUR, S., ZDELAR-TUK M., LJUBIC B.B.: Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. *Vet. Arhiv*, 75:67-73.2005.
- STOJIĆ V.: *Physiology of domestic animals.* IP "Naučna knjiga", Faculty of Veterinary Medicine, Belgrade, pp.23-67, 1996.
- STENGARDE, L., TRAVEN, M., EMANUELSON, U., HOLTENIUS, K., HULTGREN, J., NISKANEN, R.: Metabolic profile in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis, *Acta Vet. Scand.*, 50:31, 2008.
- TAINTURIER, D., BRAUN, J.P., RICO, A.G., THOUVENOT J.P.: Variation in blood composition in dairy cows during pregnancy and after calving. *Res. Vet. Sci.*, 37:129-131, 1984.

# SERUMSKE AKTIVNOSTI AST, ALT, GGT I LDH KOD KLINIČKI ZDRAVIH MLEČNIH KRAVA TOKOM TRANZICIJSKOG PERIODA I SREDINE LAKTACIJE

RADOJICA ĐOKOVIĆ, VLADIMIR KURČUBIĆ, ZORAN ILIĆ, MARKO R. CINCOVIĆ, MILUN PETROVIĆ, BILJANA DELIĆ

## Izvod

U radu je procenjavana aktivnosti aspartat-aminotransferaze (AST), alanin-aminotransferaze (ALT), gama-glutamat-transferaze (GGT) i laktat dehidrogenaze (LDH) u krvnom serumu kod 45 Simentalskih mlečnih krava, podeljenih u tri grupe u zavisnosti od stadijuma produkcijonog perioda. Prvu grupu (n=15) su činile visoko gravidne krave, drugu grupu (n=15) krave u ranoj laktaciji, a treću grupu (n=15) krave u sredini laktacije. Statistički značajno više ( $P < 0.05$ ) aktivnosti AST su utvrđene kod krava u ranoj laktaciji u odnosu na zasušene krave, dok su enzimske aktivnosti AST kod krava u sredini laktacije bile veće ( $P > 0.05$ ) u odnosu na krave u zasušenju. ALT aktivnosti su pokazale niže vrednosti ( $P > 0.05$ ) kod krava na početku laktacije u odnosu na grupe krava u zasušenju i u sredini laktacije. Veće aktivnosti GGT ( $P > 0.05$ ) i LDH ( $P < 0.05$ ) u krvnom serumu su utvrđene kod krava u ranoj laktaciji u odnosu na aktivnosti ovih enzima u serumu kod zasušenih i krava u sredini laktacije. Dobijeni rezultati ukazuju na mogućnost blagog stepena oštećenja hepatocita, odnosno masnu infiltraciju hepatocita kod krava na početku laktacije. Serumske aktivnosti AST su bile u značajnoj korelaciji ( $P < 0.05$ ) sa aktivnostima ALT, GGT i LDH u krvnom serumu i AST može biti najosteljiviji indikator u utvrđivanju funkcionalnog stanja jetre kod mlečnih krava tokom tranzicionog perioda i sredine laktacije.

**Ključne reči:** mlečne krave, serumske enzimske aktivnosti, tranzicioni period, sredina laktacije.

Received / *Primljen*: 15.04.2014.

Accepted / *Prihvaćen*: 30.04.2014.

## WHY ELIMINATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN SLOVENE FARMS FAILED?

MARINA ŠTUKELJ<sup>1</sup>

*SUMMARY: Slovenia was free of PRRS before joining European Union (EU) in 2004. In EU quarantine for animals coming from other EU members is not mandatory and thus quarantine-free import of pigs from EU member countries unfortunately worsened the health status of pigs in our country. This is how diseases were and still are introduced in Slovenia. Antibodies against PRRS were detected in Slovene domestic pigs already in the second half of the year 2004. In 2010 as part of monitoring and in 2011 according to the annual Order the status of PRRS in Slovenia had been re-established. In both studies the PRRS antibody prevalence was 48% and results from molecular epidemiology originating from the same monitoring detected high genetic diversity which revealed the circulation of numerous subtypes all belonging to genotype 1. And in year 2012 genotype 2 was detected for the first time. Regarding these data both the quarantines and biosecurity measures were not implemented in farms. The study was carried out on 24 (5 negative, 19 positive) farms to evaluate the implementation of required biosecurity measures. On the positive farms for the elimination of PRRS either natural exposure or serum inoculation and strict biosecurity measures were proposed. On the negative farms only biosecurity measures were proposed. Five of the negative farms kept the negative status as a result of the implementation of all the proposed biosecurity measures. In seven positive farms PRRS was eliminated, all these farms adopted the strict biosecurity protocol. In 12 farms neither external nor internal biosecurity measures were implemented at all or at least not to a satisfying level. Hence PRRS and probably also other diseases were easily spreading between the farms and also inside the farms. Main detected deficiencies in biosecurity measures in Slovene farms are: introducing new pigs without quarantine, different categories of pigs in the same room, no consistency with all in all out system, outside traffic in the yard, numerous outside visitors to the farm. Slovenia was not aware of its excellent health status before EU membership and did not take measures to gain the official PRRS-free status which would definitely result in the economic benefits. Now*

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Marina Štukelj, PhD, Assistant Professor, University of Ljubljana, Veterinary faculty, Institute for health care of pigs, Gerbičeva 60, 1000 Ljubljana, Slovenia, e-mail: marina.stukelj@vf.uni-lj.si, phone: + 386 1 477 92 06

*economic status in pig breeding will not improve without well planned and well thought-out action.*

**Key words:** *biosecurity, PRRS, control*

## INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is an economically significant swine disease worldwide. The total cost of productivity losses due to PRRS virus (PRRSV) in the US national breeding and growing-pig herd was estimated at US \$ 664 million annually (Holtkamp et al., 2013). The mean loss per outbreak in Europe is 126 €/sow and total PRRS cost of an infected European farm is around 100 €/sow/year (Nieuwenhuis et al., 2012). Slovenia was free of PRRS before joining European Union (EU) in 2004 (Valenčak, 2004). In EU quarantine for animals coming from EU members is not mandatory and thus quarantine-free import of pigs from EU member countries unfortunately worsened the health status of pigs in our country. This is how the PRRS and other diseases were and still are introduced in Slovenia.

The highest density of the domestic pig population is located in the eastern part of the country. Moreover, the majority of the Slovenian pig industry involved traditional small pig farms. Antibodies against PRRS were detected in Slovene domestic pigs already in the second half of the year 2004. In 2010 as part of monitoring and in 2011 according to the annual Order the status of PRRS in Slovenia had been re-established. In both studies the PRRS antibody prevalence was around 48% and results from molecular epidemiology originating from the same monitoring detected high genetic diversity which revealed the circulation of numerous subtypes all belonging to genotype 1 (Toplak et al., 2010). And in year 2012 genotype 2 was detected for the first time. Regarding these data both the quarantines and biosecurity measures were not implemented in Slovene farms.

There is no drug to stop PRRS virus replication in the host. Therefore, pig herds need to adopt strict biosecurity programs (Pitkin et al., 2009; Spronk et al., 2010) to prevent PRRS virus infection. Once infected, the options are: to keep the farm PRRSV positive and control the manifestation of clinical disease or to eliminate PRRSV from the farm. To eradicate PRRS from a pig farm, there are different strategies including: complete herd depopulation and repopulation with PRRSV-negative pigs (Dee, 2003), test and removal (Dee and Molitor, 1998), and immunisation either with herd closure (Torremorell et al., 2003) or vaccination or serum inoculation and partial depopulation (Dee et al., 1997a; Dee et al., 1997b).

Implementation of biosecurity measures is essential for successful control or elimination of PRRS. Biosecurity is the term used to describe the measures and procedures needed to protect a population against the introduction and spread of pathogens. A biosecurity plan can be implemented to attain two strategic objectives:

- external biosecurity: policies developed to prevent the introduction of a new pathogen to the farm
- internal biosecurity: a biosecurity strategy developed to reduce the spread of disease among pigs on the farm (Pitkin et al., 2009).



**External biosecurity** includes: new replacement or incoming stock should be negative, quarantine, transportation of animals with clean, disinfected and dry transport vehicles, minimize or ban visitors to the farm, mandatory shower and changing of clothes and boots. Recent research has demonstrated the ability of infectious PRRSV to be transmitted by aerosols over a distance of 120 meters. However, preliminary results from experimental studies along with field reports indicate that aerosol transmission of PRRSV can occur at least up to 3.3 km or more (Dee et al. 2005, Otake et al., 2010). To reduce the risk of airborne spread of PRRSV, the adaptation of filtration systems to swine facilities has come about. Installation of an air filtration system depends upon the individual producer's budget, the location of the site (high swine density vs. low density), the level of acceptable risk and type of production system (Otake et al., 2010; Reicks, 2010).

In order to prevent spreading the virus it is essential to know the routes of spreading the virus. Pigs can be infected either by direct contact or indirectly by animal products, biological vectors, by contaminated materials or in a contaminated environment.

### **1. Direct routes**

As stated, pigs are the only animal capable of becoming infected with PRRSV (Zimmerman et al., 1997). Once infection occurs, the virus can be shed from persistently infected pigs via blood, saliva, milk and colostrum, urine and feces, as well as contaminated semen (Rossow et al., 1994; Wills et al., 1997).

#### **1.1 Live pigs**

To prevent introduction of virus with live animals the following biosecurity measures have to be implemented:

- replacement stock should originate from PRRS negative breeding,
- replacement stock should be bought from as few as possible sources,
- isolation (quarantine) facility is a critical component of a PRRSV biosecurity program. Isolation facilities should be located more than 120 meters from the breeding herd and ideally, offsite. Incoming stock should be kept separately from the resident stock for a minimum of 30 days. Animals should be monitored daily for clinical signs by the farm personnel,
- replacement stock should be blood-tested 24-48 hours after the arrival to the isolation facility as well as 5-7 days prior to their entry to the breeding herd. Once infected, PRRSV RNA can be detected in the bloodstream 24 hours after the infection; therefore, testing of samples by PCR is recommended to enhance detection of per acute infections. Samples collected late in the isolation period can also be tested by ELISA for the presence of PRRSV antibodies,
- herd closure; interrupting the introduction of new pigs into the farm for at least 6 months (Torremorell and Christianson, 2002)
- semen for the artificial insemination must originate from the negative boars (Pitkin et al., 2011; Zimmerman et al., 2012).

### **2. Indirect routes**

PRRSV can be mechanically transmitted in a number of ways:

- transport vehicles; PRRSV can be spread to susceptible animals following contact with contaminated transport vehicles. Therefore, as with facilities, stringent compliance with cleaning/disinfection and drying protocols is critical for sanitizing the trailers of transport vehicles. Following sanitation, the vehicle must be allowed adequate drying time after disinfection.

- around the farm the fence must be sited and the traffic in the yard must be banned. In front of each entry of farm buildings the disinfection footbaths must be placed (Dee et al., 2006; et al., 2011).
- visitors should be avoided as much as possible
- personnel and visitors; the hands, coveralls and boots of personnel can serve as mechanical vehicles for PRRSV. Personnel should practice one night of downtime before entering a farm. Research has shown that extended periods of downtime are not necessary for this agent. Shower protocols have been proven to successfully decontaminate personnel contaminated with PRRSV prior to entry. The use of such a procedure upon entry to the system each day is recommended. The Danish entry system; this system utilizes the changing of coveralls and boots plus the washing of hands in designated areas prior to entering the animal air space and has been demonstrated to be very effective for reducing the risk of PRRSV spread. Clothes and boots should never leave the farm and boots should be power-washed to remove feces from the soles and disinfected routinely (Dee et al., 2003; Otake et al., 2002; Pitkin et al., 2011).
- Insects; house flies and mosquitoes can serve as mechanical vectors of PRRSV and can transport the virus at least 2.4 km from an infected farm. In order to prevent spread of PRRSV via insects windows and areas that could be accessed by insects should be covered with screens. The use of insect bait is an effective means to control the number of insects. Cutting the grass and removing weeds surrounding swine facilities as well as removal of standing water are also recommended for eliminating insect breeding areas (Otake et al., 2003; Pitkin et al., 2009; Pitkin 2011).
- aerosol; airborne spread of PRRSV was established particularly at low temperature and high humidity (Dee et al., 2005; Otake et al., 2010; Pitkin et al., 2011).
- pig meat; meat from infected pigs can harbor PRRSV for at least 7 days at 4 °C and for months when frozen at -20 °C. Therefore, fresh or frozen pig meat should not be allowed into a swine facility at any time (Pitkin et al., 2011).

**Internal biosecurity** deals with the control of movement of virus from infected to non-infected animals within the same population. PRRS has tendency for self-recovery when biosecurity measures are implemented (Pitkin et al., 2011).

### **3. Direct transmission of virus**

Direct transmission of virus could be prevented with:

- construction of pig facilities that allow that each pig category is in a separate room,
- swine facilities should be managed using all in, all out pig flow, thereby reducing the spread of PRRSV from older, infected pigs to younger, naïve animals,
- partial depopulation of critical category of pigs meaning to move weaners off-site,
- one way pig flow,
- **cross fostering** within 24 hours of birth,
- set up hospital pens in a separate room, even better choice is to euthanize animals with poor prospect of recovery. Moving these pigs to a remote area of the barn so that blood from the euthanasia process does not infect susceptible pigs,
- interrupting the introduction of own replacement females and males into the breeding herd for at least 6 months (Dee et al., 2003; Dee et al., 2006; Otake et al., 2002; Pitkin et al., 2009; Pitkin et al., 2011).

#### 4. Indirect transmission of virus

Indirect transmission of virus could be prevented with:

- technology; all-in-all-out pig flow is an essential part of internal biosecurity. It is important to properly sanitize the facilities before introducing new animals. Otherwise the virus remains in the room and is important source of re-infection,
- categories of pigs must be separated by the different rooms,
- personnel's' and visitors' hands, coveralls and boots can serve as mechanical vehicles for PRRSV. Changing boots, clothes and gloves as well as strict hand disinfection is essential for preventing transfer of the virus between the buildings,
- use of footbaths can greatly help reducing the risk of PRRSV transfer between groups of pigs. Footbaths should be placed in front of each entry. Baths should be changed at least every day to maintain disinfectant efficacy,
- clean boots and changing coveralls after each category of pig,
- change disposable gloves between litters,
- change needles between sows and change needles between litters,
- equipment should be keep on-site otherwise equipment must be cleaned and disinfected,
- insects such as house flies and mosquitoes can serve as mechanical vectors of PRRSV. Therefore all inlets, windows and areas that could be accessed by insects should be covered with screens and the use of insecticides are highly recommended,
- regularly provide rodent control,
- any water supply that is drawn from a surface reservoir or shallow well can be a significant health risk. PRRS virus likes cold, wet conditions, and its survival in water is thought to be 10-14 days. That is why only clean and fresh water should be used,
- aerosol transmission is also a potential route of PRRSV spread between farms. For that purpose Air-filtration systems should be used (Dee et al., 2003; Dee et al., 2006; Otake et al., 2002; Pitkin et al., 2009; Pitkin et al., 2011).

The objective of this study was evaluation of implemented biosecurity measures on 24 farms with the intention of both the elimination of PRRS and general improvement of health status of farms.

### MATERIAL AND METHODS

*Farms:* The study was carried out from June 2010 till April 2014 on 24 farrow to finish farms.

Table 1. Number of breeding pigs and PRRS status for each farm.  
*Tabela 1. Broj priplodnih svinja i PRRS situacija za pojedine farme.*

FARMS																								
Farm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
No. of breeding pigs	12	7	17	74	141	80	17	15	30	66	88	41	50	50	52	37	60	50	45	34	64	59	53	58
PRRS status before study	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos	neg	neg	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos

Before study 4 farms were negative for antibodies against PRRSV (table 1).

*Samplings procedure:* Blood samples for blood analysis were collected from vena cava cranialis approximately every three months. Blood samples were collected into serum separator tubes (Vacuette, Greiner Bio-one, Kremsmunster, Austria). The tubes were left on a room temperature for 2 hours to clot prior to centrifugation at 1300 x g at 4 °C for 10 minutes.

All together 3962 blood samples were collected for serology.

*Enzyme-linked immunosorbent assay (ELISA):* The HerdChek, IDEXX Laboratories, PRRS X3 ELISA test was used for detecting antibodies in serum samples. The ELISA was performed according to the instructions of the manufacturer. The results of samples were divided in two groups, samples with S/P less than 0,4 as negative and samples with S/P greater than 0,4 as positive.

*Detection of PRRSV with gel-based RT-PCR:* Total RNA was extracted from 140 µl of serum samples using the QIAamp<sup>®</sup> viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. 1837 samples were tested individually or as pools (maximum 5 samples in the pool) by one-step RT-PCR (One-Step RT-PCR Kit, Qiagen, Germany) using sequences based on the open reading frame 7 (ORF7), which detect Type 1 and Type 2 PRRSV strains respectively (Donadeu et al., 1999; Toplak et al., 2012). The PRRS strain VR-2332 (Type 2) and the Lelystad viruses (Type 1) were used as positive controls. Reaction mixtures without RNA served as a negative control.

*Biosecurity measures:* The owners got acquainted with the obligatory measures. The required biosecurity measures included the strict biosecurity protocols: herd closure (introducing of new pigs to the farm was prohibited for at least 200 days. In this period also gilts from the farm itself cannot enter the breeding herd), entering farm only after changing the clothes, for personnel changing of coveralls and boots, washing of hands, using footbaths, individual responsibility for each pig category, all in/all out system, one age category of pigs in one room, one way pig flow, cleaning and disinfection of pens, pig equipment kept on the farm, deratization and disinsection, new replacement or incoming stock should be negative, quarantine. With each visit (approximately every three months) we'd checked if owners implemented required biosecurity measures.

## RESULTS

By ELISA and RT-PCR was proved that five of negative farms kept the negative status as a result of good biosecurity practice. From seven positive farms PRRS was eliminated (proved by ELISA and RT-PCR), all these farms adopted the strict biosecurity protocol and followed all the required biosecurity measures. In 12 farms they did not implement almost any of the required biosecurity measures. Besides other required biosecurity measures, the most important biosecurity measures that were not implemented are: the herd closure was not performed as well as all in/all out protocol, each pig category was not in a separate room, visitors and workers did not change the clothes and boots, a frequent traffic in the yard.

## DISCUSSION

Preventing the introduction of disease agents is a continuous challenge for pork producers and veterinarians. When a farm or site is affected by a disease the impact

can be devastating to the health of swine and the producer's bottom line. Every farm or production system should have a written plan documenting its biosecurity protocols. Appropriate education, training and compliance strategies should be utilized so all people working on and around the farm are properly informed and trained to apply the required biosecurity measures. Personnel should review, understand and follow the applicable biosecurity protocols for their assigned tasks (Canadian Swine Health Board, 2010; Pitkin et al., 2011).

Five of the negative farms kept the negative status as a result of a good biosecurity practice. From seven positive farms PRRS was eliminated, all these farms adopted the strict biosecurity protocol. In 12 farms neither external nor internal biosecurity measures were implemented at all or at least not to a satisfying level.

