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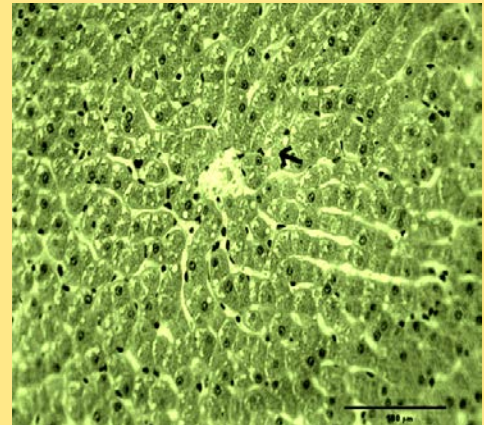
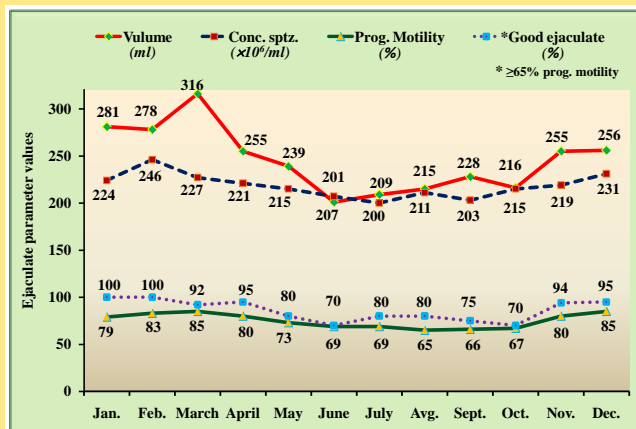
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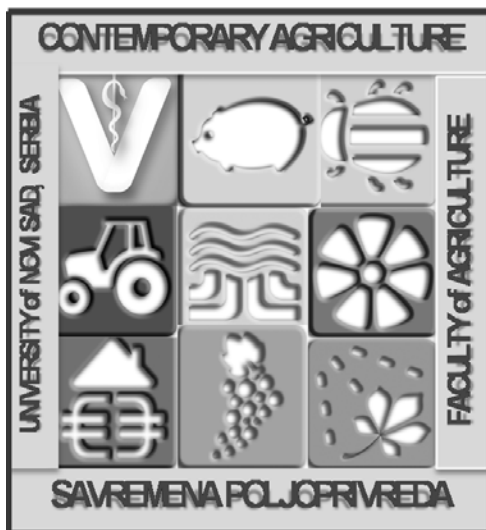
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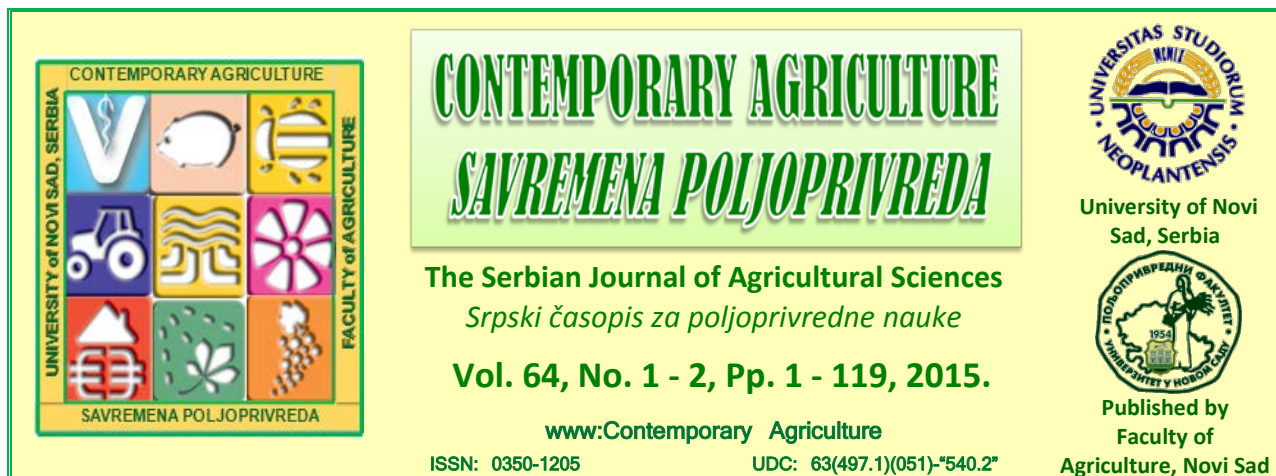
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OPTIMIZATION OF SPECIFIC α -TOCOPHEROL CONCENTRATIONS FOR *IN VITRO* CULTURES OF BOVINE SPERMATOZOA*

Eva TVRĐÁ[♦], Jana LUKÁČOVÁ, Tomáš JAMBOR, Peter MASSÁNYI, Norbert LUKÁČ¹

Summary: This study was designed to assess the dose- and time-dependent *in vitro* effects of α -tocopherol (α -TOC) on bovine spermatozoa during different time periods (Times 0h, 2h, 6h, 12h, 18h, 24h). Semen samples were collected from 20 adult breeding bulls, and diluted in physiological saline solution containing 0.5% ethanol (96%) together with 0, 1, 5, 10, 50, 100, 200, 500, 1000 and 2000 μ M/L of α -TOC. Most α -TOC concentrations had beneficial effects on the spermatozoa motion parameters, while groups supplemented with 500 and 200 μ M/L exhibiting significant differences ($P < 0.05$ and $P < 0.001$, respectively). The MTT assay indicated that none of the α -TOC concentrations had a negative or cytotoxic effect on the spermatozoa mitochondrial activity, and moreover showed a significantly ($P < 0.05$; $P < 0.01$; $P < 0.001$, respectively) improved cell viability in all the experimental groups throughout the *in vitro* culture. The NBT test showed that the addition of 1000-50 μ M/L α -TOC had an instant positive effect on the spermatozoa protection against free radical production. This protection remained present with a significant impact at all timeframes ($P < 0.001$ and $P < 0.01$, respectively). At the same time, all α -TOC concentrations exhibited significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively) protective effects on the spermatozoa free radical formation during the end-stages of spermatozoa culture. The results indicate that the addition of α -tocopherol, especially in concentrations ranging between 500 and 50 μ M to the culture medium could be beneficial for the overall stimulation of spermatozoa activity and protection against possible *in vitro* oxidative stress development.

Key words: vitamin E, spermatozoa, bulls, motility, viability, superoxide production.

INTRODUCTION

Vitamin E is a term that encompasses a group of potent, lipid-soluble tocol (tocopherol) and to-cotrienol derivatives qualitatively exhibiting the biological activity of RRR- α -tocopherol (Wen and Geffen, 2006). Structural analyses have revealed that molecules having vitamin E antioxidant activity include four tocopherols (α -, β -, γ - and

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δ-) and four tocotrienols (α-, β-, γ- and δ-) that occur naturally differing in the number and position of methyl groups on the chroman ring (Brigelius-Flohe and Traber, 1999).

α-tocopherol (α-TOC) is the most abundant form in nature and mostly available in food (Wen and Geffen, 2006), having the highest biological activity, and reversing vitamin E deficiency symptoms (Brigelius-Flohe and Traber, 1999). The molecular functions fulfilled specifically by α-TOC have yet to be fully described, but it is unlikely they are limited to general antioxidant functions, although the antioxidant feature is the flagship of the biological activity related to vitamin E (Wen and Geffen, 2006).

Vitamin E is present within the seminal plasma and plasma membrane (Agarwal et al., 2003). It is a lipid soluble, chain-breaking antioxidant, able to terminate a free radical chain reaction. Specifically, it inhibits peroxidation of polyunsaturated fatty acids (PUFA), which is especially important in spermatozoa due to their high PUFA content (Bolle et al., 2002). A broad array of studies support the role for α-TOC in reversing the pathogenesis of male infertility. A positive correlation was found between α-TOC extracted from spermatozoa membranes and the percentage of motile, living and morphologically normal sperm cells (BOLLE et al., 2002). Furthermore, α-TOC levels were found to be decreased significantly among oligo- and azoospermic subjects as compared to normospermic controls (Bhardway et al., 2004).

A significant improvement in the *in vitro* ability of spermatozoa to bind the *zona pellucida* of unfertilized oocytes was found in men with high ROS production supplemented with VIT E for 3 months (Kessopoulou et al., 1995). *In vivo* toxicology studies have shown that VIT E has proven to be an efficient in alleviating testicular and epididymal damage as well as disruption of spermatogenesis caused by environmental pollutants or chemotherapeutics (Oda and El-Maddawy, 2012). Moreover, *in vitro* experiments have revealed beneficial and protective effects of the molecule on the cryosurvival for human and animal spermatozoa (Amini-Pour et al., 2013), as well as protection of the male germ cells in environmental with increased ROS-production (O'Flaherty et al., 2006; Bansal and Bilaspury, 2009).

In order to define an optimal concentration of α-TOC for future experiments, this *in vitro* study was aimed to find out the efficacy of different α-TOC concentrations on bovine spermatozoa motility, viability and superoxide radical formation during a 24 hour *in vitro* cultivation.

MATERIAL AND METHODS

Bovine semen samples were obtained from 20 adult breeding bulls (Slovak Biological Services, Nitra, Slovak Republic). The samples had to accomplish the basic criteria given for the corresponding breed. The samples were obtained on a regular collection schedule using an artificial vagina. After collecting the samples were stored in the laboratory at room temperature (22–25°C).

Each sample was diluted in physiological saline solution (PS; sodium chloride 0.9 % w/v; Bieffe Medital, Italia) containing 0.5% ethanol (96% ethyl alcohol, EtOH; Merck Chemicals, Darmstadt, Germany), with various concentrations of α-tocopherol (α-TOC; Sigma-Aldrich, St. Louis, USA; A – 2000; B – 1000; C – 500; D – 200; E – 100; F – 50; G – 10; H – 5; I - 1 μM/L) using a dilution ratio of 1:40. The samples were cultured at room temperature (22–25°C). We compared the control (Ctrl) group (medium without CUR supplementation) with the experimental groups.

Motility and progressive motility analysis was carried out using the CASA (Computer Assisted Semen Analyzer) system equipped with the SpermVision™ program (MiniTub, Tiefenbach, Germany) and the Olympus BX 51 microscope (Olympus, Japan) at cultivation Times 0 h, 2h, 6h (models suitable for a short-term *in vitro* culture) as well as 12 h and 24 h (models suitable for a long-term *in vitro* culture). Each sample was placed into the Makler Counting Chamber (depth 10 μm, Sefi-Medical Instruments, Israel) and the percentage of motile (motility > 5 μm/s; MOT) and progressively motile spermatozoa (motility > 20 μm/s; PROG) was evaluated. 1000–1500 cells were assessed in each analysis (Massányi et al., 2008). Viability of the cells exposed to α-TOC *in vitro* was evaluated by the metabolic activity (MTT) assay (Mosmann, 1983; Knazicka et al., 2012). This colorimetric assay measures the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, USA) to purple formazan particles by mitochondrial succinate dehydrogenase of intact mitochondria of living cells.

Formazan can then be measured spectrophotometrically at a measuring wavelength of 570 nm against 620 nm as reference by a microplate ELISA reader (Multiskan FC, ThermoFisher Scientific, Finland). The data are expressed in percentage of control (i.e. optical density of formazan from cells not exposed to α -TOC). Results from the analysis were collected during five repeated experiments at each concentration.

The nitroblue-tetrazolium (NBT) test was used to assess the intracellular formation of superoxide radical (Esfandiari et al., 2003). This assay is conducted by counting the cells containing blue NBT formazan deposits, which are formed by reduction of the membrane permeable, water-soluble, yellow-colored, nitroblue tetrazolium chloride (2,2'-bis(4-Nitrophenyl)-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; Sigma-Aldrich, St. Louis, USA) and superoxide radical. Formazan can be measured spectrophotometrically at a measuring wavelength of 620 nm against 570 nm as reference by a microplate ELISA reader (Multiskan FC, ThermoFisher Scientific, Finland). The data were expressed in percentage of control (i.e. optical density of formazan from cells not exposed to α -TOC). Results from the analysis were collected during five repeated experiments at each concentration (Tvrđá et al., 2013).

Statistical analysis was carried out using the GraphPad Prism program (version 3.02 for Windows; GraphPad Software, La Jolla California USA, www.graphpad.com). Descriptive statistical characteristics (mean, standard error) were evaluated at first. One-way ANOVA with Dunnett's post test was used for statistical evaluations. The level of significance was set at ^A($P<0.001$); ^B($P<0.01$); ^C($P<0.05$).