One very important measure that was not followed was the all-in/all-out protocol. In consequence non-vacant pens could not be thoroughly cleaned and disinfected and this resulted in poor pen hygiene. It follows that both pigs and pens were the source of PRRSV. Moreover, additional staffs were not appointed to a single pig category and did not change coveralls or boots between pig categories, nor wash hands between pig categories. Hence these factors were the reason as well as the route of transmission of PRRSV between categories and between facilities. Pig equipment was not kept on the farm but rather brought to the farm without prior sterilization (tattooing pliers). From the results of serology, molecular testing and biosecurity measures, we can conclude that the owners did not follow the required biosecurity measures nor carried out a strict herd closure which all proved to be the reason for the unsuccessful elimination of PRRS from the farms. Therefore, strict biosecurity procedures have to be followed at all times and it is imperative to have isolation areas for all replacement animals (Torremorell and Christianson, 2002). In order to eliminate PRRS from the farm, the proposed measures should be strictly followed since we are dealing with a one-site farm. Farrow-to-finish farms are at a disadvantage when it comes to using herd closure and natural exposure procedure since virus infection from the grower animals cannot be kept away from the breeding herd (Torremorell and Christianson, 2002).

## CONCLUSION

It can be concluded that the elimination with herd closure did not prove itself as a successful measure for elimination of PRRS from the 12 farrow-to-finish farm. Implementation of herd closure and biosecurity measures in field conditions is a much more difficult challenge than expected. Nonetheless, further study focusing on the education of farmers must be undertaken. On the other hand elimination of PRRS is possible even in situations in which neighboring farms are PRRS-positive and on one-site farms. Pig production in Slovenia is concentrated in few regions, so a regional program for elimination of PRRS is advisable to prevent the introduction of new viruses and to prevent movement of the virus within the farm and region (Štukelj, 2013).

## REFERENCES

CANADIAN SWINE HEALTH BOARD. National swine farm-level biosecurity standard: Technical committee on Biosecurity. Ottawa, 29: 2010. <http://www.swinehealth.ca/>

CSHB\_Biosecurity\_StandardE.pdf (5.11.2012)

DEE, S.A., JOO, H.S., POLSON, D.D., MARSH, W.E.: Evaluation of the effects of nursery depopulation of the profitability of 34 pig farms. *Veterinary Record*, 140: 498-500, 1997a.

DEE, S.A., JOO, H.S., POLSON, D.D., PARK, B.K., PIJOAN, C., MOLITOR, T.W., COLLINS, J.E., KING, V.: Evaluation of the effects of nursery depopulation on the persistence of porcine reproductive and respiratory syndrome virus and the productivity of 34 farms. *Veterinary Record* 140, 247-248, 1997b.

DEE, S.A., MOLITOR, T.W.: Elimination of porcine reproductive and respiratory syndrome virus using a test and removal process. *Veterinary Record* 143, 474-476, 1998.

DEE, S.: Approaches to prevention, control and eradication, In: Zimmerman, J.J., Yoon, K.J. (Eds.) PRRS Compendium. National Pork Board, Des Moines, Iowa, 119-130, 2003.

DEE, S.A., DEEN, J., JACOBSON, L., ROSSOW, K.D., MAHLUM, C., PIJOAN, C.: Laboratory model to evaluate the role of aerosols in the transport of porcine reproductive and respiratory syndrome virus. *Veterinary Record*, 156: 501-4, 2005.

DEE, S.A., DEEN, J., PIJOAN, C.: An evaluation of an industry-based sanitation protocol for full-size PRRSV-contaminated transport vehicles. *J. Swine Health Prod.*, 14: 307-11, 2006.

DONADEU, M., ARIAS, M., GOMEZ-TEJEDOR, C., AGUERO, M., ROMERO, L.J., CHRISTIANSON, W.T., SANCHEZ-VIZCAINO, J.M.: Using polymerase chain reaction to obtain PRRSV-free piglets from epidemically infected herds. *J. Swine Health Prod.*, 7: 255-61, 1999.

HOLTKAMP, D.J., KLIEBENSTEIN, J.B., NEUMANN, E.J., ZIMMERMAN, J.J., ROTTO, H.F., YODER, T.K., WANG, C., YESKE, P.E., MOWRER, C.L., HALEY, C.A.: Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *J. Swine Health Prod.*, 21(2): 72-84, 2013.

JULIO PINTO, C., SANTIAGO URCELAY, V.: Biosecurity practices on intensive pig production systems in Chile. *Prev. Vet. Med.*, 59, 139-145, 2003.

NIEUWENHUIS N., DUINHOF, T.F., VAN NES, A.: Economic analysis of outbreaks of porcine reproductive and respiratory syndrome virus in nine sow herds. *Veterinary Record*, 170, 225, 2012.

ROSSOW, K.D., BAUTISTA, E.M., GOYAL, S.M., et al.: Experimental porcine reproductive and respiratory syndrome virus infection in one-, four-, and 10-week-old pigs. *J. Vet. Diagn. Invest.*, 6: 3-12, 1994.

OTAKE, S., DEE, S.A., ROSSOW, K.D., et al.: Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J. Swine Health Prod.*, 10(2): 59-65, 2002.

OTAKE, S., DEE, S.A., ROSSOW, K.D., MOON, R.D., TRINCADO, C., PIJOAN, C.: Transmission of porcine reproductive and respiratory syndrome virus by houseflies (*Musca domestica*). *Veterinary Record*, 152: 73-6, 2003.

OTAKE, S., DEE, S.A., CORZO, C., OLIVIERA, S., DEEN, J.: Long-distance airborne transport of infectious PRRSV and *Mycoplasma hyopneumoniae* from a swine population infected with multiple viral variants. *Vet. Microbiol.*, 145: 198-208, 2010.

PITKIN, A., OTAKE, S., DEEN, J., MOON, R.D., DEE, S.A.: Further assessment of houseflies (*Musca domestica*) as vectors for the mechanical transport and transmission of porcine reproductive and respiratory syndrome virus under field conditions. *Can. J. Vet. Res.*, 73(2): 91-6, 2009.

PITKIN, A., OTAKE, S., DEE, S. Biosecurity protocols for the prevention of spread of porcine reproductive and respiratory syndrome virus ([http://aasv.org/aasv/PRRSV\\_BiosecurityManual.pdf](http://aasv.org/aasv/PRRSV_BiosecurityManual.pdf), Swine Disease Eradication Center), 1-17, 2011.

SPRONK, G., OTAKE, S., DEE, S.: Prevention of PRRSV infection in large breeding herds using air filtration. *Veterinary Record*, 166: 758-759, 2010.

TOPLAK, I., RIHTARIČ, D., HOSTNIK, P., GROM, J., ŠTUKELJ, M., VALENČAK, Z.: Identification of genetically diverse sequence of porcine reproductive and respiratory syndrome virus in Slovenia and the impact on the sensitivity of four molecular tests. *J. Virol. Meth.*, 179: 51-6, 2012.

TORREMORELL, M., CHRISTIANSON, W.T.: PRRS eradication by herd closure. *Adv. Pork. Prod.*, 13: 169-76, 2002.

TORREMORELL, M., HENRY, S., CHRISTIANSON, W.T.: Eradication using herd closure, In: Zimmerman, J., Yoon, K.J. (Eds.) *PRRS Compendium*. National Pork Board, Des Moines, IA, 157-162, 2003.

VALENČAK, Z.: Porcine reproductive and respiratory syndrome (PRRS) in Slovenia: Evaluation of serology. *Slov. Vet. Res.*, 41(2): 99-101, 2004.

ZIMMERMAN, J.J., YOON, K.J., WILLS, R.W., SWENSON, S.L.: General overview of PRRSV: a perspective from the United States. *Vet. Microbiol.*, 55: 187-96, 1997.

ZIMMERMAN, J.J., BENFIELD, D.A., DEE, S.A., et al: Porcine reproductive and respiratory syndrome virus (porcine arterivirus). In: Zimmerman JJ, Karriker LA, Ramirez KJ, Stevenson GW. *Diseases of swine*. 10th ed. Ames: Blackwell Publishing Professional, 461-86, 2012.

WILLS, R.W., ZIMMERMAN, J.J., YOON, K.J., et al.: Porcine reproductive and respiratory virus: Routes of excretion. *Vet. Microbiol.*, 57:69-81, 1997.

## **ZAŠTO ELIMINACIJA REPRODUKTIVNOG I RESPIRATORNOG SINDROMA(PRRS) SVINJA U SLOVENAČKIM UZGOJIMA SVINJA NIJE EFIKASNA?**

MARINA ŠTUKELJ

### **Izvod**

Pre ulaska u Europsku zajednicu Slovenija bila je slobodna od Svinjskog reproduktivnog i respiratornog sindroma (PRRS) i za sve uvezene životinje obavezni su bili karantini. Prilikom ulaska Slovenije u EU naše granice postale su otvorene za uvoz živih životinja. Uvoznicima više ne treba predložiti iskaze o zdravstvenom stanju svinja. Prilikom nekontrolisanog uvoza u Sloveniju unose se različiti uzročnici bolesti. Već u drugoj polovini 2004 godine kod domaćih svinja prvi put smo utvrdili antitela protiv virusa PRRS. Proširenost PRRS u Sloveniji ponovo smo počeli istraživati tek 2010 godine u okviru monitoringa i 2011 godine u okviru zakonskog propisa o izvođenju sistematičkog praćenja stanja zaraznih bolesti i vakcinacija. U obe dve studije ustanovili smo 48 postotnu prevalenciju bolesti. U pozitivnim uzgojima ustanovili smo prisustvo virusa genotipa 1 sa mnogobrojnim podtipovima. U 2012. godini prvi put smo dokazali i viruse PRRS genotipa 2. Ovi podaci otkrivaju, kako se aljkavo izvode

karantini i kako se u uzgojima ne izvode ni najosnovnije mere biosigurnosti. U okviru urađene studije obrađivali smo 24 uzgoja, kako bi ocenili izvođenje preventivnih mera protiv unosa bolesti. Pozitivnim uzgojima za eliminaciju bolesti predložili smo ili prirodno prekuženje ili izvođenje biosigurnosnih mera. Negativnim uzgajalištima predložili smo striktno izvođenje biosigurnosnih mera. Pet uzgoja, koje su bili u početku studije negativni uspeli su održati status, jer su izvodili sve propisane biosigurnosne mere. U sedam uzgoja smo u početku studije dokazali tako protivtela protiv virusa PRRS kako i virusnu nukleinsku kiselinu. U svih sedam uzgoja bolest smo eliminisali, jer su se odgajivači držali propisanih biosigurnosnih mera. U ostalim uzgojima ustanovili smo, da uopšte ne izvode ni vanjskih ni unutrašnjih biosigurnosnih mera ili jih izvode nedovoljno. Zbog toga se PRRS a možda i druge bolesti bezgranično šire između uzgoja i unutar njih. Glavni nedostaci, koje primećujemo u slovenačkim uzgojima su unos nove prasadi u uzgoj bez karantina, različite kategorije prasadi u istim prostorijama, neizvođenje sistema »all in, all out«, prodaja prasadi u dvorištu, mnogobrojne posete ljudi na farmi. Slovenija pre ulaska u EU nije bila dovoljno svesna svog visokog zdravstvenog stanja i nije dobila zvaničnog statusa zemlje proste PRRS, što bi značilo ekonomsku prednost. Bez planskog preduzimanja mera u smislu poboljšanja stanja prasadi se neće poboljšati ni stanje u svinjogojstvu.

**Ključne reči:** biosigurnosne mere, PRRS, kontrola.

Received / *Primljen*: 16.05.2014.

Accepted / *Prihvaćen*: 27.05.2014.



## SEROLOGICAL SURVEY ON SELECTED PATHOGENS ON 16 SMALL PIG FARMS IN SLOVENIA

IRENA GOLINAR OVEN, MARINA ŠTUKELJ<sup>1</sup>

*SUMMARY: Blood samples were collected between 2012 and 2014 on 16 small one-site pig farms with 34 to 79 breeding animals. 1636 serum samples of breeding animals and 815 serum samples of fatteners were tested with IDEXX PRRS ELISA. 1054 samples (from 7 small farms) were screened with one step RT-PCR (Qiagen, Germany) using sequences based on the open reading frame 7 (ORF7), which detect Type 1 and Type 2 PRRSV strains respectively. 81 serum samples of breeding animals and 85 serum samples of fatteners were tested with Swine Salmonella Antibody Test Kit (IDEXX) and CHEKIT\*APP-ApxIV (IDEXX). 80 serum samples of sows were assayed for leptospira antibody using a microscopic agglutination test (MAT). Antibodies against porcine reproductive and respiratory virus (PRRSV) were detected in 67.5% of breeding animals and in 41.1% fatteners. By RT-PCR, PRRSV was detected in 7.9% of serum samples. The seroprevalence to salmonella in breeding animals was 21% and in fatteners 5.8%. The prevalence against Actinobacillus pleuropneumoniae (APP) antibodies was 87.6% in breeding animals and 49.4% in fatteners. Three farms were positive to leptospirosis (serovar hardjo, serovar grippotyphosa). We suggested farms free of PRRS to continue with biosecurity practices and 11 farms with PRRS we had suggested biosecurity measures and herd closure. Almost all breeding animals had antibodies against APP, though clinical signs were not present. Seroprevalence of salmonella in Slovenia is low. 3 pig farms had leptospirosis. All tree farms treated infected pigs.*

**Key words:** pig, health status, small pig farms, control measures.

---

Original scientific paper / Originalni naučni rad

<sup>1</sup> Irena Golinar Oven, PhD, Asistant, Marina Štukelj, PhD, Assistant professor, University of Ljubljana, Veterinary faculty, Institute for health care of pigs, Gerbičeva 60, 1000 Ljubljana, Slovenia.

Corresponding author: Marina Štukelj, e-mail: marina.stukelj@vf.uni-lj.si, phone: +386 1 477 92 06.

## INTRODUCTION

After 2004 a significant decrease in pig meat production in Slovenia was noticed; also the number of animals and number of large farms has been decreasing for several years (Statistical office of the Republic of Slovenia, data from 1.1.2010). Most pig farms in Slovenia are small-sized and one site production farms. We have 3909 farms with 1 to 20 breeding pigs, 206 farms with 21 to 50 breeding pigs, 31 farms with 51 to 100 breeding pigs, 12 farms with 101 to 200 breeding pigs, 2 farms with 501 to 1000 breeding pigs and only 2 farms with more than 1000 breeding pigs (base VOLOS, Ministry for a agriculture, forestry and food, UVHVVR, data from 1.2.2013).

Before Slovenia joined EU in 2004 pig production in our country was for years under systematic monitoring and control for many important diseases. Representative numbers of pig sera were tested each year for the following disease: Aujeszky's disease, transmissible gastroenteritis, porcine reproductive and respiratory syndrome (PRRS) and pig brucellosis. All testings were obligatory and paid by government. In period 1995 to 2003 seroprevalence of PRRS was stable and low (3%), Slovenia was free of PRRS (Valenčak, 2004). Epidemiological situation in our country is different now. Majority of large farms became seropositive to PRRS what is not surprising due to fact that new breeding animals and fatteners were introduced to the farms without quarantine or serological testing.

Now in Slovenia systematic monitoring is implemented only for classical swine fever and Aujeszky's disease. The health status for other diseases on small pig farms is mostly unknown.

The objective of this study was serological survey on PRRS, salmonella, *Actinobacillus pleuropneumoniae* (APP) and leptospirosis at selected small farrow-to-finish pig farms in Slovenia. After completed serological testings we had proposed farmers appropriate control measures.

## MATERIAL AND METHODS

Blood samples were collected between 2012 and 2014 on 16 small one-site pig farms with 34 to 79 breeding animals, free of classical swine fever and Aujeszky's disease. At the beginning of the research pigs from 14 small farms were vaccinated against *Mycoplasma hyopneumoniae*, pigs at 4 farms were vaccinated against porcine circovirus diseases and porcine parvovirus, pigs from 3 farms were vaccinated against atrophic rhinitis, pigs from 2 farms were vaccinated against PRRS and pigs from 1 farm were vaccinated against *Erysipelothrix rhusiopathiae*.

Blood samples were drawn from the anterior *vena cava* by venipuncture. Serum was harvested by centrifugation for 10 min at 3000 rpm and stored at  $-20^{\circ}\text{C}$  until testing for the presence of antibodies.

*PRRS*: 1636 serum samples of breeding animals and 815 serum samples of fatteners were tested with IDEXX PRRS ELISA (HerdChek X3, IDEXX Laboratories Westbrook, Maine, USA). The presence or absence of antibody to PRRS virus (PRRSV) is determined by calculating the S/P ratio for each sample. If the S/P ratio was less than 0.40, the sample was classified as negative for PRRS virus antibodies. 1054 samples (from 7 small farms) were screened with one step RT-PCR (Qiagen, Germany) using sequences based on the open reading frame 7 (ORF7), which detect Type 1 and Type 2

PRRSV strains respectively (Donadeu et al., 1999; Torremorell et al., 2002). The PRRS strain VR-2332 (Type 2) and the Lelystad viruses (Type 1) were used as positive controls. Reaction mixtures without RNA served as negative controls. PRRSV positive samples were directly sequenced in both directions using the MacroGen sequencing service (MacroGen, South Korea) and the RT-PCR amplification primers. For each sample, 258 nucleotide long sequences were aligned with the published data using BLAST (available at <http://www.ncbi.nlm.nih.gov/>) at the National Centre for Biotechnology Information (NCBI), and PRRSV sequences obtained were compared using the sequence analysis software Lasergene® (DNASTAR Inc., Madison, WI, USA).

*APP*: 81 serum samples of breeding animals and 85 serum samples of fatteners were tested with ELISA CHEKIT\*APP-ApxIV (IDEXX). The diagnostic relevance of the result was obtained by comparing the OD of the samples, with OD of the positive control. Samples were considered positive when the value (%) was equal or higher than 40%. Samples were considered negative when the value (%) was lower than 30%. If the samples were suspect ( $\geq 30\%$  to  $< 40\%$ ) they were tested in a second run.

*SALMONELLA*: 81 serum samples of breeding animals and 85 serum samples of fatteners were tested with ELISA Swine Salmonella Antibody Test Kit (IDEXX). The results were calculated in OD% referring to a set of standard sera, defined according to the Danish Mix-ELISA system. Samples with “OD%” equal or greater than 20% (S/P = 0.5) were classified as positive (more stringent screening).

*LEPTOSPIROSIS*: 80 serum samples of sows were assayed for leptospirosis antibody using a microscopic agglutination test (MAT). As a positive result the titer  $\geq 1:100$  was estimated.

## RESULTS

Table 1. Presence of antibodies against PRRS on small farms.

Farm	Tested		Positive No. / %	
	Breeding animals	Fatteners	Breeding animals	Fatteners
1	10	19	0 / 0	0 / 0
2	310	100	187 / 60.3	49 / 49
3	337	162	305 / 90.5	85 / 52.4
4	44	41	41 / 93.2	20 / 48.8
5	20	20	0 / 0	0 / 0
6	17	13	0 / 0	0 / 0
7	127	75	29 / 22.8	7 / 9.3
8	114	39	64 / 56.1	31 / 79.4
9	64	5	64 / 100	5 / 100
10	68	21	68 / 100	21 / 100
11	44	31	35 / 79.5	21 / 67.7
12	15	15	0 / 0	0 / 0
13	211	137	179 / 84.8	87 / 63.5
14	30	50	0 / 0	0 / 0
15	157	75	67 / 42.6	0 / 0
16	68	12	66 / 97	9 / 75
Total	1636	815	1105 / 67.5	335 / 41.1

Antibodies against PRRS virus were detected in 67.5% of serum samples of breeding animals and in fatteners in 41.1% (Table 1).

Table 2. Results of PRRSV detection by RT-PCR on 7 small farms.

Farm	Tested	Positive No./%
2	141	20 / 14.1
3	436	56 / 12.8
7	72	0 / 0
8	52	0 / 0
11	20	3 / 15
13	247	5 / 2
15	86	0 / 0
Total	1054	84 / 7.9

By RT-PCR, PRRSV was detected in 7.9% of serum samples (Table 2). The nucleotide identity (ORF7) between reference strain Lelystad and PRRSV strain detected from 4 farms was only 89.5% - 93.4%.

Table 3. Presence of antibodies against salmonella and APP on small farms.

Farm	Tested		Salmonella Positive No.		APP Positive No.	
	Breeding animals	Fatteners	Breeding animals	Fatteners	Breeding animals	Fatteners
1	5	5	0	0	3	0
2	5	5	3	0	5	2
3	5	5	2	0	5	1
4	5	5	0	0	0	2
5	5	5	0	0	5	4
6	5	5	0	0	5	4
7	5	5	2	0	5	2
8	5	5	0	0	5	1
9	5	5	1	0	5	4
10	6	4	1	0	3	4
11	5	5	0	0	5	1
12	5	5	0	0	5	0
13	5	11	3	1	5	8
14	5	5	0	0	5	4
15	5	5	2	1	5	0
16	5	5	3	3	5	5
Total	81	85	17 / 21%	5 / 5.8%	71 / 87.6%	42 / 49.4%

The prevalence of serum samples with salmonella antibodies in breeding animals was 21% and in fatteners 5.8%. The prevalence against APP was 87.6% in breeding animals and 49.4% in fatteners (Table 3).

Table 4. Results of leptospirosis detection.

Farm	Tested	Positive No.
1	5	0
2	5	0
3	5	2
4	5	0
5	5	1
6	5	0
7	5	0
8	5	0
9	5	0
10	5	0
11	5	0
12	5	0
13	5	0
14	5	1
15	5	0
16	5	0
Total	80	4

Three farms were serologically positive to leptospirosis (serovar hardjo, serovar grippotyphosa).

## DISCUSSION

Slovenia was free of PRRS before joining EU in 2004 (Valenčak, 2004). Only few years later the detected seroprevalence was 44.8%, as indicated by the data of a study on antibody prevalence in 194 herds in 2010 (Toplak et al., 2010). PRRS is endemic in most swine-producing countries and leads to major economic losses (Stadejak et al. 2003). Besides that, increased prevalence of endemic diseases on the farm after introduction of the PRRS to the farm is important also from welfare perspective. Therefore the elimination of the disease is the most justified decision. Elimination of a disease is disappearance of all clinical cases of a specific disease (Toma et al., 1991) which is the consequence of desistance of virus replication and circulation in the population of pigs. No single strategy for elimination will work on infected farms; therefore, the program must be individually designed based on the unit's pig flow and facility design as well as serological results (Gillespie et al., 1999). In our study 11 small pig farms had antibodies against PRRSV and PRRSV was detected in breeding animals in 67.5% of serum samples and in fatteners in 41.1%. We suggested to PRRS free farms to continue with biosecurity practices and to 11 farms with PRRS we suggested biosecurity measures and herd closure or serum inoculation. Herd closure is required to achieve herd stability. In the period of herd closure new pigs cannot be introduced to the farm. This applies also to internal replacements of gilts to the breeding herd. PRRSV elimination through herd closure is based on the fact that naturally developed immunity eliminates virus infection from the farm (Torremorell et al., 2002). Serum inoculation is intramuscular injection of virus derived from serum of viremic pigs, which contains a farm-specific strain of PRRSV. Procedures that expose pigs to the homologous herd strain have repre-

sented successful approach being implemented in many countries (Batista et al., 2002). The success of PRRS elimination depends on the biosecurity practices and the cooperative work (Toma et al., 1991). On five small farms we successfully eliminated PRRS. On one farm we eliminated PRRS by serum inoculation, herd closure (a period of year and 7 months) and biosecurity measures, on other farms only with herd closure (immunization with natural exposure) and strict biosecurity protocols. Biosecurity measures included: entering the farm after changing clothes; having personnel aid in the changing of coveralls and boots; the washing of hands; using footbaths; maintaining individual responsibility for each pig category; use of the all in/all out system; one age category of pigs in one room; one way pig flow; the cleaning and disinfection of pens, pig equipment kept on the farm; deratization and disinsection (Pitkin et al., 2011). One farm which eliminated PRRS with herd closure and biosecurity measures quit vaccination against PRRS, the nucleotide identity (ORF7) between reference strain Lelystad and PRRSV strain detected on 3 farms was only 89.5% to 93.4%. The complete protection in PRRS is only against the same or homologous type of virus (Batista et al., 2002). The detected high heterogeneity between field and vaccine strain in Slovenian pig population is likely to be the main obstacle for the effective elimination of PRRS virus by vaccination, hence the detected PRRSV in Slovenia shared an 88.0% to 93.4% nucleotide identity with the Lelystad virus (Štukelj, 2013).

If those five farms will continue with biosecurity measures the PRRS eradication will be achieved in one year. Eradication of a disease is disappearance of the clinical case of a disease and of the pathogen, as well as antibodies and virus (Toma et al., 1991).

Pleuropneumonia is one of the important bacterial diseases of the respiratory tract of the pig and occurs in most pig-keeping countries (Gottschalk et al., 2003). Its importance derives from the fact that can cause pneumonia that results in death, clinical disease that may become chronic, or subclinical disease in successive batches of pigs and causes losses from death, reduced production, and increases costs of medication or vaccination. Early identification of subclinical infected herds is important for the control of the disease because carrier animals are the main source of transmission between herds (Gottschalk and Taylor, 2006). In our research almost all breeding animals had antibodies against APP, though clinical signs were not present. The ApxIV ELISA does not differentiate serotypes of APP. The previous survey made on large farms proved that in Slovenia pathogen serotypes are present (Golinar, 2002). Vaccination against APP in Slovenia is not used.

Salmonella infections of swine are concerned for two major reasons. The first is the clinical disease (salmonellosis) in swine that may result, and second is that swine can be infected with broad range of salmonella serotypes that can be a source of infection of pork products (Griffith et al., 2006). Although salmonella contamination of poultry and beef products exceeds that of pork, salmonella control programmes in swine will continue to be a primary focus of food safety initiatives. Seroprevalence of salmonella in Slovenia is low. From 2006 to 2008 we had started the salmonella control of fatteners at one large farm. In 2008 the level of samples with optical density (OD) % equal or greater than 20% was 24.8% (Štukelj et al., 2009). In present study at small farms OD 20% in fatteners was only 5.8%. Comparison of the seroprevalence between large and small farms shows that the number of positive fatteners is higher at large farms.

Leptospirosis is a cause of reproductive loss in breeding herds and is an occupational zoonosis of those who work with pigs. Endemic infection in a herd of swine may

produce little evidence of clinical disease, but when it is first introduced into susceptible breeding herd, or during periods of waning herd immunity, it can cause very appreciable losses through abortion, the full-term birth of dead pigs or weak pigs or reduced viability, or infertility (Ellis, 2006). In our survey 3 small pig farms had antibodies against leptospira. All tree farms had started with treatment prescribed by their veterinarians and they are now free of leptospirosis. We had additionally proposed strict biosecurity and rodent control programs instigated in and around the farm complex.

## CONCLUSION

By monitoring and enforcing measures against various diseases as well as following strict biosecurity protocol we could improve the wellbeing of these animals, decrease the necessity of medications and other pharmaceutical additives which would result in a more economically sound husbandry and consequently safer food for consumers.

## ACKNOWLEDGEMENT

The authors thank associate professor Zdravko Valenčak for his proof reading and his valuable scientific suggestions.

## REFERENCES

- BATISTA, I., PIJOAN, C., TORREMORELL, M.: Experimental infection of gilts with porcine reproductive and respiratory syndrome virus (PRRSV) during acclimatization. *J. Swine Health Prod.*, 10:147–150, 2002.
- DONADEU, M., ARIAS, M., GOMEZ-TEJEDOR C. et al.: Using polymerase chain reaction to obtain PRRS-free piglets from epidemically infected herds. *J. Swine Health Prod.*, 7:255-261, 1999.
- ELLIS, W.A.: Leptospirosis. In: *Diseases of swine* (B.E. Straw, J.J. Zimmerman, S. D’Allaire, D.J. Taylor, eds.). Blackwell Publishing, Ames, pp. 691-700.
- GILLESPIE, T.G., CAROLL, A.L.: Techniques for PRRSV elimination utilizing modified live virus vaccines on single-site swine farms. *Proceedings of the 4<sup>th</sup> International symposium on emerging and re-emerging pig diseases*, Rome, Italy, 29 June -2 July, 2003, pp. 127–128.
- GOLINAR, I.: Porcine respiratory disease complex (PRDC) in Slovenia, preliminary results. *Proceedings of the 17th International Pig Veterinary Society Congress*, Ames, USA, 2-5 June, 2002, pp. 325.
- GOTTSCHALK, M., BROES, A., FITTIPALDI, N.: Recent developments on *Actinobacillus pleuropneumoniae*. *Proc. Am. Assoc. Swine Vet.*: 387-393, 2003.
- GOTTSCHALK, M., TAYLOR D.J.: *Actinobacillus pleuropneumoniae*. In: *Diseases of swine* (B.E. Straw, J.J. Zimmerman, S. D’Allaire, D.J. Taylor, eds.). Blackwell Publishing, Ames, pp.563-576, 2006.
- GRIFFITH, R.W., SCHWARTZ, K.J., MEYERHOLT, D.K.: Salmonella. In: *Diseases of swine* (B.E. Straw, J.J. Zimmerman, S. D’Allaire, D.J. Taylor, eds.). Blackwell Publishing, Ames, pp. 739-754, 2006.

PITKIN, A., OTAKE, S., DEE, S.: Biosecurity protocols for the prevention of spread of porcine reproductive and respiratory syndrome virus. Swine Disease Eradication Center, University of Minnesota College of Veterinary Medicine, 2011, pp. 17. [http://www.aasv.org/aasv/VIRUS\\_PRRS\\_BiosecurityManual.pdf](http://www.aasv.org/aasv/VIRUS_PRRS_BiosecurityManual.pdf) (5.11.2012)

STADEJEK, T., STANKIEWICZ, I., PESJAK, Z.: Concurrent circulation of PRRSV-EU and PRRS-US within swine herd in Poland. Proceedings of the 4<sup>th</sup> International symposium on emerging and re-emerging pig diseases, Rome, Italy, 29 June -2 July, 2003, pp. 67–68.

ŠTUKELJ, M., GOLINAR OVEN, I., VALENČAK, Z.: Assessment of salmonella prevalence on large pig farm with serological testing in finishing pigs. *Compt. Rend. Bulg.,Sci.*, 62(8)1031-1038, 2009.

ŠTUKELJ, M.: Elimination of porcine reproductive and respiratory syndrome (PRRS) with natural exposure, serumization and vaccination. PhD Thesis, Veterinary Faculty, Slovenia, Ljubljana, 2013.

TOMA, B., VAILLANCOURT, J.P., DUFOUR, B., et al.: Dictionary of veterinary epidemiology. Iowa State University press, Ames, pp. 83, 1991.

TOPLAK, I., ŠTUKELJ, M., ZABAVNIK PIANO, J., HOSTNIK, P., GROM, J., VALENČAK Z.: Študija o pojavnosti prašičjega reproduktivnega in respiratornega sindroma (PRRS) v Sloveniji v letu 2010. Veterinarska fakulteta, Ljubljana, pp. 1-40, 2010.

TORREMORELL, M., CHRISTIANSON, W.T.: PRRS eradication by herd closure. *Adv. Pork. Prod.*, 13:169-176, 2002.

VALENČAK, Z.: Porcine reproductive and respiratory syndrome (PRRS) in Slovenia: evaluation of serology. *Slov. Vet. Res.*, 41(2)99-101, 2006.

## SEROLOŠKE PRETRAGE ODABRANIH UZROČNIKA NA 16 MANJIH UZGOJA SVINJA U SLOVENIJI

IRENA GOLINAR OVEN, MARINA ŠTUKELJ

### Izvod

Uzorci krvi uzimani su u godinama 2012 do 2014 na 16 manjih uzgoja svinja koje drže 34 do 79 priplodne životinje. 1636 uzoraka seruma priplodnih životinja i 815 uzoraka tovljenika je bilo testirano IDEXX PRRS ELISA testom. 1054 uzoraka (sa 7 uzgoja) bilo je skenirano sa RT-PCR (Qiagen, Nemačka) upotrebom sekvencija koje temelje na "open reading frame" 7 (ORF7) koji utvrđuje tip 1 odnosno tip 2 PRRS virusni antigen. 81 uzoraka seruma priplodnih životinja i 85 uzoraka tovljenika testirali smo sa Swine Salmonella Antibody Test Kit (IDEXX) i CHEKIT\*APP-ApxIV (IDEXX). 80 seruma krmača testirali smo na prisustvo protivtela leptospire upotrebom testa mikroskopske aglutinacije (MAT). Protivtela protiv virusa reproduktivnog i respiratornog sindroma svinja (PRRS) utvrdili smo kod 67,5% priplodnih životinja i 41,1% tovljenika. Upotrebom RT-PCR, PRRS virus je bio dokazan kod 7,9% uzoraka seruma. Seroprevalencija na salmonelu kod priplodnih životinja bila je 21% a kod tovljenika 5,8%. Prevalencija na *Actinobacillus pleuropneumoniae* (APP) protivtela je bila 87,6% kod priplodnih i 49,4% kod tovnih životinja. Tri uzgoja bila su pozitivna na leptospiru (serovar hardjo, serovar



grippotyphosa). Na farmama slobodnih od PRRS svetovali smo održavanje postojećih mera biosigurnosti a na zaraženim farmama preporučili smo biosigurnosne mere i zatvaranje stada. Gotovo sve priplodne životinje su imale protivtela protiv APP ali bez kliničkih znakova. Serprevalencija salmoneloze je u Sloveniji niska. 3 uzgoja imala su leptospiru. Na svima se zaražene svinje leče.

**Ključne reči:** svinje, zdravstveni status, manji uzgoji, mere nadzora.

Received / *Primljen*: 16.05.2014.

Accepted / *Prihvaćen*: 30.05.2014.

## COMPOSITION AND GERMINATION CAPABILITY OF WEED SEED BANK IN THE SOIL UNDER MAIZE

KONSTANTINOVIĆ BRANKO, BLAGOJEVIĆ MILAN,  
KONSTANTINOVIĆ BOJAN, SAMARDŽIĆ NATAŠA<sup>1</sup>

*SUMMARY: Monitoring of quantitative and qualitative properties of weed species seeds in the arable soil layer is one of the most efficient methods for prediction of weed occurrence in the field. It provides better herbicide choice, as well as their timely application. In 2011 and 2012 in two localities with similarly applied agricultural practice in maize crop, weed seed bank was studied in the arable soil layer up to 30 cm of depth. In the first locality, seeds of 12 weed species were separated. Of these species, the most numerous were seeds of *Polygonum lapathifolium* and *Sinapis arvensis* at all studied depths, and the greatest quantity was found at the depth of 20-30 cm. The greatest number of all remaining evidenced weed species was also found at this depth. In the other locality seeds of even 15 weed species was separated, but only seeds of *Amaranthus retroflexus* occurred in greater quantity at all studied layers. In average, for all species, the greatest number of seeds was found in the deepest layer, but statistically significant differences were not recorded in number of seeds per soil depth. After determination of the separated weed seeds followed their germination in climatic chamber. The highest germination percentage of 70-80% was recorded for weed species *Sinapis arvensis* at locality Žabalj. At locality Zmajevo, the highest germination was established for weed species *Amaranthus retroflexus* and *Chenopodium album*.*

**Key words:** weed soil seed bank, maize, germination, hypocotyls, epicotyls.

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Branko Konstantinović, PhD, Full Professor, Milan Blagojević, Research Assistant, Bojan Konstantinović, PhD, Assistant Professor; Nataša Samardžić, MSc, Research Assistant, University of Novi Sad, Faculty of Agriculture, 21000 Novi Sad, Serbia.

Corresponding author: Branko Konstantinović, e-mail: brankok@polj.uns.ac.rs; phone: +381 21 485-3319

## INTRODUCTION

Well-designed and efficient crop protection from diseases, pests and weeds is significant for intensive plant protection. Weed occurrence represents constant problem in agricultural production and their control is the basic condition for gaining of optimal yields and high quality products (Vasileiadis et al., 2007). One of preconditions for efficient weed control is prediction of their occurrence in the field. This can be achieved by soil analysis, especially of the arable layer up to the depth of 30 cm, on which the greatest and for the production the most important mass of weed seeds is found. Beside soil type, presence of weeds in the field is the second factor that has impact to spatial change of agricultural areas, and this makes determination of weed seed bank very important (Walter et al., 2002). Prediction of weed occurrence in the field enables more accurate herbicide choice, as well as their timely applications. This undoubtedly brings positive consequences to yield and crop quality. In studies of the weed seed bank in the soil, it must be borne in mind that it is only part of the complex and dynamic system that consists of land (Otto et al., 2007), plants, animals and microorganisms (Chee-Sanford et al., 2006). It is exposed to different influences and changes, therefore, results of these studies provide only temporary, but not final insight into condition on the terrain. Weed seed bank is usually linked with the top, arable soil layer, up to the depth of 30 cm, although seeds of some perennial weeds can be found at even deeper layers. Weed seeds are not evenly distributed in the field, but are usually concentrated in patches (Wiles and Schwiezer, 1999). This concentration of weeds is usually result of weak dispersal and spread of new seeds from mother plant, or due to the influence of humans to weeds that mature at the same time as crop, causing spreading during harvest and picking in direction of crop rows. For these reasons, field sampling should be performed from the whole plot, by width and depth. Germination capability of weed plants is certainly is of great importance, especially for survival of annual plants (Haj Seyed Hadi and Gonzalez-Andujar, 2009). Viability studies of weed seeds are of high importance for they enable prediction of their occurrence in the field. Because of these reasons, the study of the weed seed bank included determination of seed viability, i.e. germination capability in controlled conditions.

## MATERIAL AND METHOD

During 2011-2012 composition of the weed seed bank was studied in various depths of the arable soil layer in maize crops in localities of Žabalj (45°21'N, 20°12'E) and Zmajevó (45°28'N, 19°41'E). Both sites are in moderate climate and soil type is chernozem. At locality Žabalj, sowing of maize was performed in the third decade of April. Sowing was followed by herbicide treatment by *dimethenamid-P*, and in 4<sup>th</sup> and 5<sup>th</sup> leaf phase, the additional treatment was performed by herbicide tembotrione. In Zmajevó, maize was sown in the second decade of April, and the first treatment was in the phase 3-4 leaves of maize by herbicide dicamba, after which followed the second treatment in the phase 5-6 leaves of maize by herbicide combination rimsulfuron + dicamba. Taking of the soil samples was performed by the end of vegetation period, in four replications diagonally from each plot, especially from depths of 0-10 cm, 10-20 cm and 20-30 cm (Smutný and Křen, 2002). Samples of 1.5 kg soil were rinsed by water through copper sieves of 0.25 mm in diameter. After drying of the obtained

samples followed separation of weed seeds and their determination by microscope and determinators (Kronaveter and Boža, 1994). Determination and data processing by the method of Conn (1987) and Sharratt (1998) were followed by two weeks lasted seed germination of weed species in Petri dishes, their keeping in controlled conditions of climatic chamber convenient for seed germination. Subsequently, the evaluation of the germination capabilities and measurement of hypocotyls and epicotyls lengths of shootings were performed. The aim of the paper was study weed seed bank composition and germination capability in arable soil layer under maize crop.