RESULTS AND DISCUSSION

The CASA assessment of the motion parameters showed a gradual decrease of spermatozoa motility and progressive motility in all groups over the course of a 24h *in vitro* culture (Table 1, Table 2). The initial (Time 0h) MOT was higher in all experimental groups when compared to the control group (0 μ M/L α -TOC), although without any statistical significance ($P>0.05$). Although statistically insignificant, a motion-promoting effect of α -TOC remained visible after 2h, specifically in experimental groups C (500 μ M/L α -TOC), D (200 μ M/L α -TOC), E (100 μ M/L α -TOC), F (50 μ M/L α -TOC) and G (10 μ M/L α -TOC). At the same time, 2000 μ M/L α -TOC (group A), 1000 μ M/L α -TOC (group B), 5 μ M/L α -TOC (group H) and 1 μ M/L α -TOC (group I) caused a non-significant decrease of the spermatozoa motility parameters. After 6h, the decline of spermatozoa motion characteristics was significantly ceased in the group C ($P<0.001$) alongside with an insignificant decline ($P>0.05$) in groups A, E and F in comparison with the control. Examination at 12h of *in vitro* culture showed that the spermatozoa motility and progressive motility were significantly increased in the group C ($P<0.001$), together with an insignificant ($P>0.05$) increase in groups B, D, E and F when compared to the control. At the end of the experiments (24h), the highest motility parameters were observed in all experimental groups, being significantly higher in groups C and D in comparison with the control ($P<0.05$) (Table 1, Table 2).

Table 1. Spermatozoa motility (%) in the absence (Ctrl) or presence (A-I) of α -tocopherol during different time periods (Mean \pm SEM; n=20)

	Ctrl	A	B	C	D	E	F	G	H	I
0h	90.00 \pm 1.27	86.42 \pm 1.15	90.94 \pm 1.52	93.00 \pm 1.30	92.96 \pm 1.27	88.35 \pm 0.88	89.73 \pm 1.35	88.00 \pm 1.10	85.58 \pm 1.14	88.33 \pm 0.96
2h	89.88 \pm 2.25	85.44 \pm 1.71	87.68 \pm 1.98	90.61 \pm 1.69	87.58 \pm 1.49	87.41 \pm 1.66	87.91 \pm 1.03	89.96 \pm 1.31	83.66 \pm 1.74	83.23 \pm 1.10
6h	80.61 \pm 2.14	78.85 \pm 3.53	78.71 \pm 1.35	88.52 \pm 1.57 ^C	83.75 \pm 1.57	81.02 \pm 1.68	80.96 \pm 1.12	80.59 \pm 1.98	78.19 \pm 1.79	76.49 \pm 1.54
12h	73.78 \pm 2.05	71.47 \pm 1.87	75.00 \pm 1.47	83.82 \pm 1.01 ^A	76.78 \pm 3.02	74.93 \pm 2.04	74.17 \pm 2.44	73.30 \pm 1.87	73.64 \pm 1.64	69.48 \pm 2.90
18h	62.18 \pm 1.98	60.88 \pm 3.14	68.43 \pm 1.90	74.29 \pm 1.53 ^A	69.59 \pm 1.82	67.10 \pm 1.85	65.13 \pm 2.01	64.61 \pm 3.51	63.95 \pm 3.13	60.68 \pm 1.35

24h	53.75±	55.41±	59.62±	62.75±	62.64±	59.10±	59.49±	57.20±	56.33±	54.42±
	1.84	2.67	3.61	2.74 ^A	3.28 ^A	3.02	3.27	3.06	2.61	2.74

^A P<0.001, ^B P<0.01, ^C P<0.05

Table 2. Spermatozoa progressive motility (%) in the absence (Ctrl) or presence (A-I) of α -tocopherol during different time periods (Mean±SEM; n=20)

	Ctrl	A	B	C	D	E	F	G	H	I
0h	82.51± 1.38	80.79± 1.38	79.08± 1.63	87.87± 1.25	87.96± 1.38	86.50± 1.44	83.74± 2.22	85.62± 1.49	84.79± 1.30	81.19± 1.61
2h	80.41± 2.21	79.83± 1.74	77.00± 2.70	85.97± 1.72	86.48± 1.62	82.50± 1.58	81.98± 1.13	81.70± 1.41	80.16± 2.10	79.62± 1.21
6h	74.67± 2.13	69.06± 3.45	76.70± 1.39	83.37± 1.62 ^C	80.00± 1.65	77.11± 1.64	75.01± 1.09	72.10± 1.92	69.81± 1.69	67.24± 1.52 ^C
12h	63.98± 1.95	61.84± 1.79	65.06± 1.21	75.94± 1.12 ^A	65.94± 2.82	67.03± 2.08	65.71± 2.39	64.02± 1.72	65.99± 1.68	60.14± 3.06
18h	59.48± 2.07	59.07± 3.01	65.05± 2.02	69.51± 1.43 ^A	66.10± 1.90	63.60± 1.92	62.64± 2.14	58.95± 3.22	55.18± 1.57	57.09± 3.07
24h	50.73± 3.11	46.79± 2.69	51.78± 3.44	54.10± 2.80	52.01± 3.40	51.14± 2.97	50.14± 3.27	45.73± 1.70	39.86± 2.78	44.98± 2.95

^A P<0.001, ^B P<0.01, ^C P<0.05

According to the MTT assay, the immediate α -TOC administration (Time 0h) led to a significant improvement of the sperm cell viability in groups B, C, D and E (P<0.001 in case of C, P<0.05 in case of B, D and E). At 2h it was revealed that all concentrations of α -TOC had a stimulating and vitality-promoting effect on the bovine spermatozoon, alongside with statistically significant results (P<0.001; Figure 1) when compared to the control group (0 μ M/L α -TOC). These beneficial effects remained visible and statistically relevant throughout Time 6h and Time 12h, as shown in Figure 1 (P<0.001 in all experimental groups vs. control). After 18h of *in vitro* culture, the cell viability remained significantly improved in the experimental groups B-D (P<0.05), however the beneficial impact of α -TOC extended through a broader array of concentrations with significant effects at the end of the culture (Time 24h; P<0.05 in case of group A; P<0.01 in case of groups E-H; P<0.001 in case of groups B-D; Figure 1).

The NBT test revealed that concentrations between 1000 and 50 μ M of α -TOC had an instant and significant (P<0.001 in case of groups B-E; P<0.05 in case of group F) protective effect against superoxide production in the sperm cells, when compared to the control (Figure 2). Positive effects of α -TOC remained persistent and significant (P<0.001) over the course 2h, 6h and 12h, and was subsequently joined by the experimental groups G (Time 2h; P<0.01), H (Time 6h; P<0.01) and I (Time 12h; P<0.05). NBT examinations of both representative timeframes (Time 18h and 24h) of a long-term *in vitro* spermatozoa culture showed that all α -TOC concentrations led to a significant improvement in the oxidative balance of the bovine germ cells in comparison with the control (P<0.05 in case of groups A and I; P<0.01 in case of groups B and H; P<0.001 in case of groups C, D, E, F and G; Figure 2).

In the present study, ROS-mediated damage to sperm cell with a subsequent loss of spermatozoa fertilizing potential could be reduced using various doses of α -tocopherol. ROS are generally produced under elevated oxygen tension, which may be induced when spermatozoa are transferred to and manipulated under *in vitro* conditions. Supplementation of antioxidants to semen can protect against the damaging effects of ROS on sperm motility and viability, and therefore may be of clinical value in cryopreservation and assisted reproduction protocols (Baker et al., 1996).

Our results indicate that α -TOC improves the percentage of motile and viable spermatozoa under *in vitro* conditions. It is known that normal *ex vivo* oxidative stress to spermatozoa causes a significant damage to their structural components related to the cell movement, resulting in a reduction in their motility. Nevertheless, supplementing the culture medium with different doses of α -TOC preserved the sperm motility throughout the *in vitro* incubation. Thus, we may suggest that α -TOC is effective in preventing the rapid loss of motility regularly

developing during spermatozoa incubation, thus maintains the motion characteristics under oxidative stress conditions.

This study shows that different doses of α -TOC improve the viability of bovine sperm. These results may be based on the fact that α -TOC protects the spermatozoa by preventing mitochondrial oxidative DNA and membrane damage, thereby helping the spermatozoon to overcome oxidative attacks. Thus, by maintaining the cell integrity and optimal function and activity of spermatozoa, α -TOC improves the per cent of sperm viability. Moreover, supplementing culture media with with α -TOC of variants of vitamin E improved the sperm viability in humans (Askari et al., 1994) and in rabbits (Yousef et al., 2003).

All concentrations of α -TOC significantly reduced the intracellular production of superoxide radical. It may be explained by the fact that α -TOC reduces the oxidative damage and lipid peroxidation in sperm cell membranes by disrupting oxidative chain reactions. Thus, α -TOC increases the sperm membrane integrity and promotes the protection of the spermatozoa against the formation of the superoxide radical, which is considered to be the most reactive ROS initiating further oxidative processes. Similar observations have been reported in humans (Verma and Kanwar, 1999) and boars (Slebozinska et al., 1995).

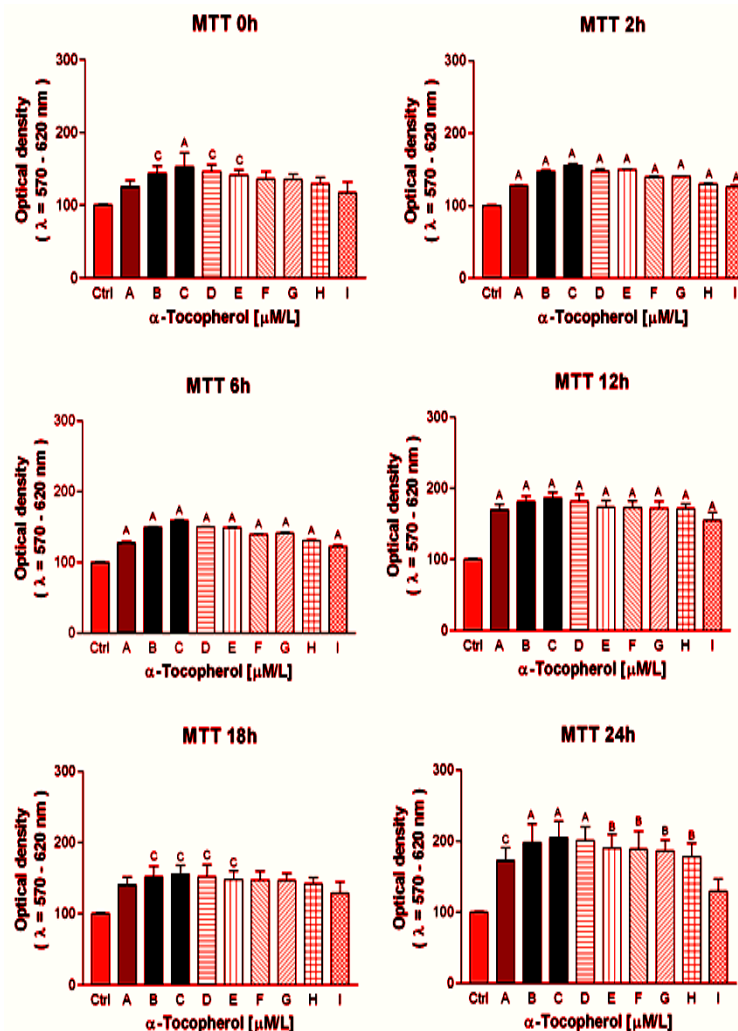


Figure 1. The effect of various doses of α -tocopherol on the viability of bovine spermatozoa at 0h, 2h, 6h, 12h and 24h. Each bar represents mean (\pm SEM) optical density as the percentage of controls ($n=20$), which symbolize 100%. The data were obtained from five independent experiments. The level of significance was set at ^A $P<0.001$; ^B $P<0.01$; ^C $P<0.05$. Ctrl – 0; A – 2000; B – 1000; C – 500; D – 200; E – 100; F – 50; G – 10; H – 5; I – 1 $\mu\text{M/L}$ α -TOC.

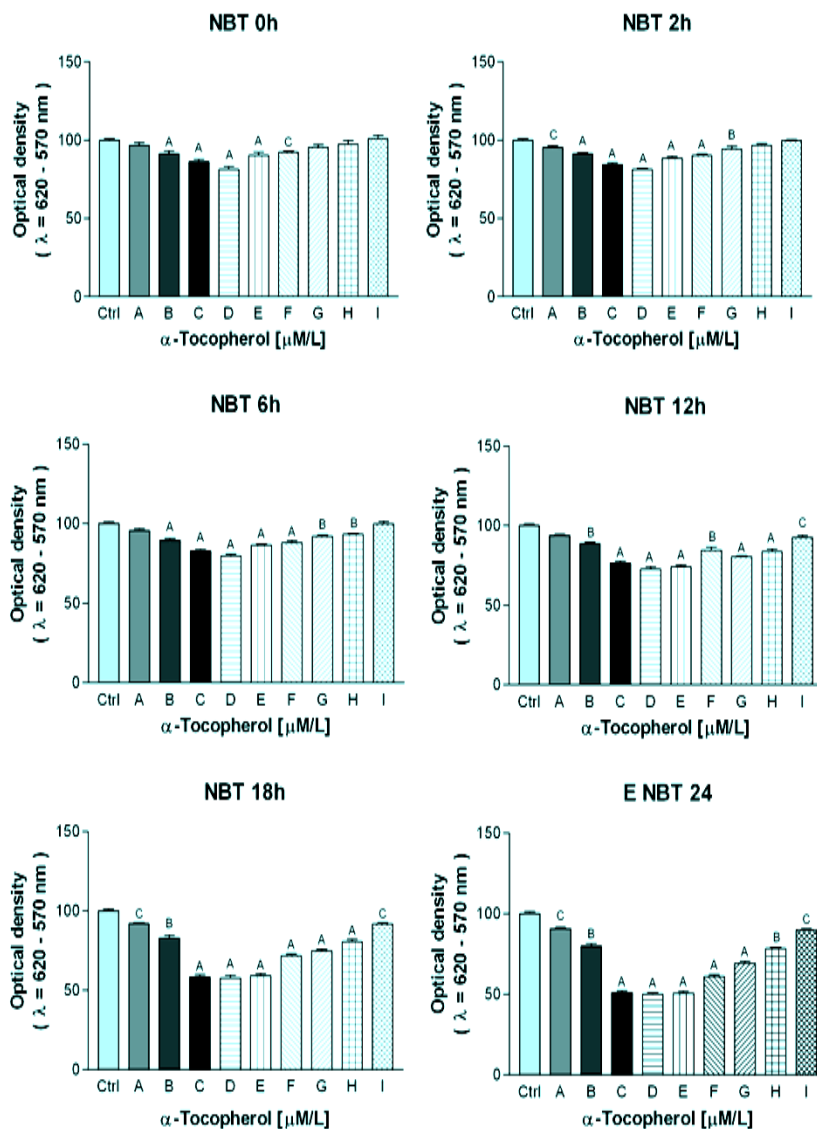


Figure 2. The effect of various doses of α -tocopherol on the spermatozoa superoxide production at 0h, 2h, 6h, 12h and 24h. Each bar represents mean (\pm SEM) optical density as the percentage of controls ($n=20$), which symbolize 100%. The data were obtained from five independent experiments. The level of significance was set at ^A $P < 0.001$; ^B $P < 0.01$; ^C $P < 0.05$. Ctrl – 0; A – 2000; B – 1000; C – 500; D – 200; E – 100; F – 50; G – 10; H – 5; I – 1 $\mu\text{M/L}$ α -TOC.

The present study shows the effectiveness of α -TOC in protecting spermatozoa motility and viability by decreasing the intracellular ROS production. Comparable events have been observed in experiments with human (Aitken et al., 1989; Agarwal et al., 2007), boar (Slebodzinska et al., 1995) and rabbit (Yousef et al., 2003) spermatozoa.

α -tocopherol has been reported to inhibit the free radical-induced damage to sensitive spermatozoa cell membranes as it is a major chain-breaking antioxidant (Sinclair, 2000). The present results suggest that α -TOC may directly quench various free radicals generated during the *in vitro* generated OS. Thus, by scavenging these radicals, it breaks the chain reaction and forms a relatively stable complex such as the tocopheroxyl radical. Similar suggestions have been made by Verma and Kanwar (1999) in a human model.

CONCLUSION

It may be concluded that all doses of α -tocopherol increased the percentage of motile and viable spermatozoa but decreased and prevented the intracellular overproduction of free radicals within the sperm mitochondrial membrane, although the most effective concentrations of α -tocopherol seem to vary between 500 and 100 μ M/L α -tocopherol seems to protect bovine spermatozoa against the damages caused by reactive oxygen species. Supplementing the semen samples with α -tocopherol could therefore be of scientific importance for extending the time of spermatozoa storage before further designated andrology experiments and clinical procedures, such as artificial insemination, *in vitro* fertilization techniques or spermatozoa cryopreservation.

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OPTIMIZACIJA SPECIFIČNE KONCENTRACIJE α -TOCOPHEROL ZA *IN VITRO* KULTIVACIJU SPERMATOZOIDA BIKA

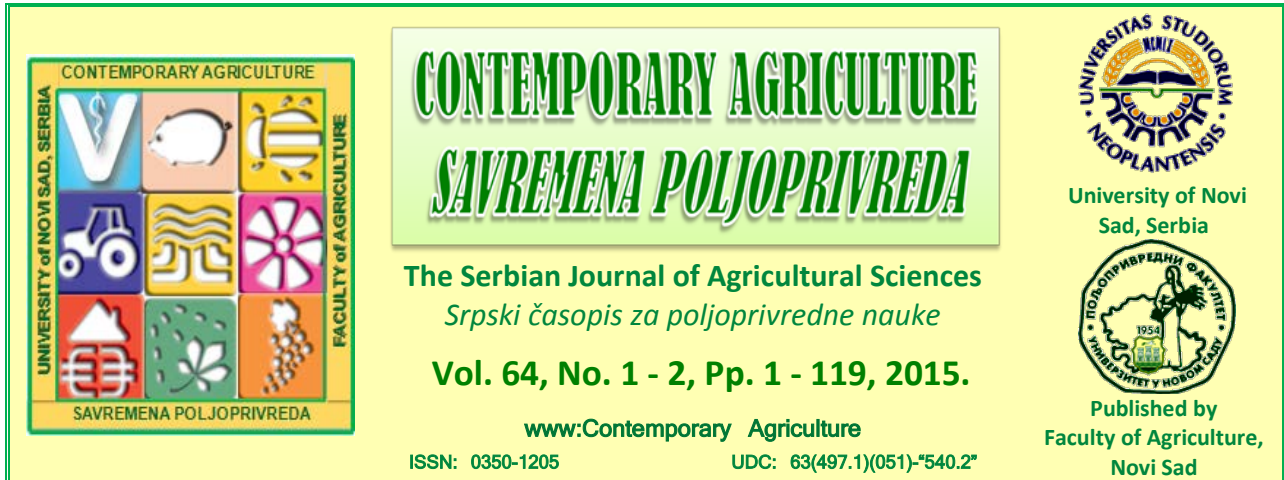
Eva TVRĐÁ, Jana LUKÁČOVÁ, Tomáš JAMBOR, Peter MASSÁNYI, Norbert LUKÁČ

Izvod: Istraživanje je izvedeno da se ustanove doze i potrebno vreme za *in vitro* efekt α -tocopherol (α -TOC) na spermatozoide bika, tokom različitih vremenskih perioda (0h, 2h, 6h, 12h, 18h, 24h). Dobijeni rezultati pokazuju da dodavanje α -tocopherola, posebno u koncentracijama od 500 do 50 μ M u medijum za kultivaciju, može biti stimulatивно za aktivnost spermatozoida i njihovu zaštitu protiv mogućih *in vitro* oksidativnih stresnih faktora.

Ključne reči: *vitamin E, spermatozoidi, pokretljivost, produkcija superoxide, bik.*

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EFFECT OF SEASON ON BOAR SEMEN QUALITY*

Jelena APIĆ[†], Slobodanka VAKANJAC, Ivan RADOVIĆ, Stoja JOTANOVIĆ,
Branislav STANKOVIĆ, Zdenko KANAČKI¹

Summary: It was demonstrated that boar sperm production and characteristics significantly decrease in the warmer period of the year. As a result, the boars reproductive exploitation efficiency decrease in the warmer seasons. Therefore, the aim of the present paper was to investigate the influence of cold and warm seasons on the boars semen quality, in the our intensive pig production conditions. The obtained results clearly show that the main semen quality parameters were significantly ($p < 0.05$ or $p < 0.01$) higher in the cold, compared with the warm season (ejaculate volume=274ml, sperm concentration=229×10⁶/ml, total sperm number=60×10⁹, progressive motility=79%, and good ejaculates=96%, vs. 218ml, 208×10⁶/ml, 45×10⁹, 69% and 78%, resp.). In conclusion, keeping boars cool during summer and frequent observation of boars to determine if they are being heat-stressed, can significantly reduce the negative influence of elevated ambient temperature on sperm production and, consequently, improve boars reproductive exploitation efficiency.

Key words: season, semen, quality, boar.

INTRODUCTION

Consistently high-quality ejaculates producing are crucial for successful reproductive exploitation of AI boars in contemporary pig production industry (Grafenau et al., 2003; Stančić and Dragin, 2011). Semen characteristics of boars is influenced by many factors, such as breed, age, nutrition, environmental effects, health status and frequency of ejaculate collection (Cheon et al., 2002; Jankevičiute and Žilinskas, 2002; Stančić et al., 2003; Wilson et al., 2004).

Season is the most important environmental factor that influence the great variations in boar semen characteristics (Ciereszko et al., 2000; Cheon et al., 2002; Stančić et al., 2003) and sows fertility (Rozeboom et al., 2000; Almond and Bilkei, 2005; Stančić et al., 2011). The decreasing fertility in both male and female pig during

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summer is known as “summer or seasonal infertility syndrome” (Okere, 2003; Quesnel et al., 2005). In boars, seasonal changes were influenced by ambient temperature and photoperiod (Corcuera et al., 2002; Sancho et al., 2004; Stančić et al., 2011). High temperature cause germ-cell destruction, which result in a temporary decrease in sperm production and fertility. On the other hand, photoperiod alter androgen synthesis or secretion, by increasing the sensitivity of the boars testes in the longer photoperiod to endogenous LH (It was demonstrated that serum testosterone response to GnRH-mediated LH release was greater in boars exposed to 16 hours of light per day (Kunavongkrit et al., 2005). Therefore, better knowledge of factors influencing seasonal variation on semen quality, may help to improve the efficiency of AI boars reproductive exploitation. So, the aim of this study was to investigate the effect of season on boar semen characteristics variations, in the Serbian intensive pig production conditions.

MATERIAL AND METHODS

The study was conducted on the one industrial pig farm in AP Vojvodina (Serbia), with a capacity of about 1,000 sows. The study included 10 Swedish Landrace boars, aged 12 to 18 month. From each boar was taken 14 ejaculate in a warm and 13 ejaculates in a cold season. The period from May to October was taken as warm season, and period from November to April was taken as cold season.

Using standard laboratory methods, basic parameters of semen quality (volume, sperm concentration, total number of sperm per ejaculate and progressive motility). The number of good ejaculates (volume ≥ 120 ml; sperm concentration $\geq 200 \times 10^6$ /ml of ejaculate; progressive motility $\geq 65\%$) was also determined.

RESULTS

Ejaculates quality parameters of tested boars, in warm and cold season of the year, are shown in Table 1. Ejaculate volume (274ml), sperm concentration (229×10^6 /ml), total sperm number per ejaculate (60×10^9) and the number of good ejaculates (96%) were statistically significantly ($P < 0.01$) higher in cold compared to the warm season of the year (218ml, 208×10^6 /ml, 45×10^9 and 78%, resp.). The average progressive motility was significantly ($P < 0.05$) higher in cold (79%) compared with the warm season (69%).

Table 1. Parameters of boar semen quality within cold and warm season ($\bar{x} \pm SD$)

Parameter	Season of the year		Total
	Cold ¹	Warm ²	
Boars (n)	10	10	20
Ejaculates (n)	140	130	270
Ejaculate per boar (n)	14	13	13.5
Ejaculate volume (ml)	274±88.93 ^A (85-650)	218±70.89 ^B (95-370)	247 (85-650)
Sperm concentration ($\times 10^6$ /ml)	229±69.81 ^A (103-483)	208±39.27 ^B (112-320)	219 (103-483)
Total sperm number per ejaculate ($\times 10^9$)	60±23.76 ^A (15-135)	45±17.18 ^B (17-90)	53 (15-135)
Progressive motility (%)	79±12.02 ^a (40-95)	69±11.11 ^b (20-90)	74 (20-95)
Good ejaculates ³ (%)	96±25.03 ^A (134/140)	78±20.88 ^B (102/130)	87 (236/270)

¹November to April; ²May to October. ³Volume ≥ 120 ml; Sperm concentration $\geq 200 \times 10^6$ /ml of ejaculate; Prog. motility $\geq 65\%$. Values with different superscripts, within the same row, differ (^{A,B} $P < 0.01$; ^{a,b} $P < 0.05$). In parenthesis: (min. - max.), or (good ejaculate number/totale ejaculate number).