## RESULTS AND DISCUSSION

In this locality of Žabalj, seeds of 12 following weed species were found: *Abutilon theophrasti* Med., *Amaranthus retroflexus* L., *Bilderdykia convolvulus* (L.) Dum., *Chenopodium album* L., *Convolvulus arvensis* L., *Panicum crus-galli* (L.) R. et Sch., *Hibiscus trionum* L., *Polygonum lapathifolium* L., *Polygonum aviculare* L., *Ranunculus repens* L., *Sinapis arvensis* L. and *Stachys annua* L. The highest number of seeds belonged to the species *Polygonum lapathifolium* and *Sinapis arvensis*, and they were present at all depths, but the most abundant were at depth of 20-30 cm. LSD test indicated statistically highly significant differences in numbers of seeds of these two species in relation to all other determined weed species, in all depths. The highest number of seeds for all determined weed species was found in the layer of 20-30 cm, somewhat lower number was determined in the layer of 10-20 cm; statistically significantly lower number was established in the layer of 0-10 cm. From soil samples taken from locality Zmajev, under maize crop, seeds of 15 weed species were segregated. Despite great variety of seeds, from soil samples taken from locality Zmajev, only seed of *Amaranthus retroflexus* occurred in higher quantity of 834-626 seeds per m<sup>2</sup>, at all studied layers. LSD test showed statistically very significant differences in number of seeds of this species in relation to all other determined weed species. *Amaranthus retroflexus* seed was evenly distributed in all examined layers, and the highest number of seeds of all other species occurred in the layer of 20-30 cm. Seed of *Amaranthus retroflexus* may remain persistent in the soil during several years period (Burnside et al. 1996). In average, for all species, the highest number of seeds was in the deepest layer, but statistically significant differences were not found in number of weed seeds concerning soil depth. After determination of segregated weed seeds they were germinated in controlled conditions of climatic chamber for two weeks. The obtained results are given in Figure 1.

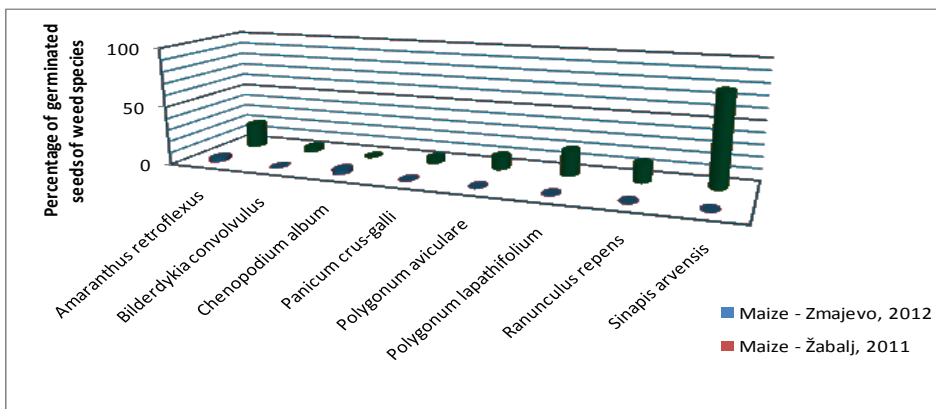


Figure 1. Weed seeds percentage of germination in all studied localities

Seed from soil samples taken in locality Zmajev, showed very low germination percentage. Only seeds of *Amaranthus retroflexus* and *Chenopodium album* germinated, mostly those from the deepest arable soil depth (Figure 1.). Hypocotyls and epicotyls lengths of germinated seeds were measured. Results are given in Figures 2. and 3.

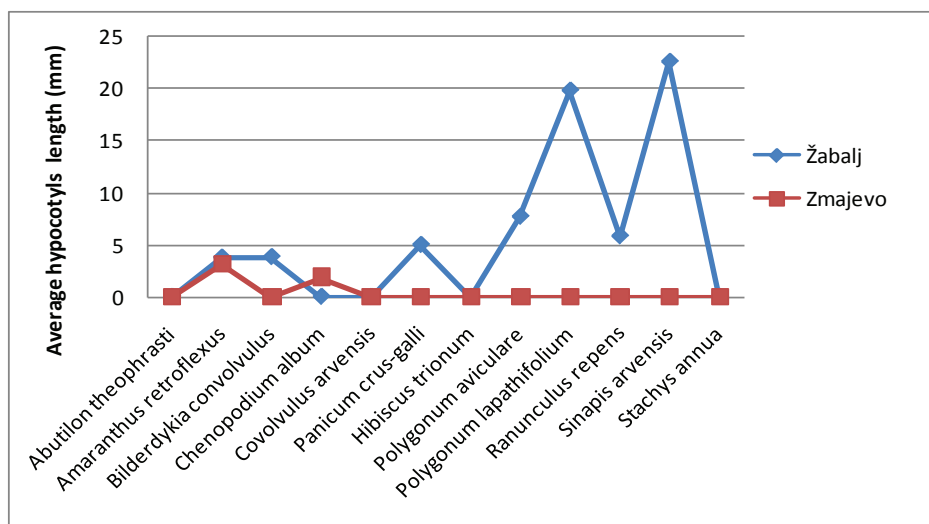


Figure 2 - The average hypocotyls length of weed species seeds in maize crop at localities Žabalj and Zmajev

The highest average length of seedlings hypocotyls had seeds of weed species *Sinapis arvensis* L. (22.63 mm) and *Polygonum lapathifolium* L. (19.86 mm). In locality Zmajev, the highest average hypocotyls length had seedlings of weed species *Amaranthus retroflexus* L. (3.13 mm).

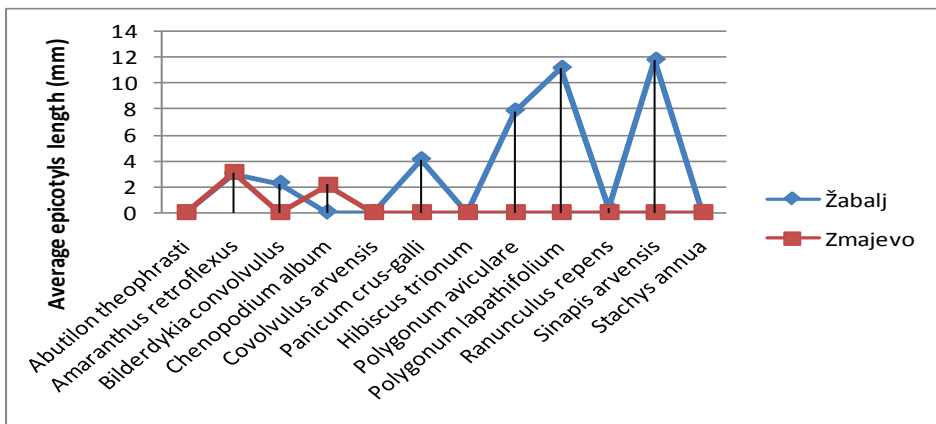


Figure 3. The average epicotyls length in maize crops in localities Žabalj and Zmajjevo

Obtained results of epicotyls length measurements of seeds from locality Žabalj (Figure 3.) indicate that the highest average length had seedlings of weed species *Polygonum lapathifolium* L. (11.17 mm) and *Sinapis arvensis* L. (11.79 mm). The average measured epicotyls length of germinated seeds at locality Zmajjevo for *Amaranthus retroflexus* L. was (3.13 mm) and for *Chenopodium album* L. (2.17 mm). During 2011-2012 in localities Žabalj and Zmajjevo, composition and germination capability of weed seed bank in various depth of the arable layer under maize crop were studied. In locality Žabalj, seeds of 12 species were segregated, and the majority belonged to the species *Polygonum lapathifolium* and *Sinapis arvensis*. Great number of seeds of these species was found in all depths, and mostly was buried in depth of 20-30 cm. LSD test showed statistically very important differences in number of seeds of these two species in regard to all other determined weed species, at all depths. Greater quantities of seeds of weed species *Polygonum lapathifolium* under maize crop represent problem in neighboring countries such as Romania (Bogdan et al., 2007). The highest number of seeds, of all determined weed species was found in the soil layer of 20-30 cm (136 seeds/m<sup>2</sup>). Somewhat lower number was found in the layer of 10-20 cm, and statistically significantly lower in the layer of 0-10 cm. Such disposal of weed seeds is characteristic for soils that are regularly ploughed, causing deeper burial of weed seeds. Significant lower number of seeds in shallow layer of 0-10 cm indicates that weed species were controlled by herbicides and surface cultivation. The obtained results were in accordance with results of Swanton (2001), Menalled (2008) and Konstantinović et al. (2008). In locality Zmajjevo, seeds of 15 weed species were segregated, of which only seed of the species *Amaranthus retroflexus* occurred in higher quantity in all studied layers. LSD test indicated statistically highly significant differences in number of seeds of this species, in relation to remaining weed species. Seed of the species *Amaranthus retroflexus* was buried in all soil depths, from 0-30 cm, while seeds of remaining species were predominantly detected in the layer of 10-30 cm. In average, the greatest quantity of weeds of all weed species was found in the deepest layer, but statistically significant differences were not recorded in the number of weed seeds in soil depth as a whole. Similar disposal of weed seeds in the soil that was regularly cultivated deeper by plough is in accordance with results of the studies of other authors (Boguzas et al., 2004; Ashrafi, 2006; Menalled, 2008). Dominance of

*Amaranthus retroflexus* seeds under maize crop was also established in studies of Wiles and Schweizer (2002). Germination tests revealed that seeds taken from soil samples in locality Žabalj, seed of weed species *Sinapis arvensis* had the highest germination rate of 70-80% regardless to the soil depth in which it was found. The highest germination capability had also seeds of *Amaranthus retroflexus*, especially those found at soil depth of 20-30 cm (70%). Significant germination rate showed also seed of weed species *Polygonum aviculare* in the top soil layer (up to 50%), whose fresh seed otherwise also shows higher germination capability. This refers also to the species *Polygonum lapathifolium* whose seed, from all studied soil levels shooted, although in somewhat lower percentage of 15-37%. The greatest quantity of germinated seeds belonged to the weed species *Sinapis arvensis* and *Polygonum aviculare*. Seeds of *Amaranthus retroflexus* had also significantly higher germination percentage. The highest average length of seedlings hypocotyls and epicotyls had seeds of weed species *Sinapis arvensis* L. and *Polygonum lapathifolium* L. from locality Žabalj. In locality Zmajevo, the highest average hypocotyls and epicotyls length had seedlings of weed species *Amaranthus retroflexus* L.

## CONCLUSION

Results of germinated seeds from locality Žabalj indicated that the highest germination percentage of 70-80% had seeds of weed species *Sinapis arvensis* regardless on the soil depth in which it was found, which is explained by fact that seed of this weed species may remain dormant for longer time period deep in the soil, without losing germination capability. Higher germination capability had also seeds of *Amaranthus retroflexus* that was found buried in depth of 20-30 cm. Significant percentage of germination of up to 50% had also seeds of weed species *Polygonum aviculare* in top soil, and *Polygonum lapathifolium* seeds that germinated from all studied soil layers in 15-37%. The highest quantity of germinated weed belonged to weed species *Sinapis arvensis* and *Polygonum aviculare*, that belong to the species of short vegetative cycles, and germinate almost through all vegetation period, which explains the obtained results. Significantly higher germination percentage had also seeds of *Amaranthus retroflexus*. This can be explained by the fact that it is widely distributed weed that grows in differing environmental conditions, due to humble life needs. Seed from soil samples taken from locality Zmajevo had very low germination capability. Shooted only seeds of *Amaranthus retroflexus* and *Chenopodium album*, predominantly those from the deepest arable layer of 20-30 cm. This is explained by the fact that seed was germinated shortly after sampling, and it is possible that majority of seeds had not passed the phase of dormancy. Measurement of the average epicotyls and hypocotyls lengths of segregated seeds it was established that seeds with the highest germination energy also had the highest average epicotyls and hypocotyls lengths, and these were seeds of weed species *Sinapis arvensis* and *Polygonum lapathifolium*.

## REFERENCES

ASHRAFI, Z.: Effect Tillage and Soil Type on Weed Seed Bank. International Union of Soil Science. 18 th World Congress of Soil Science. Philadelphia. Pennsylvania. USA., 2006.

BOGDANI, I., GUS, P., RUSU, T., POP, A., VAJU, A., MORSR, P.: Research concerning weed control in maize crop. *Cercetări Agronomice în Moldova, Anul XXXX* 1:129, 2007.

BOGUZAS, V., MARCINKEVICIENE, A., KAIRVATE, A.: Quantitative and qualitative evaluation of weed seed bank in organic farming. *Agronomy Research*, 2:13-22, 2004.

BURNSIDE, O.C., WILSON, R.G., WEISBERG, S., HUBBAR, K.G.: Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. *Weed Sci.*, 44:74–86, 1996.

CHEE-SANFORD, J.C., WILLIAMS, II M.M., DAVIS, A.S., SIMS, K.G.: Do Microorganisms Influence Seed-Bank Dynamics? *Weed Science*, 54:575-587, 2006.

CONN, J.S.: Effects of tillage and straw management on Alaskan weed vegetation: a study on newly cleared land, *Soil Tillage Res.*, 9:275–285, 1987.

HAJ SEYED HADI, M.R., GONZALEZ-ANDUJAR, J.L.: Comparison of fitting weed seedling emergence models with nonlinear regression and genetic algorithm *Computers and Electronics in Agriculture*, 65:19-25, 2009.

KONSTANTINOVIĆ, B., MESELDŽIJA, M., KONSTANTINOVIĆ, Bo., MANDIĆ, N.: Ispitivanje banke semena korova pod usevom soje. *ACTA Herbologica*, 17:171-174, 2008.

KRONAVETER, Đ., BOŽA, P.: Poznavanje semena najčešćih korova u semenarstvu, Univerzitet u Novom Sadu, Institut za ratarstvo i povrtarstvo, Novi Sad, 1994.

MENALLED, F.: Weed seed bank dynamics - Integrated Management of Agricultural Weeds. *Agriculture and Natural Resources (Weeds)*. Montana State University, 2008.

OTTO, S., ZUIN, M.C., CHISTE, G., ZANIN, G.: A modeling approach using seed bank and soil properties to predict the relative weed density in organic fields of an Italian pre-alpine valley. *Weed Research*, 47:311-326, 2007.

SHARRATT, B.S.: Barley yield and evapotranspiration governed by tillage practices in interior Alaska, *Soil Tillage Res.*, 46:225–229, 1998.

SMUTNY, V., KREN, J.: Improvement of an elutriation method for estimation of weed seed bank in the soil. *Rostlinna výroba*, 48:271-278, 2002.

SWANTON, C. J., SHRESTHA, A.: Tillage, soil type and weed seed bank dynamics. *Ontario Corn Producer*, March, 28, 2001.

VASILEIADIS, V.P., FROUD-WILLIAMS, R.J., ELEFTHEROHORINOS, I.G.: Vertical distribution, size and composition of the weed seed bank under various tillage and herbicide treatments in a sequence of industrial crops. *Weed Research*, 47:222-230, 2007.

WALTER, A.M., CHRISTENSEN, S., SIMMELSGAARD, S. E.: Spatial correlation between weed species densities and soil properties. *Weed Research*, 42:26–38, 2002.

WILES, L., SCHWEIZER, E.: Spatial dependence of weed seed banks and strategies for sampling. *Weed Science* 50: 595–606, 2002.

WILES, L.J.; SCHWEIZER, E.E. The cost of counting and identifying weed seeds and seedlings. *Weed Science*, 47:667-673, 1999.



## SASTAV I SPOSOBNOST KLIJANJA BANKE SEMENA KOROVAU ZEMLJIŠTU U USEVU KUKURUZA

KONSTANTINOVIĆ BRANKO, BLAGOJEVIĆ MILAN,  
KONSTANTINOVIĆ BOJAN, SAMARDŽIĆ NATAŠA

### Izvod

Utvrđivanje kvantitativnih i kvalitativnih osobina semena korovskih vrsta u obradivom sloju zemljišta je jedan od najefikasnijih metoda za predviđanje pojave korova na obradivim površinama. On osigurava bolji izbor herbicida, kao i njihovu pravovremenu primenu. U 2011 i 2012 na dva ispitivana lokaliteta sa sličnom obradom zemljišta u usevu kukuruza, ispitana je banka semena korova u obradivom sloju zemljišta do 30 cm dubine. Na prvom lokalitetu, determinisano je seme 12 korovskih vrsta. Od ovih vrsta, najbrojnija su bila semena *Polygonum lapathifolium* i *Sinapis arvensis* na svim ispitivanim dubinama, a najveća količina semena je pronađen na dubini od 20-30 cm. Najveći broj preostalih determinisanih korovskih vrsta je također pronađena na ovoj dubini. Na ostalim lokalitetima broj semena 15 korovskih vrsta je bila različita, ali samo seme *Amaranthus retroflexus* je zastupljena u većoj količini u svim ispitivanim slojevima. U proseku, za sve vrste najveći broj semena je pronađen u najdubljem sloju, statistički značajne razlike nisu zabilježena u broju semena po dubinama zemljišta. Nakon utvrđivanja izdvojenih korovskih semena praćena je njihova klijavost u klima komori. Procentualno najveća klijavost (70-80%) je zabeležena kod korovske vrste *Sinapis arvensis* na lokalitetu Žabalj. Na lokalitetu Zmajevu najveća klijavost je utvrđena za korovske vrste *Amaranthus retroflexus* i *Chenopodium album*.

**Ključne reči:** zemljišna banka semena korova, kukuruz, klijavost, hipokotil, epikotil.

Received / *Primljen*: 30.04.2014.

Accepted / *Prihvaćen*: 02.06.2014.

## PRODUCTIVE LIFE OF HIGH YIELDING DAIRY COWS

ŽELJKO NOVAKOVIĆ, SNEŽANA TRIVUNOVIĆ,  
RADE JOVANOVIĆ, RADMILA BESKOROVAJNI,  
DUŠICA OSTOJIĆ-ANDRIĆ, NIKOLA POPOVIĆ<sup>1</sup>

*SUMMARY: The objective of the research was to investigate the significance of the main systematic effects on productive life of high yielding Black-and-White dairy cows. The average length of productive life reached  $1494.18 \pm 463.66$  days. Observed by genotypes, the mean values were as follows: 1377.35 days (>73% HF), 1477.47 days (58-73% HF) and 1627.71 days (<58% HF). Differences in length of productive life came as a result of highly significant ( $P \leq 0.01$ ) effect of the class of HF genes, bull sires and year of culling, whereas the reason for culling had no significant effect ( $P > 0.05$ ).*

**Key words:** productive life, high yielding dairy cows, systematic effects

### INTRODUCTION

Length of productive life is an extremely complex trait. It is considered one of the best indicators for longevity of cows. Much research on average age of culling implies a relatively short productive life of high yielding dairy cows. Decision on culling is affected by numerous factors that constantly change over time (Ducrock, 2005).

More important indicators for evaluating cow longevity are length of productive life, age at culling, number of lactations and a lifespan. The lifespan of cows is precisely determined by two periods. The period from birth to the first calving is the period of growth and development. The productive period is a period from the first calving to culling and it shows potential of an animal to survive in production conditions as long as possible (Sewalem et al., 2008).