Varyng the ejaculate quality parameters by months of year, are shown in Figure 1. The greatest variation was observed in the ejaculate volume, which is the most higher during the period January to April (316ml to 255ml), the lowest in the period May to October (228ml to 201ml), to be re-started growth in the period November to December (256ml). And the other parameters (sperm concentration, progressive motility and number of good ejaculates) show the same trend of variation by month, but with much lower differences between the values.

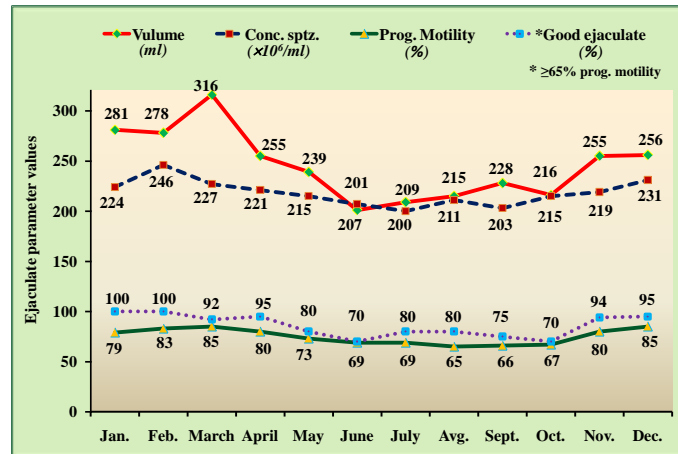


Figure 1. Ejaculate parameters variation by months of year

DISCUSSION

Significant reduction of pigs fertility is evident during the warm summer months. Summer fertility decrease is affected by the interaction of high ambient temperature and extended daily photoperiod in the warm period of the year. The most important aspect of these seasonal fertility decrease is a significant reduction of sperm production and semen characteristics in boars (Ciereszko i sar., 2000; Stančić et al., 2002; Jankevičiute and Žilinskas, 2002; Stančić et al., 2003a; Stančić et al., 2003; Chukwuemeka et al., 2005; Kunavongkrit et al., 2005; Stančić et al., 2006; Lapuste et al., 2011). Long-lasting heat stress (more than 2 weeks with ambient temperature above 30°C) decrease percentage of motile spermatozoa. Percentage of motile spermatozoa did not return to normal values until 5 weeks after the end of exposure to increased ambient temperatures (Stančić, 2006). As a result of heat treatment, normal motile sperm production decreased from control levels (1.28×10^{10} /day) to 0.15×10^{10} /day, 3 weeks after heating ceased (Stone, 1982). Percentage of morphological abnormal spermatozoa in ejaculate significantly increase in the warm season (19%) compared with cold season (25%) (Lipensky et al., 2010). The spermatozoa motility was the lowest in summer (Kozdrowski and Dubiel, 2004; Macchi i sar., 2010). The seasonal variation in sperm production and characteristics is also mediated by the changes in daily photoperiod duration (Stančić, 2006). Namely, photoperiod alter androgen synthesis or secretion, by increasing the sensitivity of the testes of boars in the longer photoperiod to endogenous LH (Kunavongkrit et al., 2005). Season had a significant effect on the intensity of spermatogenesis and qualitative sperm parameters. During the summer–autumn period, the incidence of pathological spermatozoa increased, but sperm motility and viability decreased (Šerniene et al., 2002).

The number of insemination doses per ejaculate significantly decrease in the warmer part of the year, as a result of lower semen characteristic values. Further more, the ability of semen for *in vitro* preservation is influenced by seasonal variation of native ejaculate quality (Johnson et al., 2000; Stančić et al., 2002; Stančić et al., 2003; Stančić et al., 2003; Wolf and Smital, 2009; Stančić et al., 2012). Consequently, seasonal variation in semen quality have great influence on the efficiency of AI boar exploitation (Glossop, 2000; Singleton, 2001; Stančić et al., 2009; Stančić et al., 2011).

The previous studies on the industrial pig farm in AP Vojvodina (Serbia) (Stančić et al., 2002; Stančić et al., 2003a; Stančić et al., 2003; Stančić et al., 2006) was clear demonstrated the significant seasonal variations of ejaculate volume, total sperm number per ejaculate, sperm concentration in ejaculate and sperm progressive motility. In these studies, average ejaculate volume, sperm concentration and total number of spermatozoa in ejaculate were significantly higher and percentage of sperm progressive motility were lower in the period December to May (293 to 283 ml, 319 to 284×10^6 sperm/ml, 93 to 81×10^9 sperm/ejaculate and 85% prog. motility, resp.), compared with the period June to November (213 to 232 ml, 220 to 210×10^6 sperm/ml, 47 to 49×10^9 sperm/ejaculate and 75 to 70% prog. motility, resp.)

Practical implications. Boars exposure to high environmental temperatures reduces their fertility. Boars subjected to heat stress conditions produce ejaculates that have low sperm concentrations, high percentages of abnormal sperm cells (damaged acrosomes, proximal cytoplasmic droplets, etc.) and decreased percentages of progressively motile spermatozoa. Research has indicated that the minimum exposure time and critical air temperature above which production of sperm cells is adversely affected is 29°C and 72 hours, respectively. Keeping

boars cool during summer and frequent observation of boars to determine if they are being heat-stressed, can significantly reduce the negative influence of elevated ambient temperature on sperm production. Producers can get an indication that boars are heat stressed by checking rectal temperatures and/or respiration rates. Normal values for mature boars are 38,4°C and 13 to 18, respiration rate. On this way it is possible to significantly increase the boars reproductive exploitation.

CONCLUSION

In conclusion, seasonal variation in sperm production and characteristics is mediate by changes in ambient temperature and daily photoperiod duration. Bath, while temperature direct affect the spermatogenetic process in testis, photoperiod have indirect influence on the testicular function, by changing the sensitivity of testosterone production to endogenous LH activity.

The results presented in this paper, clearly confirm the strong influence of season on the ejaculate quality parameters. Namely, the values of all parameters (volume, sperm concentration, total number of sperm in the ejaculate, progressive motility, as well as a number of good ejaculate) were significantly higher in coold, compared to the warm season of the year.

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UTICAJ SEZONE NA KVALITET SPERME NERASTA

Jelena APIĆ, Slobodanka VAKANJAC, Ivan RADOVIĆ, Stoja JOTANOVIĆ,
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Izvod: Ustanovljeno je da su produkcija i osobine sperme nerasta značajno niže tokom toplijeg perioda godine. Posledično, u ovom periodu godine značajno se smanjuje efikasnost reproduktivnog iskorištavanja nerastova. Zbog toga je cilj ovog rada bio da se ustanovi uticaj hladne i tople godišnje sezone na parametre kvaliteta sperme u našim uslovima intenzivne proizvodnje svinja. Dobijeni rezultati jasno pokazuju da su osnovni parametri kvaliteta ejakulata značajno veći ($p < 0.05$ or $p < 0.01$) u hladnoj, nego u toploj godišnjoj sezoni (volumen ejakulata=274ml, koncentracija spermatozoida= 229×10^6 /ml, ukupan broj spermatozoida= 60×10^9 , progresivna pokretljivost=79% i dobrih ejakulata=96%, prema 218ml, 208×10^6 /ml, 45×10^9 , 69% i 78%). Zaključak je da rashlađivanje nerastova tokom leta i češća observacija prisustva toplotnog stresa, može značajno smanjiti negativan uticaj povišene ambijentalne temperature na produkciju sperme i, posledično, povećati efikasnost reproduktivnog iskorištavanja nerastova.

Key words: sezona, sperma, kvalitet, nerast.

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WEED FLORA IN BASIL (*OCIMUM BASILICUM* L., LAMIACEAE MARTYNOV 1820, LAMIALES) GROWN IN CONVENTIONAL AND ORGANIC PRODUCTION*

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Summary: This study was aimed to compare weeds occurring in basil crops grown under conventional and organic production systems. Weed flora recorded in the conventional production consisted of 16 taxa, with *Setaria glauca* and *Portulaca oleracea* dominating. Concerning the organic plots, only seven taxa were noted and dominant species were *Sorghum halepense* and *Amaranthus retroflexus*. Unexpectedly lower floristic diversity in the organic agricultural system was caused by omitted application of fertilizers during three consecutive years, still unbalanced agro ecological conditions, partial isolation of the organic plots and the presence of even four invasive weed species.

Key words: *Ocimum basilicum*, organic and conventional production, weeds, ecological analysis.

INTRODUCTION

Agro ecological conditions in Serbia can be generally characterized as favorable, and the agricultural producers are experienced and skilled for collection and cultivation of medicinal, aromatic and spice plants; however, those opportunities are often underused. The branch was developing until the end of eighties, regarding both occupied areas and technology of growing. Despite a rich bio fond of these plants, the production is far below those recorded 25-30 years ago. Serbian market demands for medicinal plants are today mostly satisfied by the raw material collected from natural habitats (90%). According to Serbian Chamber of Commerce and Industry, cultivated medicinal, aromatic and spice plants occupied 1,419 ha and 1,337 ha in 2011 and 2012, respectively. Together with spices characterized as vegetables and areas attended for foreign customers, these plants are grown on approximately 20,000 ha (Filipović and Ugrrenović, 2014).

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Basil (*Ocimum basilicum* L.) is a medicinal and spice plant belonging to family Lamiaceae and originating from India. It is cultivated for centuries and widespread in many regions. Anti-inflammatory and antioxidant effects of the plant are confirmed by numerous studies, while hypoglycemic effect and possible application in treatment of diabetes remains to be confirmed in further research. Nevertheless, basil is widely used as an antiseptic, preservative, mild sedative, diuretic, against dyspepsia, diarrhea, headache and cough. Basil oil is useful in relieving mental fatigue, colds, cramps, rhinitis, as well as a first aid after wasp sting and snake bite (Özcan and Chalchat, 2002). Therefore, the plant is used as raw material in medical, dental and food industry, pharmacy and cosmetology (Budka and Khan, 2010). The biological activity is due to active compounds, mainly flavonoids and polyphenols (El-Beshbishy and Bahashwan, 2012). The main compounds responsible for typical basil aroma are chavicol methyl ether (estragol), linalool, eugenol, 1,8-cineole and methyl cinnamate. The non-volatile compounds were found to be rich in phenolic acids with the major part of caffeic and rosmarinic acid (Modnicki and Balcerek, 2009).

Basil is an annual plant; the crop establishment is done in the spring and seedlings are produced in warm nurseries (Radanović and Nastovski, 2002). Weed control is the main problem in crop protection. Because herbicides are not allowed in Serbian herb production, mechanical weeding remains as the only solution in both conventional and organic practices. Weeds occurring between rows are controlled with special tools, while those growing inside rows have to be removed by hoeing. Thermal weed control is also allowed in organic herb production; however, special tools such as infrared and burner type flame weeders are required and that equipment is difficult to obtain in our country (Radanović and Nastovski, 2002).

This study was aimed to investigate the weed flora in the basil crops, including its ecology and phytogeographical analysis, analysis of weed flowering time, and categorization according to habitat, as a necessary prerequisite for the selection of weed control measures adequate for conventional and organic production.

MATERIAL AND METHODS

The floristic-ecological study of weed flora in organic and conventional basil production systems was carried out during basil growing season of 2014, at the experimental fields of the Institute of Field and Vegetable Crops, Novi Sad, Alternative Crops Department, Bački Petrovac. Sowing was performed on April 28th. Preceding crops were onion (*Allium cepa* L.) for organic and maize (*Zea mays* L.) for conventional plots. Organic field was fertilized with farmyard manure in 2011, and mineral fertilizer was applied in the conventional field in 2013 (15:15:15 NPK, rate 400 kg/ha).

Weed species were identified according to Josifović (1970-1977). Table 1 provides an overview of the identified taxa, their life forms (Ujvárosi, 1973), flowering month and categorization by habitat (Čanak et al., 1978), floristic elements (Gajić, 1980) and ecological indices (Landolt, 1977).