The period from the first calving to culling from a herd should be a period for im-

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup> Željko Novaković, PhD, Research Associate, Rade Jovanović, Ph., Senior Research associate, Radmila Beskorovajni, MSc, Research Assistant, Nikola Popović, MSc (Eng), Associate, Institute for Science Application in Agriculture, Belgrade, Serbia. Snežana Trivunović, PhD, Associate Professor, Faculty of Agriculture, University of Novi Sad, Serbia. Dušica Ostojić Andrić, M.Sc., Research Assistant, Institute for Animal Husbandry, Belgrade, Serbia.

Corresponding author: Željko Novaković, e-mail: zeljko.novakovic013@gmail.com, phone: +381112751622.

proving total lifetime production. Enhancing the length of productive life per cow can lead to a decreased need for replacement heifers, which results in a greater proportion of cows in lactations producing on a mature level, better use of the genetic basis and fewer health and reproduction expenses (Hare et al., 2006). The goal of functional breeding (breeding of functional traits: udder structure, somatic cell score, normal movement and prolificacy) is to decrease involuntary culling and increase culling for breeding reasons (Hadley et al., 2006; Chirinos et al., 2007; Lopez de Maturana et al., 2007).

Productive life is number one indicator for longevity. As ability to survive in certain production conditions, it is the most important functional trait of high yielding dairy cows. From economic aspect, the length of productive life is a necessary condition for milk production sustainability (Berry et al., 2005). Longevity traits have low heritability, ranging from 0.03 to 0.20, depending on evaluation methods (Boettcher et al., 1999; Tsuruta et al., 2005; Páchová et al., 2005; Terawaki and Ducrocq, 2009). Good health and good prolificacy affect prolonging of productive life of cows by decreasing the culling rate, which positively impacts breeding intensity and a level of economic results (Caraviello et al., 2004; de Vries et al., 2010; Bogdanović et al., 2012).

Knowing the significance and power of systematic effects on productive life of Black-and-White cows is important in terms of including them in mathematical-statistical models. According to individual significance of systematic effects, their objective assessment was carried out in order to evaluate the obtained results as precise and correct as possible. For estimating their role in achieved results, it is necessary to determine those systematic factors that lead to considerable phenotypic variation of investigated traits.

The objective of this research is to use appropriate methods to investigate the significance of the main systematic factors on length of productive life of high yielding Black-and-White dairy cows with different proportion of genes of the Holstein breed.

## **MATERIALS AND METHODS**

Investigation and analysis of the key systematic factors on length of productive life were conducted in a herd of high yielding Black-and-White dairy cows. The cows included in the research were European type Black-and-White cattle. All the animals were in the final stage of intensive improvement using the Holstein breed. The high yielding cows included in the sample were under the same housing, nutritive and care conditions and the same method of exploitation.

The analysis of effects of particular systematic factors was done by the method of least squares (Harvey, 1987) The advantage of this method is that enables parallel and simultaneous determination of multiple effects on investigated traits. In an overview of the investigated factors shown below, animal distribution is displayed according to the previously defined classes:

Table 1. Distribution of animals according to the defined classes

Class of HF genes	<58%	58-73%	>73%
n	83	125	123
Year of culling	1	2	3
n	88	140	103
Reason for culling	1	3	4
n	278	47	6

The following statistical model was used:

$$Y_{ijklm} = \mu + O_i + HF_j + G_k + R_l + e_{ijklm}$$

Where:

$Y_{ijklm}$  - result of m-cow, daughter of i-sire, belonging to j-group according to the share of HF genes, culled in k-year for l-reason

$\mu$  - mean

$O_i$  - effect of i-sire

$HF_j$  - effect of j-group of HF genes

$G_k$  - effect of k-year of culling

$R_l$  - effect of l-reason for culling

$e_{ijklm}$  - random error

Within the applied model the following codes were used:

For reason of culling: 1-economical culling, 3-involuntary slaughtering and 4-death.

## RESULTS AND DISCUSSION

The analysis of the mean value (lsm), the mean value error (Slsm) of least squares and the significance of the investigated traits on productive life of high yielding Black-and-White cows at birth are shown in Table 1.

Table 2. Mean values (lsm) and mean value errors (Slsm) of least squares for the investigated traits on productive life of cows (in days)

Effects	N	lsm	Slsm
Total			
$\mu$	331	1494.18	463.66
<i>Class of HF genes (<math>df_1=2, df_2=307, f_{-exp}=8.112^{**}</math>)</i>			
<58%	83	1627.71	465.09
58-73%	125	1477.47	464.50
>73%	123	1377.35	464.79
<i>Sires (<math>df_1=17, df_2=307, f_{-exp}=54.962^{**}</math>)</i>			
23	42	2068.41	67.67
28	20	1561.39	91.44

33	3	2506.01	204.60
35	29	926.45	82.32
36	45	1134.36	70.71
38	11	515.28	116.98
270	22	2324.17	87.62
283	5	2764.67	161.20
293	8	136.73	132.07
337	7	327.09	137.87
762	21	2232.88	89.69
795	6	2949.36	149.08
816	33	1884.92	78.06
879	7	638.95	136.93
927	31	1220.64	74.59
1040	19	617.63	91.79
1304	15	1545.38	101.52
5368	7	1859.38	137.48
<i>Year of culling (df<sub>1</sub>=2, df<sub>2</sub>=307, f<sub>-exp</sub>=49.747**)</i>			
1	88	1218.08	464.25
2	140	1508.68	464.64
3	103	1755.76	464.82
<i>Reason for culling (df<sub>1</sub>=2, df<sub>2</sub>=307, f<sub>-exp</sub>=2.603ns)</i>			
1	278	1581.63	461.65
3	47	1623.12	463.81
4	6	1277.78	481.99

NS (P>0.05), \*P≤0.05, \*\*P≤0.01.

The average length of productive life reached 1494.18±463.66 days (49.08±15.24 months or 4.09±1.27 years).

Observed by genotypes, the mean values varied from 1377.35 days (>73% HF), 1477.47 days (58-73% HF) to 1627.71 days (<58% HF). Differences determined in length of productive life came as a result of highly significant (P≤0.01) effect of the class of HF genes, bull sires and year of culling, whereas the reason for culling had no significant effect (P>0.05).

The research has shown that high yielding Black-and-White dairy cows of different genotypes have a relatively short lifespan. The results of other researchers support this. In milking cows, days of productive life was analyzed as an alternative to the current trait lifespan score. Cows that died in 2009 on average lived for 6.8 years with an average production of 4.3 years. (Pritchard et al., 2013)

While investigating the productive life of the Brown Swiss breed, Vukašinović et al. (1997) determined a productive life of 29.5 months (based on full data), and 38.5 months (incomplete data). Moreover, it was determined that the average length of productive life of all heads was 32.4 (± 25.5) months.

De Vries (2003) in his results stated that about 20% of total number of cows culled in 365 days from the day of the first calving, 50% was culled after 827 days (mid-productive life), whereas 10% of cows were retained in herd after 1580 days.

Van Raden et al. (2006) stated the following values of productive life of the cows born in 1997 and standard deviation (in months): Ayrshire  $25.9 \pm 12.5$ , Brown Swiss  $25.3 \pm 12.7$ , Guernsey  $23.0 \pm 12.9$ , Holstein  $24.6 \pm 12.7$ , Jersey  $29.3 \pm 13.3$ , and Milking Shorthorn  $25.5 \pm 13.0$ .

In research that included 13.8 million dairy cows, which calved for the first time in the interval of 15 years, the sample consisted of cows of different dairy breeds (Ayrshire 0.5 %, Brown Swiss 0.9 %, Guernsey 1.3 %, Holstein 92.6 % and Jersey 4.7 %). The average rate of survival was 73 % to the second lactation, 50 % to the third lactation, 32 % to the fourth, 19 % to the fifth, 10 % to the sixth, 5 % to the seventh, whilst 2 % of cows reached the eighth lactation (Hare et al., 2006).

In some countries with developed cattle breeding, analysis of survival has become an official way to estimate longevity of cows. This method enables one to include all the cows in research, regardless they are present in herd or culled. The main limiting factors in direct longevity are time for collecting data necessary for accurate estimation of breeding value of studs and low heritability. With improving genetic potential of dairy cows, the significance of body traits related to length of productive life has also increased.

## CONCLUSION

The average productive life reached  $1494.18 \pm 463.66$  days ( $49.08 \pm 15.24$  months or  $4.09 \pm 1.27$  years). Observed by genotypes, the mean values were 1377.35 days (>73% HF), 1477.47 days (58-73% HF) and 1627.71 days (<58% HF). Differences in length of productive life came as a result of highly significant ( $P \leq 0.01$ ) effect of the class of HF genes, sires and year of culling, whereas the reason for culling had no significant effect ( $P > 0.05$ ). The research has shown that different genotype of high-yielding Black-and-White cows had a short productive life. The expressed length of productive life was not satisfactory, which contributed to a low level of lifetime milk production and an increase in culling, having direct consequences on economic and breeding effects. One should pay more attention to having continuous insight into length of productive life of cows in complex technological conditions present on cattle farms. Body traits that are evaluated in first-calving cows could make the breeding process more efficient, since they can have higher heritability than other longevity traits, and in respect to that they could be a starting point for indirect breeding for longevity traits.

## REFERENCES

- BERRY, D. P., HARRIS, B. L., WINKELMAN, A. M., MONTGOMERIE, W.: Phenotypic associations between traits other than production and longevity in New Zealand dairy cattle. *Journal of Dairy Science*, 88:2962–2974, 2005.
- BOETTCHER, P. J., JAIRATH, L. K., DEKKERS, J.C.M.: Comparison of methods for genetic evaluation of sires for survival of their daughters in the first three lactations. *Journal of Dairy Science*, 825:1034–1044, 1999.

- BOGDANOVIĆ, V., ĐEDOVIĆ, R., PERIŠIĆ, P., STANOJEVIĆ, D., BESKOROVANI, R., PETROVIĆ, M.D.: Karakteristike dugovečnosti kao funkcionalne osobine mlečnih krava. Radovi sa XXVI savetovanja agronoma, veterinara, tehnologa i agroekonomista Vol. 18. br. 3-4, str.23-33, Beograd, 2012.
- CARAVIELLO, D. Z., WEIGEL, K. A., GIANOLA, D.: Prediction of longevity breeding values for us holstein sires using survival analysis methodology. *Journal of Dairy Science*, 8710:3518–3525, 2004.
- CHIRINOS, Z., CARABAÑO, M. J., HERNÁNDEZ, D.: Genetic evaluation of length of productive life in the Spanish Holstein-Friesian population. Model validation and genetic parameters estimation. *Livestock Science*, 106, 120–131, 2007.
- DE VRIES, A., OLSON, J.D., PINEDO, P.J.: Reproductive risk factors for culling and productive life in large dairy herds in the eastern United States between 2001 and 2006. *Journal of Dairy Science*, 93: 613-623, 2010.
- DE VRIES, A.: Productive life of dairy cows in Florida. Department of Animal Sciences University of Florida, Gainesville, 2003.
- DUCROCQ, V.: An improved model for the French genetic evaluation of dairy bulls on length of productive life of their daughters. *Animal Science*, 80: 249-256, 2005.
- HADLEY, G. L., WOLF, C. A., HARSH, S. B.: Dairy cattle culling patterns, explanations, and implications. *Journal of Dairy Science*, 89: 2286-2296, 2006.
- HARE, E., NORMAN, H. D., WRIGHT, J. R.: Survival rates and productive herd life of dairy cattle in the United States. *Journal of Dairy Science*, 89:3713–3720, 2006.
- HARVEY, W.R. :Mixed model least squares and maximum likelihood computer program: User's guide for LSMLMW and MIXMDL, 1987.
- LOPEZ DE MATURANA, E., UGARTE, E., GONZALEZ-RECIO, O.: Impact of Calving Ease on Functional Longevity and Herd Amortization Costs in Basque Holsteins Using Survival Analysis. *Journal of Dairy Science*, 90:4451–4457, 2007.
- PÁCHOVÁ, E., ZAVADILOVÁ, L., SÖLKNER, J.: Genetic evaluation of the length of productive life in Holstein cattle in the Czech Republic. *Czech J. Anim. Sci.*, 50, 493–498, 2005.
- PRITCHARD, T., COFFEY, M., MRODE, R., WALL, E.: Understanding the genetics of survival in dairy cows. *Journal of Dairy Science*, 96: 3296-3309, 2013.
- SEWALEM, A., MIGLIOR, F., KISTEMAKER, G. J., SULLIVAN, P., VAN DOORMAAL, B. J.: Relationship Between Reproduction Traits and Functional Longevity in Canadian Dairy Cattle. *Journal of Dairy Science*, 91:1660–1668, 2008.
- TERAWAKI, Y., DUCROCQ, V.: Nongenetic effects and genetic parameters for length of productive life of Holstein cows in Hokkaido, Japan. *Journal of Dairy Science*, 92: 2144-2150, 2009.
- TSURUTA, S., MISZTAL, I., LAWLOR, T. J.: Changing Definition of Productive Life in US Holsteins: Effect on Genetic Correlations. *Journal of Dairy Science*, 88: 1156–1165, 2005.
- VAN RADEN, P. M., DEMATAWEWA, C. M. B., PEARSON, R. E., TOOKER, M. E.: Productive life including all lactations and longer lactations with diminishing credits. *Journal of Dairy Science*, 89: 3213-3220, 2006.
- VUKAŠINOVIĆ, N., MOLL, J., KUNZI, N.: Analysis of productive life in Swiss Brown cattle. *Journal of Dairy Science*, 8010:2572–2579, 1997.

## PRODUKTIVNI ŽIVOT VISOKOMLEČNIH KRAVA

ŽELJKO NOVAKOVIĆ, SNEŽANA TRIVUNOVIĆ,  
RADE JOVANOVIĆ, RADMILA BESKOROVAJNI,  
DUŠICA OSTOJIĆ-ANDRIĆ, NIKOLA POPOVIĆ

### Izvod

Cilj istraživanja bio je ispitivanje značajnosti najvažnijih sistematskih uticaja na produktivni život visokomlečnih crno-belih krava. Krave u okviru ispitivanog uzorka imale su različitu proporciju gena holštajn rase. Analiza uticaja sistematskih faktora na produktivni život obavljeno je u stadu visokomlečnih krava, koje su po poreklu pripadale evropskom tipu crno-belih goveda, u fazi intenzivnog oplemenjivanja holštajn rasom. Izvršena je determinacija onih sistematskih uticaja koji su u okviru istraživanja doveli do značajnijeg fenotipskog variranja ispitivane osobine. Prosečno trajanje produktivnog veka iznosilo je  $1494.18 \pm 463.66$  dana ( $49.08 \pm 15.24$  meseci ili  $4.09 \pm 1.27$  godina). Posmatrano po genotipovima krava srednje vrednosti iznosile su  $1377.35$  dana ( $> 73\%$  HF),  $1477.47$  dana ( $58-73\%$  HF) i  $1627.71$  dana ( $< 58\%$  HF). Utvrđene razlike između grla u pogledu trajanja produktivnog života nastale su kao posledica visoko značajnog uticaja ( $P \leq 0.01$ ) klase HF gena krava, bikova-očeva krava i godine izlučenja krava, dok razlog izlučenja nije imao značajan uticaj ( $P > 0.05$ ).

**Ključne reči:** produktivni život, visokomlečne krave, sistematski uticaji.

Received/ *Primljen*: 13.05.2014.

Accepted / *Prihvaćen*: 29.05.2014.



## WINE MARKET IN THE EUROPEAN UNION\*

BRANISLAV VLAHOVIĆ, ANTON PUŠKARIĆ, MARINA NACKA<sup>1</sup>

*SUMMARY: In the paper were analyzed the wine market in the European Union. The basic data source is Global Trade Atlas, in the time interval from 2010 to 2012. The wine production in the European Union is around 164 thousand hectolitres. In export structure dominate the bottled wines (67.9%), and then follow the wine in bulk (22.7%), the lowest share is of the sparkling wine with 9.4%. The largest wine export has been directed to USA market (23.3%), and then follows the Russian Federation (16.3%) and China (9.9%). The biggest wine exporter in the European Union is Italy, then Spain and France. The European Union represents compellingly the biggest world wine importer, while the average import amounted 13.607.000 hectolitres. In import structure dominate the wine in bulk (57.2%), then the bottled wines (41.7%), while the lowest share has the sparkling wines, with share of totally one percentage. The largest import is from Australia (25.3%), then Chile (21.6%) and South-African Republic (20.3%). The biggest importer is Germany, and then follows Great Britain and France. The European Union has a positive balance of wine foreign trade exchange, approximately over six thousand hectolitres. In the following period can be expected a trend of export growth from the Union, but with significantly more moderate tempo, owing to a notable saturation of the international market.*

**Key words:** wine, European Union, export, import.

---

Review paper / Pregledni rad

<sup>1</sup> Branislav Vlahović, PhD, Full Professor, University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia. Anton Puškarić, PhD, Research Assistant, Institute of Agricultural Economics, Volgina 15, Belgrade, Serbia.

Marina Nacka, MSc, University "Ss. Cyril and Methodius", Skopje, Faculty of Agricultural Sciences and Food, Institute of Agricultural Economics, Blvd . Aleksandar Makedonski nn, Skopje, Republic of Macedonia.

Corresponding author: Branislav Vlahović, E-mail: vlahovic@polj.uns.ac.rs; Phone: +381 21 4853500.

\* The paper represents a part of the research at the project III - 46006 – Sustainable agriculture and rural development in terms of the Republic of Serbia strategic goals' realization within the Danube region, funded by the Ministry of Education and Science.

## INTRODUCTION

Viniculture represents an important branch within the European Union. The wine production in the European Union amounted 164 thousand hectolitres (2010-2012), with tendency of slight increase. The basic aim of the research is to perceive the basic characteristics, as well as the changes on the wine market in the European Union. The European Union was researched, while it represents the greatest world producer, exporter and importer of wine.

## MATERIAL AND METHODS

The basic data sources were downloaded from the statistical data base of the Global Trade Atlas (GTA), for the time period from 2010 to 2012, along with apply of the standard statistical-mathematical methods. There were also used the results of the previous researches of the mentioned problems.

## RESULTS AND DISCUSSION

Wine export from the European Union - The average wine export from the EU-countries was 21.666.000 hectolitres (2010-2012). Valuably expressed, export was, approximately, 10.5 milliard US dollars. The average export price is 2 USD per a litre. It varies depending on a vintage, a quality category (premium wines, wines with protected geographic origin, table wines etc.). The European Union is the biggest world exporter of wine, with two thirds shares. In export structure dominate the bottled wines (67.9%), and then in bulk (22.7%), while the lowest share is of the sparkling wines (9.4%). The highest export is directed toward the European Union countries (72%). Externally, i.e. outside the European Union, exports 28% wines. The highest export was directed to USA market (23.3%), then follows the Russian Federation (16.3%) and China (9.9%), the above mentioned countries have participated a half of the totally exported wine from the European Union (Table 1). Not all of the EU countries have the same significance on the international wine market.

Table 1. Wine export from the European Union (2010-2012), 000 hl

Country	Average quantity		Average value 000 \$
	000 hl	%	
USA	5.060	23,3	2.965
Russian Federation	3.535	16,3	597
China	2.142	9,9	819
Canada	1.792	8,3	927
Switzerland	1.696	7,8	1.047
Japan	1.381	6,4	2.558
Other countries	6.060	28,0	1.579
Total	21.666	100,00	10.492

*Source: Global Trade Atlas ( www.gtis.com/gta/)*

The biggest wine exporters within the European Union are *Italy*, which average export was 2.223.000 hectolitres. It represents also the biggest wine exporter in the

world (Vlahović et al., 2013). It exports almost 40% of the total domestic wine production. The export has significant support of the government and appropriate institutions, and thanks to a good quality and adequate marketing activities, it permanently expands on new markets ([www.bizlife.rs/m/vesti/36111-italijanski-izvoz-vina-na-rekordnom-nivou](http://www.bizlife.rs/m/vesti/36111-italijanski-izvoz-vina-na-rekordnom-nivou)). In the observed period, wine export from Italy was approximately 5.79 milliard US dollars annually, while export price was 2.6 USD per a litre. It is a result of production structure, where dominate quality wines. Two categories make table wines and two categories – quality wines. *Indicazione Geografica Tipica (IGT)* are wines with a precise mark of origin. It refers to the wines, which do not follow strict, classic wine laws, but have a top quality. *Denominazione di Origine Controllata (DOC) and Denominazione di Origine Controllata e Garantita (DOCG)* refer to the fields, more concrete defined. These wines have to pass a rigorous quality testing, in order to satisfy strict market requirements. The most famous export commercial red wines are: „Chianti Classico“, „Barolo“, „Brunello di Montalciano“. Of white wines dominate “Fiano di Avellino” and “Greco di Tufo”, which origin from the region Alto Adige, i.e. Campanie. From the wine region Toscana export mainly white wines, as “Gavi”, “Arneis” i “Vernaccio di San Gimignano”. The most important export realizes in Germany (30.4%), then Great Britain (13.4%), USA (12.7%), France (4.7%) and Hungary (3.1%). They absorb two thirds (64%) of the total wine export from Italy (2010-2012). It is evident that dominate the European Union countries.

In the second place is *Spain* with the average export of 2.150.000 hectolitres. The export value amounts 2.96 milliard US dollars annually. Spain, by the wine production size, lags behind Italy and France, but to significant export contributes lower domestic consumption in regard to the mentioned countries. In the last researched year (2012), it came out to the first place among the biggest wine exporters in the world. The wines are in three quality groups: *Crianza* – red wines which had aged for minimum two years, of which six months in oak barrels, white and rose, aged at least one year, of which half in barrels. *Reserva* - aged for three years in barrels and one year in oak barrels for red wines and two years in barrels for white and rose, and six months in oak barrels. *Gran Reserva* – at least five years aged and 1.5 years in oak barrels for red wine, four years in a barrel and six months in oak barrels for white and rose wine ([www.vino.rs/svet-vina/regioni/spania.html](http://www.vino.rs/svet-vina/regioni/spania.html)). Two third of export is directed to the EU countries. The most significant export realizes in France (18.7%), Germany (17.3%), Italy (8.8%), Great Britain (7.5%) and Portugal (6.9%). Those mentioned countries had absorbed more than half of the total wine export (59%). In households in Spain, consuming the quality wines decreases, owing to high unemployment and indebtedness. That is the reason why vintners turn to export, mainly to France, and from recently to Germany, too (<http://trend.seebiz.eu/gastro/njemacka-postaje-najbolje-trziste-za-spanjolsko-vino/ar53046/>).

In the third place is *France*, which average export amounts 1.368.000 hectolitres. It is also the biggest wine producer in the world (FAO, 2013). The export value is 9.48 milliard US dollars annually, thanks to a high export price of 6.9 USD per a litre. It is, at the same time, the highest price in regard to other exporters. In export structure dominate *Appellation d'Origine Controlee* – wines of controlled geographic origin, which have the highest quality level and *Vin Delimite de Qualite Superieure* quality wines with protected geographic origin. The most significant export realizes in Germany (18.3%), and then follows Great Britain (14.9%), Belgium (11.1%), Holland (8.6%) and China (7.6%). They absorb more than a half (60%) of the total wine export from France.

The biggest part of export is directed to the European Union countries.

Then comes *Germany*, which has an average export of 399 thousand hectolitres. It is in the seventh place among leading world wine exporters. The export value is 1.25 milliard US dollars annually. The average export price is 3.13 US dollars per a litre. Progress in export is indubitably connected to the German wine industry efforts to improve the quality and to focus its marketing strategy to the leading German sort – Riesling (Wines of Germany, 2012). Of the total production exports 41%, which indicates a high export-orientation of wine sector. In export structure, dominant places have the white wines (88%), while significantly less is a share of the red wines. The most significant export realizes in Holland (19.3%), and then follows Great Britain (15.2%), USA (7.8%), Sweden (6.5%), and Poland (4.6%). They absorb more than half (60%) of the total wine export from Germany.

Although the global wine market has been significantly saturated, in the next period can be expected a tendency of a slight export increase from the European Union countries, mainly to USA, China and the Russian Federation.

Import of wine from the European Union - The average wine import to the European Union was 13.607.000 hectolitres (2010-2012). It represents surely the biggest wine exporter in the world. In wine import structure dominates the wine in bulk (57.2%), and then follows the bottled wines (41.7%), and the lowest share has the sparkling wine, with one percentage share. The wines in bulk have a low price and often serve for blending with the local wine. The highest import realizes from Australia (25.3%), then Chile (21.6%) and South-African Republic (20.3%), they are more than two third (67.2%) of the total wine import of the European Union (table 2). From Serbia was imported 50 thousand hectolitres of wine, which makes 0.43% of the total import.

The leading wine importer within the Union is Germany, with average import of 1.525.000 hectolitres. The wine import value amounts 3.01 milliard US dollars. Import is a resultant of high consumption of 26 litres per capita (2011), as well as by improvement of assortment with the quality sorts of wines. German consumers prefer red wines. In consumption structure, a dominant place has red wines (63%); the share of white wines is 29% and rose around 8% ([www.wineinstitute.org/resources/statistics](http://www.wineinstitute.org/resources/statistics)). In import structure dominate the bottled wines (62%), and then the wines in bulk (22%), while the lowest share is of the sparkling wines (16%). The biggest part of import participates from the European Union countries (84%). There dominates Italy with 42.7%, follows Spain with 18.4% and France with 15.9%. The above mentioned countries are main suppliers of the German market, with the share of three fourth. The biggest import out of the European Union realizes from the South-African Republic with 4.9%. In import structure dominates red wine (60%); significantly lower share is of white wine (25%), while the lowest share is of rose wine (7%) (Ammerman, Danieliane Illma, 2013).

In the second place is *Great Britain*, with slightly lower import than Germany, of 1.329.000 hectolitres. The average wine import amounts 4.76 milliard US dollars. The wine production, owing to the climatic factors, is small, so it has to import significant amounts of wine. During last years has been present a slight stagnation of import and consumption of wine. With achieved consumption of 22 litres per capita, it lags behind the European average, for around five litres (FAO, 2011). In consumption structure, a dominant place take the white wines (46%), the share of red wines is 42% and rose 12% (Observatorio Español del Mercado del Vino (2012). In import structure dominates the bottled wine (71%), then the sparkling wine (17%), while the lowest share is of bulk

wine (16%). In import dominate wines from the European Union (53.5%). The most represented are wines from Italy (18.9%), Australia (18%), France (15.5%), Spain (10.5%) and USA (9.1%), which make almost three quarters of the total import.

Table 2. Wine import into the European Union (2010-2012), 000 hl

Country	Average quantity		Average value 000 \$
	000 hl	%	
Australia	3.446	25,3	739
Chile	2.946	21,6	769
South Africa	2.762	20,3	537
USA	2.370	17,4	469
New Zealand	633	4,6	325
Argentina	574	4,2	187
Other countries	860	6,6	197
Total	13.607	100,00	3.223

Source: *Global Trade Atlas* ([www.gtis.com/gta/](http://www.gtis.com/gta/))

France comes after, with average import of 603 thousand hectolitres. The wine import value amounts 792 million US dollars. With achieved consumption of 55 litres per capita represents the leading global consumer (FAO, 2011.). In import structure dominate the bottled wines (59%); there is a high share of wines in bulk (34%), while the lowest share is of the sparkling wines (7%). In import has been the most present the wines from Spain (63.9%), then follows Italy (16.7%), Portugal (9.2%), Chile (2.4%) and USA (2.1%) which makes more than 90% of the total import. It is evident that the most imports from the European Union. The most of wines which import from USA are from California (Bettini and Ornella, 2013). The wine policy of the European Union in the following period will base on next elements (Comité Européen des Entreprises Vins, 2013).

Deepening the modernisation of the European sector and its market orientation, further pursuing dynamic and market-oriented measures, with the objective of reinforcing the competitiveness of the EU wine sector, by favouring solutions allowing to better structure the sector instead of dispersed measures.

Supporting a new regime, that frames the growth of plantings in the EU, with the objective of ensuring the orderly growth of new plantings to reinforce the competitiveness of the EU wine sector. It should allow for a balanced evolution of the EU vineyard in the future which, however, would not undermine the competitive potential of the EU wine supply on the market place, nor be an obstacle to the development of competitive production strategies that allow improving the market situation of the EU wines and other vine products across the world and meet future increase of demand(s).

Supporting an update of definitions and processing practices for aromatized wine products adapted to technological innovation and new consumer expectations, in line with the OIV standards.

Improving the definition of appropriate production conditions across the EU to satisfy the consumer's demand on quality organic wines, while ensuring smooth functioning of the internal market and the international trade, through appropriate equivalence, solutions between the EU and Third Countries' organic wine standards.

Promoting appropriate EU & international harmonised standards for alcohol-

reduced wines and alcohol-free wines, allowing the EU wine sector to respond to the dynamic consumer demand, expand the outlets for the EU production, and ensure the smooth market deployment of these new segments without unnecessary barriers to the internal and international trade.

Preserving the specificities of wine geographical indications, strengthening their protection at international level, but also its complementarity, as regards a company's brands and trademarks, so that one should not prevail upon the other. Improving economic intelligence for increasing the market legibility for the EU wine economic operators in order to better forecast future trends in production, consumption and trade.

## CONCLUSION

The European Union represents one of the biggest wine exporters in the world. The average export of wine is 21.666.000 hectolitres. The highest export has been directed to USA market (23.3%), and then follows the Russian Federation (16.3%) and China (9.9%). The biggest wine exporter within the European Union is Italy, then Spain and France, which dominate in export of the stated group. The average wine import was 13.607.000 hectolitres. It represents compellingly the most important wine importer in the world. The biggest import is from Australia (25.3%), and then follows Chile (21.6%) and South-African Republic (20.3%). The average import from Serbia has amounted 50 thousand hectolitres, i.e. 0.43% of the total EU import. The biggest importer is Germany, as also the biggest wine importer in the world, too. Then follow Great Britain and France. The European Union has a positive balance of foreign trade exchange of wine, approximately something over six thousand hectolitres. In the next period can be expected a trend of export increase from the European Union, but with significantly more moderate tempo owing to a significant saturation of the international market.

## REFERENCES

- AMMERMAN, P., DANIELIANE ILLMA: German Wine Market Opportunities. 2013. [www.navigator-consulting.com/articles/german-wine-market-opportunities/32](http://www.navigator-consulting.com/articles/german-wine-market-opportunities/32) [accessed: august 2013].
- CEEV – Comité Européen des Entreprises Vins: EU Wine Policy - Common Market Organisation and Wine Regulatory. Issues, 2013. [www.ceev.be/policy-dossiers/eu-wine-policy](http://www.ceev.be/policy-dossiers/eu-wine-policy) [accessed: november 2013].
- DEUTSCHE WELLE: Nemačka postaje najbolje tržište za španjolsko vino, 2012. <http://trend.seebiz.eu/gastro/njemacka-postaje-najbolje-trziste-za-spanjolsko-vino/ar-53046/> [accessed: october 2013].
- GLOBAL TRADE ATLAS. [www.gtis.com/gta/](http://www.gtis.com/gta/) [accessed: october 2013].
- OBSERVATORIO ESPAÑOL DEL MERCADO DEL VINO: El mercado del vino tranquilo en Reino Unido, 2011. [www.oemv.es/esp/el-mercado-delvino-tranquilo-en-reino-unido-2011-359k.php](http://www.oemv.es/esp/el-mercado-delvino-tranquilo-en-reino-unido-2011-359k.php) [accessed: november 2013].
- ORNELLA, BETTINI: Wine Annual Report and Statistics. USDA foreign agricultural service, 2013. [www.calwinexport.com/files/Wine\\_Annual\\_Rome\\_EU-27\\_2-22-2013](http://www.calwinexport.com/files/Wine_Annual_Rome_EU-27_2-22-2013).

pdf [accessed: november 2013].

VLAHOVIĆ, B., PUŠKARIĆ, A., TOMAŠEVIĆ, D.: Changes of international wine market. 135<sup>th</sup> EAAE Seminar Challenges for the Global Agricultural Trade Regime after Doha, Belgrade, pp. 349-359, 2013.

WINES OF GERMANY: German Wine Exports Boom, 2012. [www.germanwineusa.com/news-events/news/german-wine-exports-boom.html](http://www.germanwineusa.com/news-events/news/german-wine-exports-boom.html) [accessed: november 2013].  
[www.bizlife.rs/m/vesti/36111-italijanski-izvoz-vina-na-rekordnom-nivou](http://www.bizlife.rs/m/vesti/36111-italijanski-izvoz-vina-na-rekordnom-nivou) [ september 2013].

[www.vino.rs/svet-vina/regioni/spania.html](http://www.vino.rs/svet-vina/regioni/spania.html) [accessed: october 2013].

[www.germanwineusa.com/news-events/news/german-wine-exports-boom.html](http://www.germanwineusa.com/news-events/news/german-wine-exports-boom.html) [accessed: october 2013].

[www.wineinstitute.org/resources/statistics](http://www.wineinstitute.org/resources/statistics) [accessed: novembar 2013].

## TRŽIŠTE VINA U EVROPSKOJ UNIJI

BRANISLAV VLAHOVIĆ, ANTON PUŠKARIĆ, MARINA NACKA

### Izvod

U radu se analizira tržište vina u Evropskoj uniji. Determinisane su promene u prometu, i apostrofirani su regionalni najveći izvoznici i uvoznici vina. Osnovne izvore podataka predstavlja baza podataka *Global Trade Atlas*, u vremenskom periodu 2010-2012. godina. Proizvodnja vina u Evropskoj uniji na nivou je od 164 hiljada hektolitara. U strukturi izvoza dominira vino u bocama (67,9%), sledi u rinfuzi (22,7%), najmanje je učešće penušavog vina sa 9,4%. Najveći izvoz vina usmeren je na tržište SAD (23,3%), sledi Ruska Federacija (16,3%) i Kina (9,9%). Najveći izvoznik vina u okviru Unije jeste Italija, slede Španija i Francuska. Prosečan uvoz vina Evropske unije iznosio je 13.607 hiljada hektolitara. Ona predstavlja ubedljivo najvećeg svetskog uvoznika vina. U strukturi uvoza dominira vino u rinfuzi (57,2%), sledi vino u bocama (41,7%), najmanje učešće ima penušavo vino, sa učešćem od svega 1,0%. Najveći uvoz ostvaruje se iz Australije (25,3%), sledi Čile (21,6%) i Južnoafrička Republika (20,3%). Najveći uvoznik je Nemačka slede Velika Britanija i Francuska. Evropska unija ima pozitivan bilans spoljnotrgovineke razmene vina, prosečno nešto preko šest hiljada hektolitara. U narednom periodu može se očekivati trend rasta izvoza iz Unije ali sa znatno umerenijim tempom usled značajne zasićenosti međunarodnog tržišta.

**Ključne reči:** vino, Evropska unija, izvoz, uvoz.

Received / *Primljen*: 17.03.2014.

Accepted / *Prihvaćen*: 16.04.2014.

## ORGANIC DAIRY PRODUCTION – MOST COMMON ISSUES CONCERNING HEALTH CARE AND NUTRITION\*

ANKA POPOVIĆ VRANJEŠ, ŽELJKA JURAKIĆ,  
BRANISLAVA BELIĆ, IVANA DAVIDOV,  
ALEKSANDAR BOŽIĆ, MIHAJLO OSTOJIĆ, GORAN JEŽ<sup>1</sup>

*SUMMARY: In most countries transition from conventional to organic dairy production came as a result of two influences: first, as a way of additional employment, and second, in regions with problems that came as a result of pollution of soil. There are several problems in encouraging producers to practice organic dairy. One of them is health care – prevention and treatment and the other is feeding of cows, both with their limitations and prohibitions. This paper gives a brief review of most common health issues in organic dairy production and feeding through experiences in some countries.*

**Key words:** organic dairy production, udder health, mastitis, feeding.

### INTRODUCTION

Organic farming is a developing production, not only a radical fringe that attracts only the most innovative and risk taking farmers. Although organic dairy farming is still a minority faction within the dairy industry, there is certainly a lot more interest in it by various agricultural professionals who work with farmers. Full support to an organic dairy sector comes from linked producers in area of providing seeds, soil, animal nutrition and veterinary sector. Of course, it all has to have a result on a market. Organic dairy market has many typical characteristics:

---

Review paper / Pregledni rad

<sup>1</sup> Anka Popović Vranješ, PhD, Full Professor, Željka Jurakić, MSc, Teaching Assistant, Branislava Belić, PhD Full Professor, Ivana Davidov, DVM, PhD, Assistant Professor and Aleksandar Božić, PhD, Full Professor, Faculty of Agriculture, Novi Sad, University of Novi Sad. Mihajlo Ostojić, PhD, Full Professor, Goran Jež, MSc, Faculty of Agriculture Zemun, University of Belgrade.

Corresponding author: Anka Popović Vranješ, e-mail: anka.popovicvranjes@stocarstvo.edu.rs; phone: +381 21 485-33-72.

\* The paper is a part of the research work on the project: Production of hard cheese with added value of milk produced in organic and self-sustaining systems. File no.TR31095, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, from 2011. to 2014.



- 1) Typical consumer has extra incomes and is in socio-economic status which allows him to buy this products;
- 2) A deep conviction regarding the natural resources;
- 3) A health condition that demands the consumption of such products;
- 4) Religious reasons.

Issues such as health, environment and animal welfare become more and more important for consumer as a guarantee that he pays for healthy and safe product and that makes this production totally consumer driven. (Karreman, H.J., 2007). Organic milk production system can bring mutually beneficial to the animals and for the environment compared to conventional production. For many people this is of great importance in making decisions about buying organic milk. However, for the rest of the population the main reason for buying organic food is the fact that this food is nutritionally more valuable compared to the conventional. Organically produced milk and dairy products for consumers represent healthier and safer alternative (Popovic-Vranjes et al, 2012). Tendencies in development of agricultural production in Serbia include greater participation of organic production. Our country has considerable areas suitable for organic production, particularly in the parts of protected areas where legislation restricts the conventional agricultural production. Also, the structure of farms (small farms with complete production - plant and animal) form the basis for the implementation of the principles of organic production. (Popovic-Vranjes et al., 2010a).

## HEALTH STATUS

One of the conditions for the establishment of organic livestock production is a spatial isolation of livestock farm and coordinated development of plant and animal production. In organic farming, animal welfare occupies a high priority. Better milk quality from organic production is associated with feeding that is mostly based on pasture, then with free grazing, improved level of fitness which all results with healthier and more satisfied animals (Popovic-Vranjes et al., 2011; Krajinović et al., 2011). First of all, animals should be provided with conditions for their growth and development in harmony with the natural genetic potential (Popovic-Vranjes et al., 2010b).