RESULTS AND DISCUSSION

Weed flora in basil consisted of 18 taxa, counting both growing systems (Table 1). Sixteen taxa were recorded in conventional (K), and seven in organic (O) production. Five plant species were found in both growing systems: *Amaranthus retroflexus*, *Bilderdykia convolvulus*, *Chenopodium album*, *Datura stramonium* and *Senecio vulgaris*. Differential plant species found in conventional system were: *Ambrosia artemisiifolia*, *Anagallis arvensis*, *Cirsium arvense*, *Convolvulus arvensis*, *Hibiscum trionum*, *Portulaca oleracea*, *Setaria glauca*, *Sonchus arvensis*, *Stachys annua*, *Veronica hederifolia* and *Veronica persica*, with the highest average number of individuals per square meter (ind/m²) counted for *Setaria glauca* (28.00) and *Portulaca oleracea* (8.25). *Polygonum lapathifolium* and *Sorghum halepense* were differential in plots maintained according to organic principles. The highest weed infestation was noted for *Sorghum halepense* and *Amaranthus retroflexus*, with 10.00 and 9.00 ind/m², respectively.

Table 1. Weed flora in basil in conventional(K) and organic (O) production (with life form, time of flowering, characterization according to the site, floral elements and ecological indices)

Plant species	K	O	Life form	Time of flowering	Category acc. to site	Floral element	Ecological index									
	ind/m ²	ind/m ²					F	R	N	H	D	S	L	T	K	
<i>Amaranthus retroflexus</i> L.	3.25	9.00	T ₄	VI-IX	KR	Adv	2	3	4	3	3	-	4	4	3	
<i>Ambrosia artemisiifolia</i> L.	0.25	-	T ₄	VIII-IX	R	Adv	2	3	4	2	2	+	4	5	3	
<i>Anagallis arvensis</i> L.	0.25	-	T ₄	V-X	KR	Kosm	3	3	3	3	4	-	4	4	3	
<i>Bilderdykia convolvulus</i> (L)D	0.50	2.00	T ₄	VI-IX	S	Subevr	2	3	3	3	4	-	4	4	3	
<i>Chenopodium album</i> L.	0.25	1.00	T ₄	VI-IX	KR	Kosm	2	3	4	3	4	-	4	3	3	

<i>Cirsium arvense</i> (L.) Scop.	4.00	-	G ₃	VI-VIII	KR	Subevr	3	3	4	3	4	+	3	4	3
<i>Convolvulus arvensis</i> L.	0.25	-	G ₃	VI-IX	KR	Kosm	2	4	3	3	4	-	4	4	3
<i>Datura stramonium</i> L.	2.50	2.00	T ₄	VI-IX	R	Kosm	3	3	4	4	4	+	4	5	2
<i>Hibiscus trionum</i> L.	0.25	-	T ₄	VI-VIII	KR	Pont-e.subm	3	3	3	3	4	-	4	5	4
<i>Polygonum lapathifolium</i> L.	-	1.00	T ₄	VI-IX	KR	Subcirk	3	3	4	3	3	-	5	3	3
<i>Portulaca oleracea</i> L.	8.25	-	T ₄	VI-VIII	KR	Kosm	3	3	4	3	4	-	4	4	3
<i>Senecio vulgaris</i> L.	1.00	2.00	T ₁	III-XI	KR	Evr	3	3	4	3	4	-	4	4	3
<i>Setaria glauca</i> (L.) P.B.	28.00	-	T ₄	VI	KR	Kosm	2	3	4	2	3	-	4	4	3
<i>Sonchus arvensis</i> L.	5.00	-	G ₃	VII-IX	KR	Evr	³ w	3	4	4	4	+	3	4	3
<i>Sorghum halepense</i> (L.) Pers.	-	10.00	G ₁	VI-VII	KR	Kosm	1	2	3	3	3	-	4	5	3
<i>Stachys annua</i> L.	1.00	-	T ₄	VI-X	KR	Subpont-subm	2	4	2	3	4	-	4	4	4
<i>Veronica hederifolia</i> L.	3.00	-	T ₁	III-V	KR	Subse	3	3	4	3	4	-	3	4	3
<i>Veronica persica</i>	3.00	-	T ₁	III-IV(X)	KR	Adv	3	4	4	3	4	-	4	4	3

T –Therophyte, G – Geophyte;KR – Weed-ruderal, R – Ruderal, S – Segetal weed; Adv – Adventive,Kosm – Cosmopolitan, Subevr – Subeurasian, Pont-e.subm – PonticEast Submediterranean,Subcirk – Subcirkumpolar,Evr – Eurasian, Subpont-subm – Subpontic-Submediterranean,Subse– Submiddle European.

Similar reduction in weed floristic diversity (eight taxa) was reported by Ljevnaić-Mašić et al. (2014) for basil grown in certified organic farm “Bio salaš Farago” in Orom, North Banat. Three species were common for the two sites: *Amaranthus retroflexus*, *Bilderdykia convolvulus* and *Sorghum halepense*.

Generally, basil crop weed infestation on conventionally maintained plots was approximately 5% and 80% between and inside rows, respectively. Organic plots were infested less than 1% between and approximately 5% inside rows.

The results of this study oppose to the numerous reports (Menalled et al, 2001; Boguzas et al., 2004) on comparatively high weed floristic diversity in organically maintained crops. According to Nikolić et al. (2013), still not fully balanced ecological conditions on the organic plots, as well as their partial space isolation may be the explanation of the reduction in weed variety. Roschevitz et al. (2005) suggested the influence of dissemination from the surrounding area on floristic diversity of conventional plots. In addition, higher infestation of conventional plots is at least partially due to the fertilizing performed in 2013. The applied mineral fertilizer fostered weed development; on the other hand, organic plots remained unfertilized for three consecutive years.

Since invasive plant species may exhibit undesirable effects on autochthonous flora (Vrbničanin et al., 2004; Nikolić et al., 2011), it is important to note even four such plants: *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Portulaca oleracea* and *Sorghum halepense*. The possible spreading of this species should be monitored and controlled.

The majority of the analyzed weeds flowers from June to September. Weed species *Senecio vulgaris*, *Veronica hederifolia* and *V. persica* flower in March, while *Anagallis arvensis* flowers in May. The longest vegetation period have *Senecio vulgaris*, *Veronica persica* and *Stachys annua*. Except *Senecio vulgaris*, which grows in both growing systems, the mentioned species were found on conventional plots only and imply longer vegetation period of this vegetation.

Out of 18 taxa recorded in basil crops, 15 are weed-ruderal (83.33%), two (11.11%) are ruderal, and one belong to segetal weeds (5.55%) – *Bilderdykia convolvulus*. The percentages of the categories according to the site are: weed-ruderal K-81.25%, O-71.43%; ruderal K-12.50%, O-14.28% and segetal K-6.25%, O-14.28%.

Concerning the spectrum of areal types; characteristic of weeds, taxa with wide spatial distribution predominate (K-81.25%,O-100.00%). The cosmopolitan floral elements were dominant (K-37.50%, O-42.86%), followed by Adventive (K-18.75%, O-14.28%), Eurasian (K-12.50%, O-14.28%), Subeurasian (K-12.50%, O-14.28%) and Subcirkumpolar (O-14.28%). Taxa with narrow distribution were found in conventional crops only (18.75%), with one taxon of Submiddle European, Pontic East Submediterranean and Subpontic-Submediterraneanfloral element.

Therophyte life form predominates in both production systems (K-81.25%, O-85.71%, Figure 1). T₄ therophytes with germinating in spring and seed maturing by the end of summer were the most numerous (K-62.50%, O-71.43%). Therophytes T₁ were less abundant (K-18.75%, O-14.28%). Geophytes G₃ were represented with 18.75% in conventional and G₁ with 14.28% in organic basil production. Such a biological floristic spectrum is probably related to mechanical weed control (hoeing) that favors survival of therophytes, which is a characteristic for the ecosystems under strong anthropogenic influence (Nikolić et al., 2011; Džigurski et al., 2012; Ljevnaić-Mašić et al., 2013). Despite of small differences between the two growing systems, therophyte *Setaria glauca* dominates in conventional and geophyte *Sorghum halepense* in organic crops.

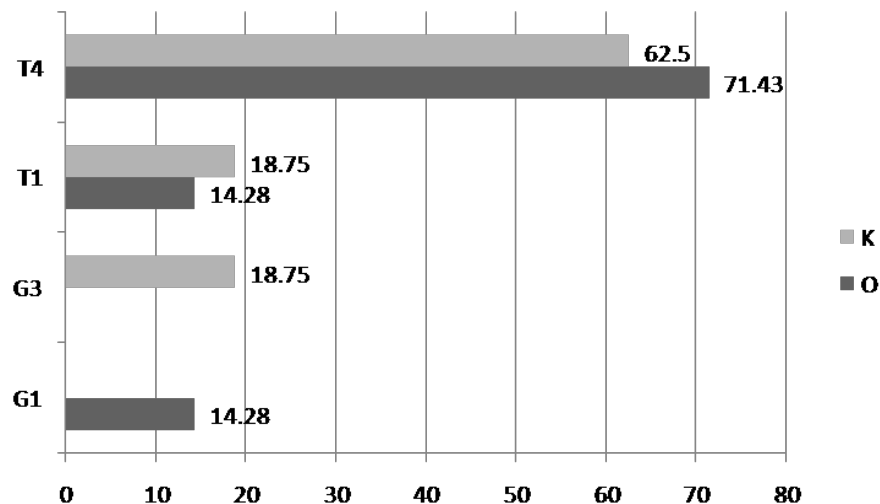


Figure 1. Biological spectrum of weed flora of basil in conventional (K) and organic (O) production

The analysis of ecological indices for the weed flora in terms of humidity showed the domination of mesophytes which are adapted to moderately humid sites (F_3) in conventional crops (K-56.25%, O-42.86%). On the other hand, presence of sub xerophytes adapted to moderately dry sites (F_2) is almost equal in the two production systems (K-43.75%, O-42.86%, Figure 2). *Sorghum halepense*, adapted to dry sites (F_1), was registered on organic plots only (14.28%). The mean values of the ecological index for humidity were 2.56 for conventional and 2.28 for organic plots, indicating somewhat wetter soil in conventional plots, which is related to higher weed infestation.

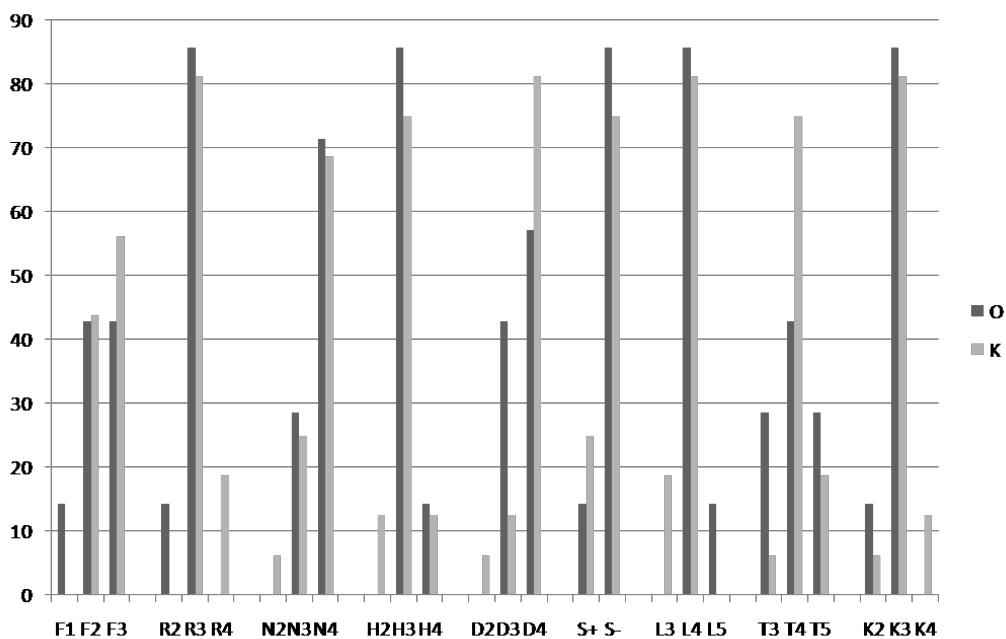


Figure 2. Percentage of ecological indices of weed flora in basil crop in conventional (K) and organic (O) production

The ecological analysis of substrate chemical reaction indices showed the predominance of neutrophilic (R_3) plants (K-81.25%, O-85.71%). The indicators of neutral to slightly alkaline soil (R_4) were present in conventional production only (18.75%). Concerning organic plots, one taxon (14.28%) adapted to acid soil (R_2) was found. The mean values of the ecological indices indicate neutral to slightly acid reaction (3.18) on conventional and neutral to slightly alkaline reaction (2.86) on organic plots.

The analysis of ecological indices for nitrogen compounds showed the dominance of the indicators of eutrophic (N_4 ; K-68.75%, O-71.43%) and moderately productive (N_3 ; K-25.00%, O-28.57%) ecosystems. One indicator of low amount of soil nitrogen compounds was present in conventional production. The mean values of the indices (K-3.62, O-3.71) characterize the agro ecosystems as eu-mesotrophic, with somewhat higher nitrogen content in plots maintained organically.

The indices concerning organomineral compounds indicated medium soil humus content (H_3) in both production systems (K-75.00%, O-14.28%). The indicators of soil with high (H_4 ; K-12.50%, O-14.28%) and low (H_2 ; K-12.50%) humus content were less abundant. Slightly higher humus content was estimated for organic production (3.14) when compared to conventional (3.00).

Regarding dispersion (aeration) of soil, dominate the indicators of moderately aerated substrate (D_4 ; K-81.25%, O-57.14%). The indicators of well aerated substrate are less abundant (D_3 ; K-12.50%, O-42.86%). One indicator of well aerated sites (D_2) was present on conventional plots. All analyzed plots were well aerated, with a slightly more favorable situation in organic system (K-3.75, O-3.57).

The weed species that are the indicators of non-saline soils (S_-) were dominated on both conventionally (75.00%) and organically (85.71%) maintained plots. However, taxa adapted to higher Na^+ ion content (S_+) were more frequent on conventional (25.00%) than on organic (14.28%) plots.

The site was characterized by high light intensity (L_4 ; K-81.25%, O-85.71%). Semi-sciophytes (L_3) were found in conventional (18.75%), and heliophytes (L_5) in organic (14.28%) system. The mean values of the ecological indices for light confirm favorable light regime (K-3.81, O-4.14).

Concerning temperature, the most abundant were the indicators of warm sites (T_4 ; K-75.00%, O-42.86%). Less numerous were the indicators of moderately warm (T_3 ; K-6.25%, O-28.57%) and very warm sites (T_5 ; K-18.75%, O-28.57%). Temperature regime was favorable, with mean values of the ecological indices of 4.12 (K) and 4.00 (O).

The analyzed site was characterized by moderately continental conditions (K_3 ; K-81.25%, O-85.71%). The mean values of the ecological indices were 3.06 (K) and 2.86 (O).

CONCLUSION

Weed flora in conventional basil production system consisted of 16 taxa. Differential plant species were: *Ambrosia artemisiifolia*, *Anagallis arvensis*, *Cirsium arvense*, *Convolvulus arvensis*, *Hibiscum trionum*, *Portulaca oleracea*, *Setaria glauca*, *Sonchus arvensis*, *Stachys annua*, *Veronica hederifolia* and *V. persica*, with *Setaria glauca* as the dominant one. Weed infestation was 5% and 80% between and inside rows, respectively.

Only seven taxa were found in organic production system. Differential species were: *Polygonum lapathifolium* and *Sorghum halepense*, with *Sorghum halepense* dominating. Weed infestation was less than 1% and 5% between and inside rows, respectively.

The analysis of weed flowering showed that the flora found in conventional growing system had longer vegetation period.

The weed flora was of therophyte-geophyte character in both production systems. Therophyte *Setaria glauca* dominates on conventional and geophyte *Sorghum halepense* on organic plots.

The ecological indices of the weed flora were similar for the two growing systems.

The relatively small differences concerning weed flora are related to the agro technical procedures specific for growing medicinal and spice plants that are similar for conventional and organic plots. Manual weeding was not performed any of the conventional or the organic plots, providing similar conditions for weed development. The higher floristic diversity recorded for the conventional plots was related to mineral fertilizer applied during the previous year, in contrast to organic plots which remained unfertilized for three consecutive years. In addition, the reduction in weed diversity on organic plots may be partially explained by still not fully balanced ecological conditions, as well as their partial space isolation.

Invasive plant species in weed flora are important from the ecological and agronomical aspect, as well as because their possible undesirable effects on autochthonous flora, therefore *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Portulaca oleracea* and *Sorghum halepense* should be monitored and controlled.

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KOROVI U KONVENCIONALNOJ I ORGANSKOJ PROIZVODNJI BOSILJKA (*OCIMUM BASILICUM*, LAMIACEAE MARTYNOV 1820, LAMIALES)

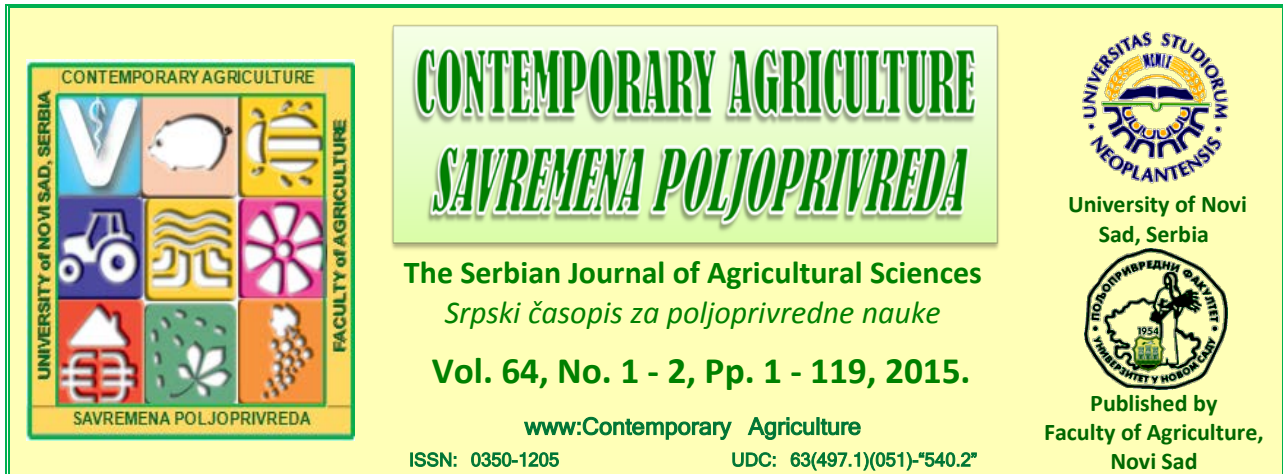
Dejana DŽIGURSKI, Branka LJEVNAIĆ-MAŠIĆ, Ljiljana NIKOLIĆ,
MILKA BRDAR-JOKANOVIĆ, Dušan ADAMOVIĆ

Izvod: Cilj rada bio je da se ukaže na razlike u korovskoj flori bosiljka u konvencionalnom i organskom sistemu gajenja. Floru korova pri konvencionalnoj proizvodnji čini 16 taksona, a dominiraju *Setaria glauca* i *Portulaca oleracea*. U organskoj proizvodnji zabeleženo je svega sedam taksona, a dominiraju *Sorghum halepense* i *Amaranthus retroflexus*. Neočekivano manji floristički diverzitet u organskom sistemu gajenja uzrokovan je izostankom đubrenja u poslednje tri godine, još sasvim neuravnoteženim ekološkim uslovima, delimičnom izolovanošću parcela i prisustvom čak četiri invazivne vrste.

Key words: *Ocimum basilicum*, organska i konvencionalna proizvodnja, korovi, ekološka analiza.

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URBAN STRESS TOLERANCE OF EVERGREEN TREES ON GREEN AREAS OF NOVI SAD CITY

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Summary: The Novi Sad area belongs to hygrophilic, mesophilic and xerophilic forest habitats. This environment is unsuitable for coniferous species. In the studied area, longitudinal monitoring has indicated a significant reduction in life expectancy of many species, especially of the representatives of conifers. Among the introduced representatives, *Cupressus arizonica* Green, *Taxodium distichum* (L.) Rich, *Tsuga canadensis* Carr. and *Cedrus atlantica* G.Don have demonstrated exceptional adaptability, condition and vitality in the urban Novi Sad environment.

Keywords: conifers, ecological conditions, urban stress, adaptability, monitoring.

INTRODUCTION

According to Craul (1999) and Unger et al. (2001), urban environment is characterized by increased air temperature, greater concentration of exhaust gasses and unbalanced groundwater regimen. The findings of numerous studies in this field (Kramer and Kozłowski, 1979; Kabata-Pendias and Pendias, 1986; Bargagli, 1998; Rajšić et al., 2008; Kastori and Milošević, 2011) confirm that deposition of pollutants from the air adversely affects physiological processes in plants. Moreover, Chen (2004) and Ninić-Todorović et al. (2010) noted that green areas are essential for the urban life quality, as they make living conditions more humane. In this context, Gerhold and Porter (2007) favor the species with strong trunk and developed canopy, due to their better adaptation to the stresses induced by negative environmental factors, as well as better resistance to pests and illnesses and minimal maintenance requirements.

Airborne pollutants, according to Hinrichsen (1987), can have both synergic and antagonistic effect on plant growth. They typically disrupt the nutrient transport mechanism from the roots to the tree trunk. Heliotis (1988)

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further noted that pollutant deposition on conifers could cause their extinction. In a more recent study, Donovan et al. (2005) determined that presence of *Pinus nigra* Arn. in urban environments significantly improved air quality. According to the findings reported by Kastori and Milošević (2011), increased occurrence of acid rains during the 20th century contributed to the greater quantity of soluble matter and gasses in the atmosphere.

Findings of the study conducted by Oćokoljić and Ninić-Todorović (2007) indicate that *Cupressus arizonica* Greene is highly adaptable to the Novi Sad urban conditions. These results were subsequently confirmed by Todorović (2012), who also highlights the resistance of *Cupressus arizonica* Green to the stress induced by climatic and edaphic factors commonly affecting the green areas in Novi Sad.

Swamp cypress (*Taxodium distichum* [L.] Rich.) was first introduced to Europe around 1640 from southeastern and flooded areas of the US. In Novi Sad, it is grown as an ornamental and resistant species in public parks, and is used within the reforestation initiatives (Ninić-Todorović and Oćokoljić, 2001).

MATERIALS AND METHODS

Novi Sad is located on a plain, at an average altitude of 72 to 80 m asl. Its climate is temperate continental to modified continental. The mean annual temperature and precipitation are 11.1 °C and 603.1 mm, respectively. The soil is mostly under anthropogenic influence, of the urbisol type (Oćokoljić and Ninić-Todorović, 2002). The methodology adopted in this study included longitudinal monitoring of the existing genotypes and studying their adaptability. Eco-physiological adaptability was assessed at the individual level by observing and analyzing autochthonic and introduced trees. Using the methodology described by Anastasijević (2012), for the creation of Futoški Park cadastre (Ninić-Todorović et al., 2010), as well as that pertaining to the conifers in the Novi Sad district Bistrica (Žugić, 2013), biometric parameters for the conifers present in these green areas were obtained.

RESULTS

More extensive and detailed studies of exogenous tree species in Serbia commenced in the mid-20th century and have, thus far, yielded numerous important findings and conclusions (Ninić, 1981; Vidaković, 1982; Vukićević, 1996; Jovanović, 2000). In the last 35 years, at the Faculty of Agriculture, University in Novi Sad, the work on monitoring autochthonic and introduced dendroflora within urban cenoses has been conducted. Green areas in Novi Sad abound with a wide spectrum of autochthonic dendroflora, with *Cupressus arizonica* Greene as one of its representatives. The species has been introduced into this area, and is currently grown for its highly decorative value across different green space categories. The analysis of *Cupressus arizonica* Greene trees, cultivated in the park of the Institute for cardiovascular diseases in Sremska Kamenica (Ninić-Todorović and Oćokoljić, 2007; Oćokoljić et al., 2005; Todorović, 2012) revealed that they possess exceptional eco-physiological characteristics. These favorable traits are exhibited through overall appearance, resistance to illnesses and pests, general functionality and visual and esthetic value. Their longitudinal monitoring has indicated that these trees periodically produce abundant yield, thus manifesting their reproductive function, significant for the production of seed material that can be used in horticulture and landscape architecture.

By analyzing *Tsuga canadensis* Carr. growth and productivity, significant productivity within the area of the Novi Sad City cemetery was noted, at the habitat of the maple and ash association. According to the statistical parameters of the analyzed trees, their height ranged from 8.50 m to 11.50 m, and the chest-level trunk diameter from 22.20 cm to 30.20 cm, while crown volume ranged from 9.42 m to 13.50 m. For a more detailed knowledge about adaptability of decorative evergreen species it was performed a comparative analysis of climate similar sites in the city of Belgrade, at localities Kalemegdan and Arboretum of Faculty of Forestry. Quantitative measurements of *Tsuga canadensis* Carr. adult trees were carried out on the former habitat of *Quercetum farnetto-cerris* Rud. in Belgrade. The height of these trees ranged from 7.56 m to 8.90 m, the chest-level trunk diameter from 9.39 cm to 12.80 cm, and the crown volume from 16.48 m to 21.20 m. In both habitats, cones were also analyzed and revealed significant variability with respect to the studied parameters (Table 1). In Belgrade, the cone length ranged from 13.58 mm to 16.87 mm, while it measured 17.68 mm to 20.46 mm in Novi Sad. The mean cone width for the trees grown in Belgrade was 6.59 mm to 7.76 mm, while those in Novi Sad measured 7.99 mm to 9.26 mm in width.