In their work, Rosati and Aumaitre (2004) analyzed all aspects of organic dairy production, their mutual dependence and interaction. They emphasize that organic production does not automatically mean reduced risk to animal health and that health care in organic farming is usually criticized. Authors point out to the importance of, so called, "natural management" considering that good environmental conditions and their good management are essential for preserving the health of animals especially in organic production, which limits the application of conventional therapy. In an effort to meet market requirements, which creates an image of organic farming as "satisfied cow on a green pasture" producers often forget the real welfare of the animals for which improvement there is always a space (Rosati and Aumaitre, 2004).

Organic animal husbandry has been strongly criticized, especially by veterinarians, who have claimed that organically bred animals are not treated properly when they are sick because of longer withdrawal time prescribed by the organic standards and because alternative medicine is preferred (including methods not recognized by science).

Organic farming and the specific requirements for the veterinary care and application of therapy significantly limits the use of drugs and conditions that are com-

monly treated with antibiotics now represent health problem. Mastitis represents a major health issue in dairy cows in conventional as well as organic production (*Roderick et al., 1996*). Mastitis is the most common and economically most burdening contagious disease affecting dairy farms. The largest number of mastitis pathogens is endemic in some countries and therefore it is essential that, in purpose to prevent the entry and spread of pathogens, all bio-security measures should be carried out at local or farm level (*Barkema et al., 2009*). They indicate that maintaining a closed herd decreases the risk of introduction of pathogens that affect udder health (directly or indirectly) and especially, if animals are purchased, their udder health history should be known and they should be examined and tested. Limitations of organic production, primarily due to the use of conventional therapy, require the strict observance of the rules in respect of quarantine (if the animal is purchased) and the implementation of prophylactic measures.

Results that indicate more or less occurrence of mastitis in organic farming compared to conventional are extremely contradictory. While some authors speak in favor of the less occurrence of mastitis in herds where is milk produced according to the principles of organic production like some earlier results from Norway (*Ebbesvik and Loes, 1994*) and Denmark (*Vaarst and Enevoldsen, 1994*), the results of other researchers suggest the contrary, as we can see in a survey conducted in Great Britain which reported higher incidence of dry mastitis and subclinical mastitis in organic comparing to conventionally managed herds (*Hovi and Roderick, 1999*). Most authors, however, agree that the potential for pathogens appearance and the spread and incidence of mastitis in organic herds is in most parts equal to those data's obtained from conventional production (*Augustburger et al., 1988; Krutzinna et al., 1996; Weller and Cooper, 1996; Smolders, 2001*). *Barkema et al. (1999)* in their work underscores the risk in the occurrence of pathogens and the creation of conditions for their growth is the environment, which is crucial in the prevention of disease and clearly points to the importance of good environmental management in organic production. Therefore, control program that includes prevention of clinical mastitis caused by environmental pathogens should focus on the environment as much as on cow's defense mechanisms.

Some studies have indicated that use of veterinary medicine is lower in organic herd. A survey conducted in U.S. by *Stiglebauer et al. (2013)* reports that organic farmers had less regular veterinary visits (36%) comparing to conventional non-grazing farmers (77%) or conventional grazing producers (56%). At the same time, 64% of organic farmers reported vaccinating adult cows as a part of prevention compared with 100% of conventional grazing and 97% of conventional non-grazing farmers.

Survey conducted in Norway showed that cows in conventional herds were found to be treated for mastitis about three times more often than cows in organic herds and somatic cell counts were at the same level in the two observed groups (*Hardeng and Edge, 2001*). Variations in udder health that were determined between herds as well as between years could be a result of the choice made - using antibiotics for infection treatment or not, and it is highly related to the management of the herd (*Bartlett et al., 1992*). That generally brings us to simple conclusion – besides feeding good health management and the choices made considering health issues are crucial factors of good organic production.

Besides mastitis, a significant item related to the health status of the animals is the number of somatic cells in milk. Research conducted in the UK to over 5700 cows on 80 farms (40 organic and 40 conventional) showed no significant difference in number

of somatic cells in milk of cows that on average was about 214,000 cells / ml. At the same time, milk production was higher in conventional farm (8436 l per cow) compared to an organic farm (7311.5 l per cow) (*Haskell et al., 2009*). *Weller and Bowling (2000)* in a study conducted in US report similar results – according to them udder health in organic herds or bulk tank somatic cell counts is not different from conventional herds. In support of these results goes research conducted in Switzerland by *Roesch et al. (2006)* in 120 farms (60 organic and 60 conventional farms with integrated production) where the value of somatic cell count ranged from 119 000 cell/ml in organic farms to 117 000 cell/ml in conventional production. On the other side, a survey conducted in UK for a period of two years, reported that the risk of mastitis was similar in organic and conventional herds, while the occurrence of mastitis during the dry period was higher in organic herds. Also, increased presence of somatic cells in organically produced milk was determined (*Roderick and Hovi, 1999; Hovi and Roderick, 2000*).

Differences between organic and conventional herds in somatic cell counts and use of disease treatment may be a result of the individual herd manager independently of production system, or a result of general differences between regulation and production principles (*Bennedsgaard et al., 2003*).

Health is an important aspect when evaluating animal welfare in organic dairy production but it should be kept in mind that organic production itself does not automatically mean better welfare and health status of animals (*Hovi et al., 2003*). When it comes to the treatment of sick animals that are grown in an organic production system, it should be noted that the EU allowed the application of certain animal health products that allow animals returning to production while maintaining the organic status. On the other side, the U.S. strictly prohibited the use of conventional veterinary products and after treatment animal loses its organic status. At the same time, the veterinary regulations clearly indicate that animal must not be denied of medical care. All this put producers in a very difficult situation and requires the development of precise plans of prevention and their consistent application (*Stiglbauer et al., 2013*). How *Hovi et al. (2003)* conclude in their work, different data's regarding animal health on organic farms come from a limited number of farms applying organic milk production, and individual differences between managers are manifested. Also, *Sundrum (2001)* considered that the problem lies in the fact that most of the organic farms are mixed character (plant and animal production) and that producers pay more attention to one or the other production and this causes fluctuations in achieved production results.

## FEEDING

Adequate nutrition is the foundation of the organization and management of organic milk production. The meal must be designed in a way that provides all necessary substances for animals. Nutrients must originate in organic crop production, it is desirable to be produced on the farm or in the region and portions should be balanced and always available. If it is impossible to provide a completely organic meal for animals, it is allowed to use of nutrients from conventional production with limited participation up to 5 % in the diet per annum calculated on dry matter feed. The concept of organic milk production is based on the pasture way of keeping and feeding the cows. Grazing is economical and rational, pastures should not be too far from stall (no more than 1 to 1.5 kilometers) to avoid too much energy been spent on walking to and from the pasture.

It is particularly suitable combined usage of grassland for grazing and mowing, but it requires a larger area of grassland per cow (*Pantelić et al., 2008*).

Basic characteristic of meal for dairy cows is to be designed to fulfill all production requirements of the organism. Meal must be sufficient by the amount, well balanced, versatile, economical, easy to manipulate. Feeding cows in organic milk production varies during the winter and summer period. Winter nutrition is based on the use of dry and succulent forage - hay (grasses and legumes) and silage (maize). The lack of certain nutrients can be compensated by adding concentrated mixtures. One of the major issues in animal feeding in organic dairy farming is protein, mineral, and trace elements deficiencies, especially for animals reaching high production (*Coonan et al., 2002*). Due to the restricted type of feeding, as imposed by EU regulations, in organic dairy farms, a clear deficiency in zinc, molybdenum, selenium, copper, and iodine can occur. The correct balance of minerals and trace elements must be constantly achieved.

The whole idea of organic production is based on the open stance and the pastoral way of feeding cows. Cows spend up to seven months during the year grazing depending on the region. Animals on pasture are in constant motion and energy consumption is much higher in this way of keeping in comparison to conventional. Also, in highly productive animals this diet is not sufficient and it is necessary to provide additional meal after returning from grazing.

With regard to dairy cow feeding, the European organic standards require forage based (60% of daily dry matter intake) and primarily home-grown diets and restrict the use of feed supplements and synthetic vitamins.

## REGULATION IN SERBIA

Organic animal production limits the use of chemical synthesized veterinary preparations, antibiotics, hormones and substances used to control reproduction. The legal framework in Serbia which regulates the organic livestock production clearly define in what cases is allowed to use conventional veterinary preparations, the duration of the withdrawal period, in this case, and if the situation repeats again translating the animals over a period of conversion into a new cycle of organic production (*Law on Organic Production, 2010*).

The essence of health care in organic production is in prevention and it strictly prohibits the use of any veterinary products for the prevention of disease. If the disease develops or animals get injured it is desirable to isolate such animal and immediately begin appropriate treatment. The advantage in this case have phytotherapeutic, homeopathic or immunological preparations, as well as certain elements and additives allowed in feeding under condition that their application has effect with certain types of animals and certain diseases. The use of phytotherapeutic preparations must be under strict control since some plants whose medicinal effect is already known may also have undesirable influence on products (milk discoloration or odor).

Homeopathic treatment is based on the provision of small amounts of substances that in high concentrations cause the same or similar symptoms that have the disease itself. The EU has stipulated the permissible concentration of substance in a solvent (1 part active substance in 10,000 parts of solvent) and these preparations are considered safe for use in the treatment of humans and animals. Immune preparations are used for activation of immune cells of the body and enhanced immune response to pathogens.

In addition to these substances it's permitted and the application of certain mineral supplements for animal feed and additives (vitamins (from natural sources), micronutrients - Fe, I, Co, Cu, Mn, Zn, Mo, Se, antioxidant elements, etc.) (*Regulation, 2011, 2012*).

In case when illness develops or, to prevent the suffering of animals it's permitted the use of conventional veterinary preparations or antibiotics with maximum responsibility of a veterinarian. After using these substances in the treatment of animals, should be kept in mind that the withdrawal period allowed in this case, is twice as long compared to the period of the withdrawal period in the conventional livestock production and can't be shorter than 48 hours.

In the case animals received therapy of chemically synthesized drugs or antibiotics for more than three times for a period of 12 months (more than one therapy in the case of animals whose productive cycle is shorter than one year), they lose status of the animal in organic production, and they should be re-through the conversion period (*Regulation, 2011, 2012*).

For veterinary interventions that are subjects to these restrictions, implementation of regular measures – programs of animal health protection, vaccination or treatment of parasites should be excepted.

## CONCLUSION

Disease prevention based on a selection of appropriate breeds and strains of animals, appropriate methods of breeding, feeding, number of animals per unit of production area, proper pasture and the hygiene conditions are of great importance in organic dairy production.

The producer of an organic livestock operation has a difficult task to fulfill all the requirements of an organic production concerning medical treatment on one hand, and on the other, he must not denied appropriate medical treatment for an animal.

The whole concept of organic production is based on the good management of health care and animal nutrition. Both of components have its strict limitations and good management to them is what brings results in this kind of production. The experiences and results that vary in some items (such as mastitis appearance in cows) clearly indicate that a good result in the production of organic milk exclusively dependent on the site where the production is carried out and the herd management.

## REFERENCES

- AUGUSTBURGER, F., ZEMP, J., HEUSSER, H.: Vergleich der Fruchtbarkeit, Gesundheit und Leistung von Milchkuchen in biologisch und konventionell bewirtschafteten Betrieben. Land wirtschaft Schweiz, 1:427–431, 1988.
- BARKEMA, H.W., SCHUKKEN, Y.H., LAM, T.J.G.M., BEIBOER, M.L., BENEDICTUS, G., BRAND, A.: Management practices associated with the incidence rate of clinical mastitis. J. Dairy Sci., 82(8)1643 – 1654, 1999.
- BARKEMA, H.W., GREEN, M.J., BRADLEY, A.J., ZADOKS, R.N.: The role of contagious disease in udder health. J. Dairy Sci., 92(10)4717-4729, 2009.
- BARTLETT, P.C., MILLER, G.Y., LANCE, S.E., HEIDER, L.E., ANDERSON, C.R.: Environmental and managerial risk factors of intramammary infection with coagulase-

negative staphylococci in Ohio dairy herds. *Prev. Vet. Med.*, 14(1-2)129–142, 1992.

BENNEDSGAARD, T.W., THAMSBORG, S.M., VAARST, M., ENEVOLDSEN, C.: Eleven years of organic dairy production in Denmark: herd health and production related to time of conversion and compared to conventional production. *Livest. Prod. Sci.*, 80(1-2)121–131, 2003.

COONAN, C., FREESTONE-SMITH, C., ALLEN, J., WILDE, D.: Determination of the major mineral and trace element balance of dairy cows in organic production systems. In: *Proceeding of Organic Meat and Milk from Ruminants*, Athens, (Kyriazakis Zervas, eds). EAAP Publ., 106:181–183, 2002.

EBBESVIK, M., LOES, A.K.: Organic dairy production in Norway—feeding, health, fodder production nutrient balance and economy—results from the '30-farm-project, 1989–1992. In: *Converting to Organic Agriculture* (Granstedt, A., Koistinen, R., eds.). Scand. Assoc. Agric. Sci. Rapp., 93:35-42, 1994.

HARDENG, F., EDGE, V.L.: Mastitis, ketosis, and milk fever in 31 organic and 93 conventional Norwegian dairy herds. *J. Dairy Sci.*, 84(12)2673–2679, 2001.

HASKELL, M.J., LANGFORD, F.M., JACK, M.C., SHERWOOD, L., LAWRENCE, A.B., RUTHERFORD, K.M.D.: The effect of organic status and management practices on somatic cell counts in UK dairy farms. *J. Dairy Sci.*, 92(8)3775-3780, 2009.

HOVI, M., RODERICK, S.: An investigation for the incidence, treatment strategies and financial implications of mastitis in organic and conventionally managed UK dairy herds. An attachment to a Scientific Report to the Ministry of Agriculture, Fisheries and Food, UK. University of Reading. 1999.

HOVI, M., RODERICK, S.: Mastitis and mastitis control strategies in organic milk. *Cattle Practice*, 8(3)259–264, 2000.

HOVI, M., SUNDRUM, A., THAMSBORG, S.M.: Animal health and welfare in organic livestock production in Europe: Current state and future challenges. *Livest. Prod. Sci.*, 80(1-2)41-53, 2003.

KARREMAN, H.J.: *Treating Dairy Cows Naturally – Thoughts and Strategies*. Publisher Acres USA. Austin, Texas, USA, 2007.

KRAJINOVIĆ, M., POPOVIĆ-VRANJEŠ, A., CVETANOVIĆ, D., JEŽ, G., JEVTIĆ, M., NOVAKOVIĆ, I.: Držanje i ishrana muznih krava u organskoj proizvodnji mleka. *Savremena poljop.*, 60(3-4)438-444, 2011.

KRUTZINNA, C., BOEHNCKE, E., HERMANN, H.-J.: Die Milch. viehhaltung im Ökologischen Landbau. *Ber. Ldw.*, 74:461–480, 1996.

LAW ON ORGANIC PRODUCTION. Official Gazette of Republic of Serbia, No.30, 2010.

PANTELIĆ, B., VIDAKOVIĆ, O., NOVAKOVIĆ, N., HORVAT SKENDEROVIĆ, T.: Osnovi organske proizvodnje. Agrozavod AD, Subotica, 2008.

POPOVIĆ-VRANJEŠ, A., GRUBAČIĆ, M., PEJANOVIĆ, R., KRALJ, A., KASALIĆ, A., MIOČINOVIĆ, D., NIKETIĆ, G.: Ispitivanje mogućnosti organske proizvodnje mleka u Srbiji. *Savremena poljop.*, 59(1-2)119-125, 2010a.

POPOVIĆ-VRANJEŠ, A., PEJANOVIĆ, R., JOVANOVIĆ, S., SAVIĆ, M., OSTOJIĆ, M., GRUBAČIĆ, M., CVETANOVIĆ, D.: Upravljanje kvalitetom u organskoj proizvodnji i preradi mleka. *Prehrambena industrija - mleko i mlečni proizvodi*, 21(1-2)56-62, 2010b.

POPOVIĆ-VRANJEŠ, A., SAVIĆ, M., PEJANOVIĆ, R., JOVANOVIĆ, S., KRAJINOVIĆ, G.: Efekat organske proizvodnje mleka na neke parametre kvaliteta

mleka. *Acta Veterinaria*, 61(4)415-421, 2011.

POPOVIĆ-VRANJEŠ, A., PEJANOVIĆ, R., KRAJINOVIĆ, M., KASALICA, A., CVETANOVIĆ, D., KRALJ, A.: Chemical parameters and hygienic quality of raw milk from conversion period in organic production. The Forth Joint UNS - PSU International Conference; University of Novi Sad, Serbia, pp. 34-37, 2012.

REGULATION OF CONTROL AND CERTIFICATION IN ORGANIC PRODUCTION AND METHODS OF ORGANIC PRODUCTION. Official Gazette of Republic of Serbia, No. 48/2011.

REGULATION OF CONTROL AND CERTIFICATION IN ORGANIC PRODUCTION AND METHODS OF ORGANIC PRODUCTION. Official Gazette of Republic of Serbia, No. 40/2012.

RODERICK, S., SHORT, N., HOVI, M.: Organic Livestock Production - Animal Health and Welfare Research Priorities. Technical Report - VEERU, University of Reading. pp. 29, 1996.

RODERICK, S., HOVI, M.: Animal Health and Welfare in Organic Livestock Systems: Identification of Constraints and Priorities. Technical report—VEERU for Ministry of Agriculture, Fishery and Food. University of Reading. 1999.

ROESCH, M., DOHERR, M.G., BLUM, J.W.: Management, feeding, production, reproduction and udder health on organic and conventional Swiss dairy farms. *Schweiz Arch. Tierheilkd.*, 148(8)387-395, 2006.

ROSATI, A., AUMAITRE, A.: Organic dairy farming in Europe. *Livest. Prod. Sci.*, 90(1)41–51, 2004.

SMOLDERS, G.A.: Preventive measures for animal health and practical means for management support on organic dairy organic in the Netherlands. In: Proceedings of the 5th NAHWOA Workshop. Rodding, Denmark, pp. 113–125, 2001.

STIGLBAUER, K.E., CICONI – HOGAN, K.M., RICHERT, R., SCHUKKEN, Y.H., RUEGG, P.L., GAMROTH, M.: Assesment of Herd Management on Organic and Conventional Dairy Farms in the United States. *J. of Dairy Sci.*, 96(2) 1290-1300, 2013.

SUNDRUM A.: Organic livestock farming – A critical review. *Livest. Prod. Sci.*, 67(3)207–215, 2001. VAARST, M., ENEVOLDSEN, C.: Disease control and health in Danish organic dairy herds. In: *Biological Basis of Sustainable Animal Production* (E. A. Huisman, J. W. M. Osse, D. van der Heide, S. Tamminga, B. J. Tolkamp, W. G. Schouten, C. E. Hollingworth and G. L. van der Winkel, Eds.). EAAP Publ., 67:211–217, 1994.

WELLER, R.F., COOPER, A.: Health status of dairy herds converting from conventional to organic dairy farming. *Vet. Rec.*, 139(6)141–142, 1996.

WELLER, R.F., BOWLING, P.J.: Health status of dairy herds in organic farming. *Vet. Rec.*, 146(3)80–81, 2000.