The data obtained indicate variability between the cones of *Tsuga canadensis* Carr. parent trees located in Belgrade and Novi Sad. Given the adaptability of the *Tsuga canadensis* Carr. species in both habitats, it is possible to improve their yields in the later stages of tree growth, with the aim of applying the obtained reproductive material in horticulture and landscape architecture (Oćokoljić et al., 2005).

In the parks within the Novi Sad area, *Taxodium distichum* /L./Rich. is of particular ecological value. For the purpose of this study, 10 trees grown in Futoški park and 18 located in Dunavski park were selected. These

individuals possess exceptional eco-physiological characteristics, as evident in their overall appearance, resistance to pests and illnesses, and visual and esthetic qualities. The distance among the chosen trees is about 5 meters. In both parks, the soil belongs to the hydromorph order, fluvial and fluvial-gley class, fluvial meadow soil - humofluvisol (Ninić-Todorović and Ocokoljić, 2001).

Table 1. Statistical parameters pertaining to the *Tsuga canadensis* Carr. cone quantitative characteristics

Characteristics	Tree	X±S _x	S±S _s	V±S _v
Belgrade				
Cone length (mm)	1	16.87-0.17	1.79-0.12	10.63-0.72
Cone width (mm)		7.08-0.07	0.77-0.05	10.86-0.74
Cone length (mm)	2	15.85-0.14	1.64-0.09	10.32-0.63
Cone width (mm)		7.76-0.07	0.82-0.05	10.62-0.64
Cone length (mm)	3	13.58-2.20	1.72-0.14	12.64-1.06
Cone width (mm)		6.59-0.08	0.74-0.06	11.30-0.94
Novi Sad				
Cone length (mm)	4	20.46-0.10	1.21-0.03	5.91-0.35
Cone width (mm)		9.26-0.04	0.48-0.02	5.17-0.36
Cone length (mm)	5	18.27-0.08	1.03-0.06	5.62-0.34
Cone width (mm)		8.35-0.03	0.39-0.02	4.76-0.29
Cone length (mm)	6	17.68-0.19	2.24-0.13	12.66-0.77
Cone width (mm)		7.99-0.04	0.50-0.01	6.28-0.38
Cone length (mm)	7	19.06-0.09	1.09-0.06	5.70-0.34
Cone width (mm)		9.04-0.02	0.29-0.01	3.23-0.20

The mean tree height among the *Taxodium distichum* /L./Rich. individuals located in Futoški park is 17.6 m, and the chest-level trunk diameter is 56.22 cm (Table 2). In Dunavski park, the mean tree height of 22.55 m and the chest-level trunk diameter of 59.72 cm were measured. At both locations, the tree crown was well developed, with mean height and width of 14.72 m and 8.05 m, respectively, measured in Futoški park, with slightly higher values of 19.63 m and 9.67 m for the meant tree crown height and width for individuals in Dunavski park. The coefficient of variation values satisfactorily reflect the hereditary potential.

Table 2. Statistical parameters for five characteristics of 28 *Taxodium distichum* /L./Rich trees grown in Novi Sad

FUTOŠKI PARK			
Boundary values Min.-max.	X±S _x	S±S _s	V±S _v
Tree height(m)			
16-21	17.6±1.05	1.84±0.75	10.44±4.26
Trunk diameter at 1.30 m height (cm)/ Prečnik debla na 1,30m			
36-90	56.22±10.11	17.69±7.22	31.47±12.85
Trunk height below the crown (m)			
2-4.5	2.88±0.45	0.78±0.32	27.13±11.07
Crown height (m)			
13-17.5	14.72±0.87	1.53±0.62	10.39±4.24
Crown diameter (m)			
4.5-10	8.05±1.27	2.21±0.90	27.54±11.24
DUNAVSKI PARK			
Tree height (m)			
18-28	22.55±1.71	2.99±1.22	13.28±5.42
Trunk diameter at 1.30 m height (cm)			
38-79	59.72±6.22	10.89±4.44	18.23±7.44
Trunk height below the crown (m)			
1.8-5	2.95±0.65	1.15±0.47	38.90±15.88
Crown height (m)			
6-14	9.67±1.41	2.47±1.00	25.59±10.44
Crown diameter (m)			
16-26	19.63±1.61	2.82±1.15	14.38±5.87

Field studies conducted over the last two decades in the Novo Naselje, Slobodana Jovanovića Boulevard (GUP Novog Sada, 2000), revealed magnificent changes in the number of conifer species. Namely, there was a significant reduction of conifer species in the street profiles (Figure 1). It was identified that 64.52% of the existing conifer trees are exotic. As a part of the development of the Novi Sad General Urban Plan (GUP), during 1994, conifer cadastre for the Novo Naselje district (Bistrica area) was created. It revealed that most abundant genotype representatives in 1994 were *Pinus nigra* Arn. with 32%, *Cedrus atlantica* G.Don with 16% and *Sequoiadendron giganteum* Lindl. with 12% participation in the total conifer number. A more recent field study, conducted in 2013, revealed some important changes in the conifer structure and number. Currently, *Picea abies* Karst. is most abundant, with 19.27%, followed by *Thuja orientalis* L. with 17.35% and *Pinus nigra* Arn., the percentage of which declined in comparison with the earlier study findings (Graph 1). *Picea abies* Karst. has become more numerous in the Novo Naselje district as a result of the initiative, whereby this species is planted with the roots following the New Year’s celebrations.

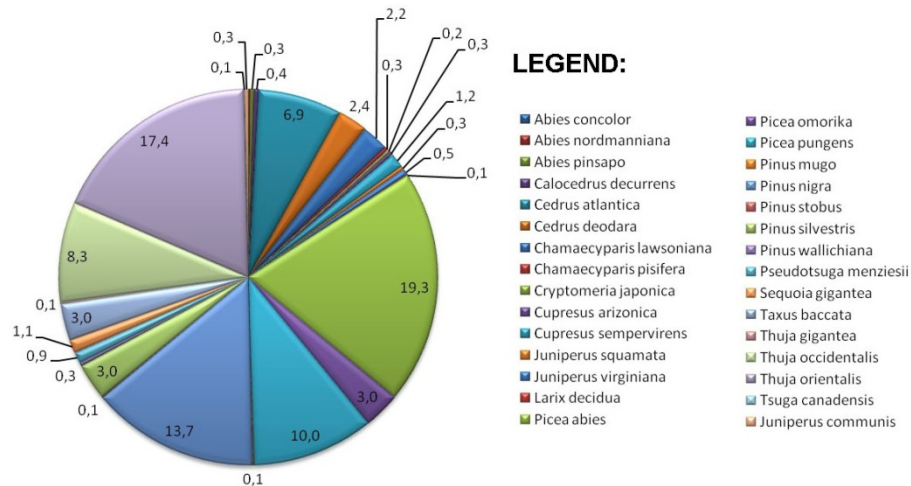


Figure 1. Prevalence of various coniferous species in the Novi Sad district Novo Naselje in 2013.

Significant reduction in the number of *Sequoiadendron giganteum* Lindl. trees occurred between 1994 and 2013, as presently only 21 individuals remain, corresponding to 34.37% of the total conifer number (Figure 2). In addition, within the residential green spaces, *Abies concolor* Lindl. et Gord suffered severe degradation with 62.96% of total. Due to the stress caused by a wide amplitude of absolute extremes and inadequate maintenance measures, nine *Picea omorika* /Panč/Purkyne were uprooted in the Dušana Danilovića street. The best adaptability was noted for *Cedrus atlantica* G. Don and *Pinus nigra* Arn.



Image 1. Slobodana Jovanovića Boulevard in the Novi Sad district Novo Naselje



Figure 2. Dried-out *Sequoiadendron giganteum* Lindl.
Trees at the corner of Slobodana Jovanovića Boulevard and Raše Radujkova

DISCUSSION

The Novi Sad area belongs to hygrophilic, mesophilic and xerophilic forest habitats and is subjected to the steppe vegetation effects. In the city green spaces, reproductive material obtained from tree nurseries aimed at reforestation is commonly used. In addition, the reproductive material is also sourced from horticultural nurseries, where the ecological conditions significantly differ from those within the Novi Sad area. These factors, compounded by the minimal maintenance measures, due to financial constraints, have resulted in a significantly diminished conifer function in the Novi Sad area. Terrain configuration, edaphic and microclimate factors, in interaction with environmental conditions and genotype, have exhibited weaker or stronger reaction levels among the individuals tolerant of stress induced by urban cenoses.

CONCLUSION

In the Novi Sad district Novo Naselje, conifer genotypes of European, American and Asian origin are mostly cultivated, with the exception of *Cedrus atlantica* G. Don, which originates from the northern parts of Africa, and has adapted to the ecological conditions prevalent in the city. Longitudinal monitoring of physiological and ecological characteristics of gymnosperm and angiosperm genotypes within the Novi Sad green areas enables reaching some conclusions regarding the cultivation and longevity potential of these species. Based on the analysis of growth and productivity of *Tsuga canadensis* in Belgrade and Novi Sad, trees are characterized by rapid and stable growth, and it can be concluded that the terms of the former habitat of oak and ash (Novi Sad) are more conducive to this species than habitat of *Quercetum farnetto-cerris* Rud. (Belgrade). These findings are valuable for the conservation of sanitary, hygienic and visual authenticity of the city environment. Owing to the methodology adopted in this study for the development of the green areas cadastre, whereby a pilot study was conducted for the Futoški park, the direction for the use of GIS—a software tool for data collection and analysis—was defined with the aim of better management of the green spaces in Novi Sad.

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TOLERANCIJA ZIMZELENOG DRVEĆA NA GRADSKI STRES NA ZELENIM POVRŠINAMA U GRADU NOVOM SADU

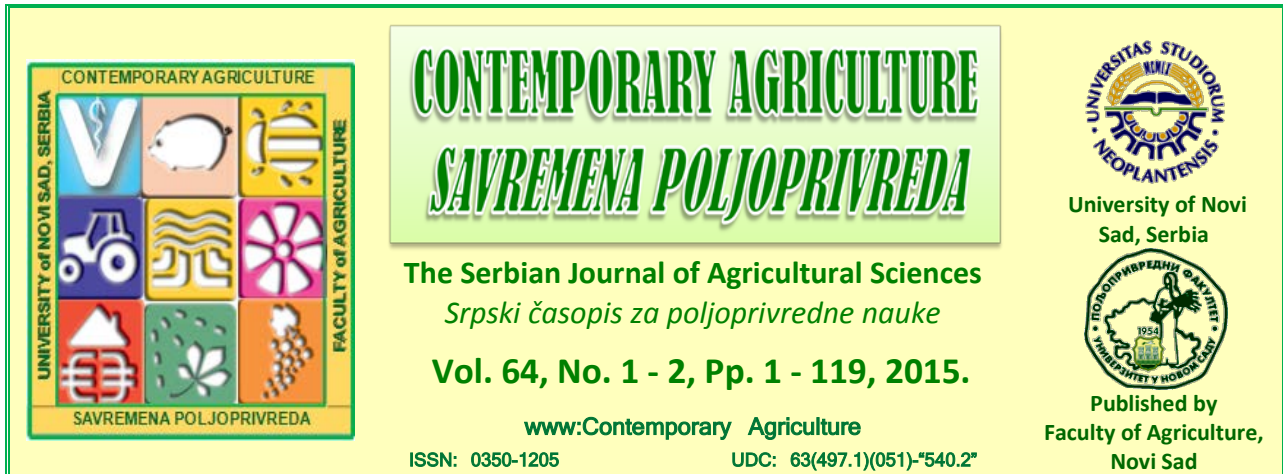
Jelena NINIĆ-TODOROVIĆ, Mirjana OCOKOLJIĆ, Ivan TODOROVIĆ,
Jelena ČUKANOVIĆ, Ivana SENTIĆ, Aleksandar KURJAKOV, Edvina ŽUGIĆ

Izvod: Područje grada Novog Sada pripada staništima higrofilnih, mezofilnih i kserofilnih šuma. Kao takvo nije pogodno za gajenje četinara. Značajna je sanitarna i higijenska funkcija koju četinari ostvaruju u gradu i vizuelni efekat na zelenim prostorima. Dugogodišnji monitoring ukazao je na značajno smanjenje dužine života, posebno predstavnika konifera, na osmatranom prostoru. Od introdukovanih predstavnika *Cupressus arizonica* Green, *Taxodium distichum* [L.] Rich, *Tsuga canadensis* Carr., *Cedrus atlantica* G. Don pokazali su izuzetnu adaptivnost, kondiciju i vitalnost u urbanim uslovima Novog Sada.