# ORGANSKA PROIZVODNJA MLEKA – NAJČEŠĆA PITANJA OBZIROM NA ZDRAVSTVENU ZAŠTITU I ISHRANU

ANKA POPOVIĆ VRANJEŠ, ŽELJKA JURAKIĆ,  
BRANISLAVA BELIĆ, IVANA DAVIDOV,  
ALEKSANDAR BOŽIĆ, MIHAJLO OSTOJIĆ, GORAN JEŽ

## Izvod

U većini zemalja tranzicija u organsku iz konvencionalne proizvodnje mleka došla je kao rezultat dva uticaja: kao način dodatnog zapošljavanja ruralnog stanovništva, i kao rešenje za područja sa zagađenim zemljištem. Postoji nekoliko problema u podsticanju proizvođača da prihvate organsku proizvodnju mleka. Jedan je zdravstvena nega - prevencija i lečenje a drugi ishrana, oba sa svojim ograničenjima i zabranama. Rad daje kratak pregled najčešćih zdravstvenih problema u organskoj proizvodnji mleka i ishrani muznih grla kroz iskustva u nekim zemljama.

**Ključne reči:** organska proizvodnja mleka, zdravlje mlečne žlezde, mastitis, ishrana.

Received / *Primljen*: 20.12.2013.

Accepted / *Prihvaćen*: 29.05.2014.



## **PRIKAZ MONOGRAFIJE „OBRAZOVANJE, NAUKA I PROIZVODNJA HRANE“**

AUTORA AKADEMIKA  
VASKRSIJA JANJIĆA<sup>1</sup>

Ovih dana, u izdanju Akademije nauka i umjetnosti Republike Srpske, izašla je iz štampe monografija „OBRAZOVANJE, NAUKA I PROIZVODNJA HRANE“, autora akademika Vaskrsija Janjića. Radi se o izuzetno vrijednom dijelu u koje je autor uložio ogroman rad i znanje, pa je, tako, stvorio djelo neobično, kompleksno i sadržajno, kako po koncepciji, tako i po sadržini i prilazu u obradi pojedinih, izuzetno značajnih, dijelova knjige. Takva djela nisam imao priliku nikada da pročitam i zato mi je posebno drago da je akademik Janjić našao vremena i snage da jedno takvo djelo napiše i objavi. Ujedno mislim da takvo djelo rijetko ko može da napiše, jer je za takvo pisanje potreban poseban dar, ogromno znanje i iskustvo, a rijetki su istraživači koji raspolažu sa svim tim. Ono je pisano sa takvim jezikom i stilom, a plus toga u njega su utkana sva, nemala, autorova znanja i ogromno eksperimentalno iskustvo, kao i iskustvo u vrijednovanju naučnog rada u različitim naučnim disciplinama, tako da će svako onaj ko bude čitao ovo djelo osijetiti zadovoljstvo i samopouzdanje. Zahvaljujući višedecenijskom radu na ovom djelu, autor je prikazao cjelokupno stanje u obrazovanju, nauci i proizvodnji hrane ne samo u našoj zemlji nego i u okruženju, na cijelom evropskom i svijetskom prostoru. Za svaku i najmanju stvar o kojoj se danas vode žučne akademske rasprave, autor je dao i obrazložio svoj stav i tako stvorio djelo koje i trebaju da stvaraju naši akademici. Djelo je tako bogato snabdjeveno podacima, da će ono biti nepresušan i trajan izvor podataka za sve one koji se ovim problemima bave, ali i cjelokupnoj našoj naučnoj, stručnoj i upravnoj javnosti.

Monografija se sastoji od tri povezane cijeline: obrazovanja, nauke i proizvodnje hrane. Naslov i sadržina djela su neobični. Ali, autor polazi od toga da je obrazovanje neophodno svakom našem čovjeku, a isto tako da, danas izuzetno, obrazovani ljudi mogu da se bave naukom. Isto tako, sve što se istraži danas se želi što pre primijeniti, a primjena se zasniva na nauci i njenoj što potpunijoj i efikasnijoj aktivnosti. Ovo naročito važi za proizvodnju hrane koja je danas zasnovna na najvišim tehnološkim dostignućima. Ako samo pogledamo proizvodnju hrane zasnovane na korišćenju genetički modifikovanih biljaka, onda ćemo se upoznati sa kakvim se problemima današnji svijet sreo. Taj izazovni problem koji danas zaokuplja najveće autorite i najbogatije kompanije u svijetu predmet su najozbiljnijih razmatranja autora, pogotovo što su genetički modifikovane

---

Book review / Prikaz knjige

<sup>1</sup> Dr Vaskrsija Janjić, akademik, profesor, naučni saradnik, u penziji,  
e-mail: janjic.vaskrsija@gmail.com.

biljke molekularno mijenjane zbog upotrebe herbicida, a u toj oblasti akademik Janjić je poznat izvan granice naše zemlje.

Monografija je napisana na 678 stranica teksta (skraćeni A4 format). Ona ima više od 300 tabela, 157 grafikona, 21 shemu, 8 mapa i 74 slike. Bogato je literaturno obrađena i sadrži preko 778 literaturnih izvora i najnoviju statističku građu OECD, FAO, UNESCO, naših statističkih Zavoda, Zavoda za intelektualnu svojinu i mnogih drugih. Zbog bogate i specifične ilustracije i grafičke obrade monografija je štampana u boji na 90 gramskom mat kunsdruk papiru. Autor je ovo djelo posvetio Akademiji u povodu ovogodišnje proslave 20 godina od formiranja i rada Akademije.

Na kraju, može se istaći da se radi o izuzetnom djelu, velike vrijednosti, neophodnog svakom naučnom radniku i istraživaču ma koje on struke bio. Ono će, takođe, popuniti prazninu koja u ovoj oblasti već duži niz postoji, a pomoći će i mladim istraživačima da se upoznaju sa problemima, izazovima i perspektivama obrazovanja, nauke i proizvodnje hrane. Svako onaj ko bude čitao ovo djelo osijetiće snagu autora, ali i njegovu volju da ga najiskrenije i najpouzdanije upozna sa svim onim sa čime će sresti u životu ili odmah poslije zaposlenja. Akademija ima sreću i posebno zadovoljstvo što se ovo djelo pojavilo u vrijeme proslave 20 godina od njenog osnivanja i rada.

7. aprila 2014. god  
*Akademik Branko Škundrić*

## INTRODUCTIONS TO AUTHORS ON WRITING PAPERS FOR THE JOURNAL “CONTEMPORARY AGRICULTURE”

The journal “Contemporary Agriculture” publishes original scientific papers, review papers and short communications. *Review paper must have a minim 10 auto-citations.*

A paper is written in English. It should comprise a short summary in Serbian. The whole script of the paper, including tables, graphs, schemes, drawings and photographs, can have maximum 6 typed pages, Portrait, in single spacing. Margins: Top 2.5cm, Left 3.0 cm, Bottom 2.0 cm, Right 2.0 cm. For typing the paper the Times New Roman font, 10 pt, should be used. Justify with the indent of the first line 0.6 cm. (Portrait - Paragraph – Indents and Spacing – Special – First Line 0.6. No pagination.

**THE PAPER TITLE** is written in bold letters, Font Size 11, centred. The title should be lowered below the upper margin clicking enter 4 times and writing should be commenced in the fifth line.

THE NAME AND SURNAME of the authors are written in normal letters, Font Size 10, centred, with a single space below the paper title. With mark 1, in superscript, (click Insert Footnote) above the name of the last author, the Footnote is marked, stating the title, the name and surname, the rank and the institution in which the respective authors are employed.

SUMMARY: (italic), Font Size 10 (Justify) with a single space below the name and surname of the author of the paper. The summary presents the basic objective, the material and method of the study, the significant results and the conclusion (500 characters maximum).

**Key words** : minimum 3 and maximum 6 words. Below the summary, Font Size 10.

Footnote (Insert Footnote):

<sup>1</sup>Titula, first name and surname, address for the all of autors (Font Size 9).

Corresponding author: first name and surname, e-mail, phone (Font Size 9).

**INTRODUCTION** (bold), centred, Font Size 10. Text normal, Justify, with a single space below the title.

**MATERIAL AND METHOD OF THE STUDY** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the title.

**RESULTS** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the table.

**DISCUSSION** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the title.

**CONCLUSION** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the title.

**REFERENCES** (bold), centred, Font Size 10.

*In scientific Journals:*

PELTONIEMI, O.A., HEINONEN, M., LEPPÄVUORI, A., LOVE, R.J.: Seasonal effects on reproduction in the domestic sow in Finland: a herd record study. Acta Vet. Scand., 40(2)133-44, 1999.

STANČIĆ, B., BOŽIĆ, A., RADOVIĆ, I., GRAFENAU, P. sen., PIVKO, J., HRENEK, P., STANČIĆ, I.: Veštačko osemenjavanje svinja dozama sa samanjanim brojem spermatozoida (pregled). Savremena poljop., 56(1-2)1-11, 2007.

***PhD Thesis:***

TAST, A.: Endocrinological basis of seasonal infertility in pigs. PhD Thesis, Faculty of Veterinary Medicine, Finland, Helsinki, 2002.

***In Scientific Books:***

TOMES, J.G., NIELSEN, H.E.: Factors affecting reproductive efficiency of the breeding herd. In: Control of Pig Reproduction (D.J.A Cole and G.R. Foxcroft, eds.). Butterworths, London, pp.527-540,1982.

***At Scientific Meetings:***

DEEB, N. and CAHANER, A.: The effect of naked neck (Na) gene on broiler stocks differing in growth rate. Proceedings of the XX World's Congress, New Delhi, India, 2-5 September, 1996. Vol. IV, pp. 11.

The order of papers is arranged according to the alphabetical order of the initial letter of the surname of the first author, without numbering. References text Font Size 9.

The tables should be clear, as simple and neat as possible. The titles, headings (text) and subtext in tables, should be in English (normal), Font Size 9. The tables are set in a specific place in the text.

The photographs, drawings, graphs and schemes, should be given in a separate supplement (an original file format –TIF, JPG, with 300 dpi, or a vector format with letters turned into curves – CDR, AI) and the place where they are to be printed should be marked in the text, by writing in the text the caption of the photograph, drawing or scheme.

For example:

Graph. 1. Sperm concentration in ejaculates according to seasons of year (Font Size 9, italic).

Citing the authors in the paper: (Stančić et al., 2005) – if there are more than two authors. If there are only two authors, then - ( Stančić and Šahinović , 1995). Or - Stančić et al. (2005).

The paper is submitted to the editor's office of the journal in **2 printed copies** and **CD**, with all the supplements (photographs, schemes, drawings, graphs) by the regular post mail, and in electronic form at **e-mail: blagoje.stancic@stocarstvo.edu.rs**

***The papers which have not been done in accordance with these instructions will not be considered for publishing.***

These introductions to authors and one sample of the correct printed paper in the Journal "Contemporary Agriculture", you can find on the web site: <http://polj.uns.ac.rs> (Faculty of Agriculture in Novi Sad).

<https://sites.google.com/site/savremenapoljoprivreda/uputstvo-autorima>

**The printed copy of papers should be sent to the following address :**

**The editor's office of the journal "Contemporary Agriculture"**

**The Faculty of Agriculture**

**8 D. Obradovića 8 Square**

**21000 Novi Sad**

**Phone: ++ 021/485-3482**

We are grateful to all the authors for their cooperation.

**Editor**

**Prof.dr Blagoje Stančić**

**Editor –in-chief**

**Prof.dr Milan Popović**

## UPUTSTVO AUTORIMA ZA PISANJE RADOVA U ČASOPISU »SVREMENA POLJOPRIVREDA«

U časopisu »Savremena poljoprivreda«, objavljuju se originalni naučni radovi, pregledni radovi i prethodna saopštenja. **Pregledni radovi treba da imaju minimalno 10 autocitata svih autora rada.**

Rad se piše na engleskom jeziku. Treba da sadrži i kratak izvod na srpskom jeziku (**Izvod**). Celokupan tekst rada, uključujući tabele, grafikone, sheme, crteže i fotografije, može da ima maksimalno 6 kucanih stranica, A4 formata (Portrait), normalnog proreda (Single Space). Margine: Top 2,5cm, Left 2,5cm, Bottom 2,5cm, Right 2,0cm. Za kucanje rada koristiti font Times New Roman, 10pt. Justify poravnanje sa uvlakom prvog reda 0,6cm (Format → Paragraph → Indents and Spacing → Special → First Line 0,6). Bez paginacije (numerisanja stranica rada).

**NASLOV RADA** se piše velikim slovima (**bold**), Font Size 11, centrirano. Naslov spustiti ispod gornje margine sa 4 entera, a pisanje početi u petom redu.

IME I PREZIME autora se pišu velikim slovima (normal), Font Size 10, centrirano, sa jednim razmakom ispod naslova rada. Oznakom 1, u superskriptu, (komandom Insert Footnote, AB<sup>1</sup>), iznad imena zadnjeg autora, označava se Footnote, u kojoj se navodi titula, ime i prezime, zvanje i ustanova u kojoj rade pojedini autori.

*SUMMARY*: (*italik*), Font Size 10 (Justify), sa jednim razmakom ispod imena i prezimena autora rada. U izvodu se daju osnovni cilj, materijal i metod rada, važniji rezultati i zaključak (maksimalno 500 znakova).

**Key words**: minimalno 3, a maksimalno 6 reči. Ispod izvoda, Font Size 10.

Footnote (Insert Footnote):

<sup>1</sup>Titula, puno ime i prezime, sa adresama svih autora.

Autor za kontakt: Ime i prezime, adresa, telefon, e-mail.

\* Ako je rad deo istraživanja u okviru nekog projekta, obavezno navesti ko finansira, broj projekta i period u kome se finansira (\* se stavlja iznad zadnje reči naslova rada).

**UVOD (bold)**, centrirano, Font Size 10. Tekst normal, Justify, sa jednim razmakom ispod naslova.

**MATERIJAL I METOD RADA (bold)**, centrirano, Font Size 10. Tekst normal, Font Size 10, Justify, sa jednim razmakom ispod naslova.

**REZULTATI (bold)**, centrirano, Font Size 10. Tekst normal, Font Size 10, Justify, sa jednim razmakom ispod naslova.

**DISKUSIJA (bold)**, centrirano, Font Size 10. Tekst normal, Font Size 10, Justify, sa jednim razmakom ispod naslova.

**ZAKLJUČAK (bold)**, centrirano, Font Size 10. Tekst normal, Font Size 10, Justify, sa jednim razmakom ispod naslova.

**LITERATURA (bold)**, centrirano, Font Size 10.

*U naučnim časopisima:*

PELTONIEMI, O.A., HEINONEN, M., LEPPÄVUORI, A., LOVE, R.J.: Seasonal effects on reproduction in the domestic sow in Finland: a herd record study. Acta Vet. Scand., 40(2)133-44, 1999.

STANČIĆ, B., BOŽIĆ, A., RADOVIĆ, I., GRAFENAU, P. sen., PIVKO, J., HRENEK, P., STANČIĆ, I.: Veštačko osemenjavanje svinja dozama sa samanjanim brojem spermatozoida

(pregled). Savremena poljop., 56(1-2)1-11, 2007.

**Doktorska disertacija:**

TAST, A.: Endocrinological basis of seasonal infertility in pigs. PhD Thesis, Faculty of Veterinary Medicine, Finland, Helsinki, 2002.

**U naučnim knjigama:**

TOMES, J.G., NIELSEN, H.E.: Factors affecting reproductive efficiency of the breeding herd. In: Control of Pig Reproduction (D.J.A Cole and G.R. Foxcroft, eds.). Butterworths, London, pp.527-540,1982.

**Na naučnim skupovima:**

DEEB, N. and CAHANER, A.: The effect of naked neck (Na) gene on broiler stocks differing in growth rate. Proceedings of the XX World's Congress, New Delhi, India, 2-5 September, 1996. Vol. IV, pp. 11.

Redosled radova je po abecednom redu početnog slova prezimena prvog autora, bez numeracije! Tekst literature Font Size 9, normal.

Posle literature, napisati kratak izvod na srpskom jeziku (identičan prevod teksta Summary) i to:

**NASLOV**, velikim slovima (**bold**), centrirano, Font Size 10.

IME I PREZIMA AUTORA, velikim slovima (normal), centrirano, Font Size 10.

**Izvod**, malim slovima, (**bold**), centrirano, Font Size 10.

Tekst, Font Size 10, (normal) Justify.

**Ključne reči:** malim slovima.

Tabele treba da budu jasne, što jednostavnije i pregledne. Naslov, zaglavlja (tekst) i podtekst u tabelama, treba da budu napisani na engleskom jeziku (normal). Font Size 9. Tabele se stavljaju na određeno mesto u tekstu.

Fotografije, crteže, grafikone i sheme, dati u posebnom prilogu (izvorni format faila - TIF, JPG sa 300 dpi, ili vektorski format sa slovima pretvorenim u krive – CDR, AI), a u tekstu rada naznačiti mesto na kome treba da budu štampane, tako što će se, u tekstu, napisati naslov ili opis fotografije, crteža, sheme. Na primer:

Graph. 1. Sperm concentration in ejaculates according to seasons of year (Font Size 9, normal)

*Graf. 1. Koncentracije spermatozoida u ejakulatu nerast, zavisno od godišnje sezone (Font Size 9, italik).*

Citiranje autora u tekstu radu: (Stančić i sar., 2005). – ako je više od dva autora. Ako su samo dva autora, onda (Stančić i Šahinović, 1995). Ili, Stančić i sar. (2005).

Rad se dostavlja uredništvu časopisa u **2 štampana primerka i na CD**, sa svim priložima (fotografije, sheme, crteži, grafikoni), redovnom poštom na adresu uredništava, i u elektronskoj formi na **e-mail: blagoje.stancic@stocarstvo.edu.rs**

**Radovi, koji nisu napisani striktno po ovom uputstvu, neće biti prihvaćeni za štampu!**

Ovo uputstvo, kao i jedan primer pravilno odštampanog rada u časopisu »Savremena poljoprivreda«, možete naći i na sajt-u Poljoprivrednog fakulteta u Novom Sadu (<http://polj.uns.ac.rs/>)

<https://sites.google.com/site/savremenapoljoprivreda/uputstvo-autorima>

**Radove poslati na adresu:**

*Uredništvo časopisa »Savremena poljoprivreda«*

*Poljoprivredni fakultet*

*Trg D. Obradovića 8*

*21000 Novi Sad*

*Tel.: ++021/485-3482*

Svim autorima se zahvaljujemo na saradnji.

Novi Sad, 01.04.2014. god.

**Urednik časopisa**

*Prof. dr Blagoje Stančić*

**Glavni i odgovorni urednik**

*Prof. dr Milan Popović*

CIP – Каталогизacija y publikaciji  
Библиотека Матице српске, Нови Сад

631

SAVREMENA POLJOPRIVREDA = Contemporary  
agriculture : Srpski časopis za poljoprivredne nauke / glavni i  
odgovorni urednik Milan Krajinović. – God. 7, br. 1 (1959) - .- Novi  
Sad: Poljoprivredni fakultet, 1959-.– 24cm.  
Tromesečno. – Engleski, izvodi na srpskom jeziku. – Raniji naziv  
publikacije: Poljoprivreda Vojvodine

ISSN 0350-1205

COBISS.SR-ID 256386





ISSN 0350-1205

<http://polj.uns.ac.rs/>