Ključne reči: konifere, ekološki uslovi, urbani stres, adaptivnost, monitoring.

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PERSISTENCE OF HUMAN ISOLATE OF *P. AERUGINOSA* IN VEGETABLE CROPS*

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Simonida DJURIĆ, Stevan MAŠIREVIĆ, Ana TAKAČ¹

Summary: Increased usage of raw vegetables, without heat treatment, in human nutrition carries the risk of possible infection by pathogenic bacteria. The possibility of persistence of human isolates in vegetable crops represents, in epidemiological sense, a serious source of infection. The aim of this research was to study the possibilities of persistence of human isolates of *Pseudomonas aeruginosa* in selected vegetable crops, as well as the possibility of bacterial transport from roots to the above-ground part of the plant. Obtained results confirmed the possibility of persistence of human isolates of *P. aeruginosa* in the aforementioned vegetables.

Key words: *Pseudomonas aeruginosa*, persistence, vegetable crops.

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous bacterium that is one of most frequent causes of opportunistic human infections (Stover et al., 2000). It causes alimentary infections and it is considered as one of the main causes of nosocomial infections in immunocompromised patients (Bodey et al., 1983). The ability of some strains of *P. aeruginosa* to cause infections in humans, animals and plants is based on a large number of virulence factors, the possibility of forming a biofilm (Costerton et al., 1999) and different mechanisms of resistance to antimicrobial drugs (Chuan-Chuen et al., 2001). Biofilm makes bacteria resistant to disinfectants, antibiotics and mechanisms of host immune response (Koch and Høiby, 1993; Costerton et al., 1999).

Pseudomonas aeruginosa was also identified as a cause of pathological changes in lettuce, tobacco, bananas, onion and other plants (Lebeda et al., 1984). The occurrence of pseudotuberculosis, yersiniosis, listeriosis and

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intestinal infections in the usage of raw vegetables, fruits and meals prepared of them was reported in epidemiological studies (Djukic et al., 2011). Research carried by Kominos et al. (1972) shows the high level of contamination by this bacterium in raw vegetables prepared in hospital kitchens which leads to the conclusion that the vegetables are the primary source of infection by this bacterium. Significant reduction of alimentary infections by *P. aeruginosa* in hospitals followed the elimination of fresh vegetables from the patients' diets (Green et al., 1974). Therefore, the consumption of vegetables contaminated by this bacterium can seriously impact human health.

The aim of this study was to investigate the possibility of persistence of human isolates of *P. aeruginosa* in plants that can be used unprocessed in human nutrition.

MATERIAL AND METHOD

The study included four types of vegetable plants: cucumber (*Cucumis sativus* L.), cabbage (*Brassica oleracea* var. *capitata* L.), tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.), grown from seeds of the Institute of Field and Vegetable Crops in Novi Sad. The used strain of *P. aeruginosa* was isolated from human throat swabs and identified in the Center for Microbiology of the Institute of Public Health of AP Vojvodina. The experiment was set up in the Laboratory of Microbiology and Parasitology, Faculty of Agriculture in Novi Sad.

Sowing containers with 56 places filled with sterile substrate (autoclaved for 45 minutes at 121 °C at pressure of 1.2 atm) were used for performing the experiment. The experiment was set up in three replications with 4 plants. Before sowing the seeds were disinfected by immersion in 75% alcohol for 15 minutes, then in sterile distilled water for 10 minutes and after that rinsed with distilled water for 5 minutes. Seeds of all species were sown on the 16th of December 2013 and by occurrence of cotyledon leaves (cabbage and cucumber after 10 days, 19 days of tomatoes, and peppers 25 days after sowing) injecting of bacterial suspensions in the zone of the root system of plants was performed. For the preparation of bacterial suspension concentration of 6×10^8 cfu ml⁻¹ (2th scale by McFarland (Clement et al., 1990) of the overnight culture were used. Quantity of the bacterial suspension were 2 ml and the injection was carried out by using a syringe and medical needle. Plants were grown in phytotron with regular irrigation with sterile water.

After 15 days of injection of the suspension into the zone of the root system, the separation of the above-ground parts of plants without touching the substrate was carried out. Removed plant material was macerated with 1 ml of sterile distilled water and then inoculated on nutrient agar and incubated for 24 hours at 37 °C. Identification of isolated bacteria was done using standard biochemical tests. Both human and plant isolates were tested for susceptibility to the following antimicrobials drugs: piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, doripenem, gentamycin, amikacin and ciprofloxacin using disk diffusion technique by Kirby-Bauer on Muller-Hinton agar according to the recommendation of Clinical and Laboratory Standards Institute (CLSI, 2012).

Data processing was performed using the Statistica software 10, by using Tukey HSD test, level of significance of $p < 0.05$.

RESULTS

The research indicates that the bacteria penetrate into the above-ground portions of the plants through the root system, without making pathological changes in plants. The isolated strains were identified as *P. aeruginosa*. The presence of *P. aeruginosa* was found in all 12 plants of cucumber and cabbage (100%), in 9 tomato plants (75%) and in 7 plants of pepper (58.3%) (Figure 1). By analyzing the infected plant a statistically significant difference ($p < 0.05$) in the presence of *P. aeruginosa* between peppers and the other crops examined was found (Figure 1).

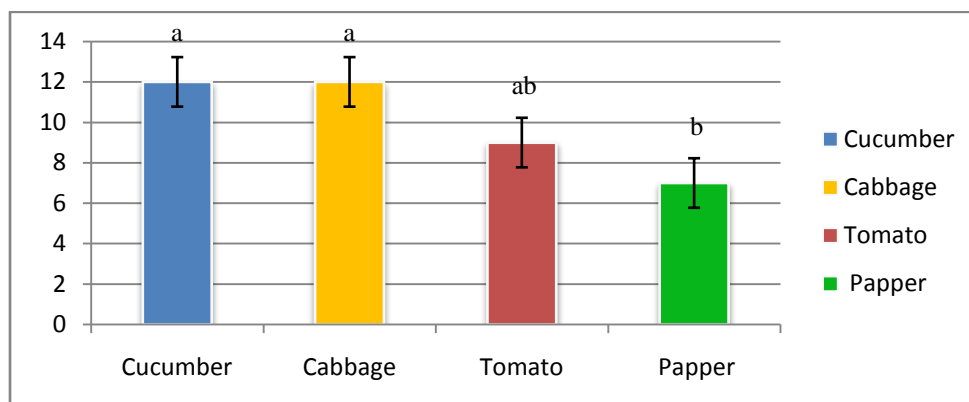


Figure 1. Number of infected plants per culture

Susceptibility of isolates originated from the inoculated plants, was identical to susceptibility of original human isolate. Based on these facts it may be considered that isolates obtained from plants and human isolate belong to the same strain.

DISCUSSION

The range of bacteria capable of living both in plants and in the human body is quite wide (Djukic et al., 2011). The same species of bacteria have the ability to cause the infection in humans, animals and plants. The examples are specific strains of *P. aeruginosa* as the strain UCBPP-PA14 (PA14) which causes disease in plants of *Arabidopsis thaliana* (Rahme et al., 1997), insect *Drosophila melanogaster* (Jander et al., 2000), mice (Rahme et al., 1995), and nematodes *Caenorhabditis elegans* (Mahajan-Mikloset et al., 1999). The ability of bacteria to survive in plants, raises the question whether plants can be reservoirs of human and animal pathogens? This fact is very significant, particularly for vegetable crops which are used without heat treatment, and therefore can be a direct source of alimentary infections. Transmission of pathogenic bacteria to plants is possible by using contaminated water, inadequate compost, fresh manure, liquid manure and feces of wild animals (Natvig et al., 2002, Santamaria and Toranzos, 2003; Tyrrel et al., 2006).

It is considered that the two factors of the virulence of *P. aeruginosa*, exotoxin A and phospholipase C, influence the occurrence of systemic spread of infection in mammals and *Arabidopsis*. By the analysis of the genome of *P. aeruginosa* it has been established that at least three genes encoding the virulence factors are involved in the pathogenesis of plants and animals (Rahme et al., 1995). The research carried out by Jander et al. (2000) indicates that the gene MucD is an important virulence factor for plants, insects, nematodes and mammals, and that the MucD gene is required for production of extracellular toxins C (Yorgey et al., 2001). The opinion is that trehalose plays a crucial role as a virulence factor during infection of leaves of plants (Djonović et al., 2013). It is assumed that *P. aeruginosa* parasitizes in parenchymal tissue of the plants (Plotnikova et al., 2000).

CONCLUSION

On the basis of our experiment, it can be concluded that *P. aeruginosa* has the ability to persist in cucumbers, cabbage, tomato and pepper. All isolates from the inoculated plants originate from the injected human isolate, which suggests the possibility of migration of bacteria from the roots in aboveground vegetative part. Consumption of infected plants may be an important way of infecting humans and domestic animals with *P. aeruginosa*.

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PREZISTENCIJA HUMANOG IZOLATA *PSEUDOMONAS AERUGINOSA* U POVRĆU

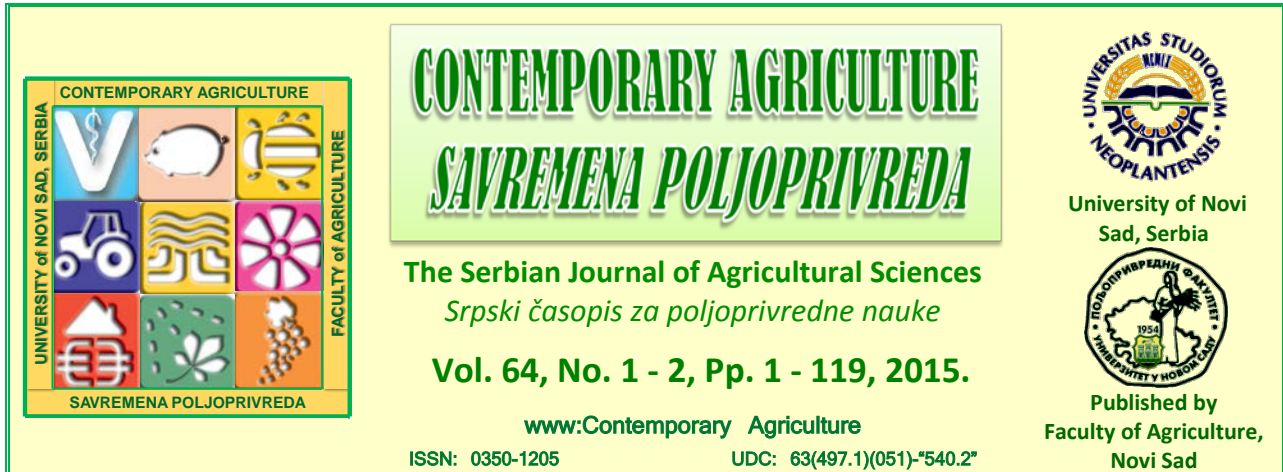
Slobodan VLAJIĆ*, Vesna LALOŠEVIĆ, Mira MIHAJLOVIĆ-UKROPINA,
Simonida DJURIĆ, Stevan MAŠIREVIĆ, Ana TAKAČ

Izvod: Povećana upotreba sirovog povrća, bez termičke obrade, u ljudskoj shrani nosi rizik od moguće infekcije sa patogenim bakterijama. Mogućnost perzistencije humanog izolata u biljnim kulturama predstavlja u epidemiološkom smislu, ozbiljan izvor zaraze. Cilj ovog istraživanja je bio ispitivanje mogućnosti perzistencije humanog izolata *Pseudomonas aeruginosa* u odabranim povrtarskim kulturama, kao i mogućnost transporta bakterije iz korena u nadzemni deobiljke. Dobijeni rezultati potvrđuju mogućnost perzistentnosti humanog izolata u odabranim povrtarskim kulturama.

Ključnereči: *Pseudomonas aeruginosa*, perzistencija, povrće.

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Novi Sad

Original scientific paper

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HARVESTING OPTIMISATION AND POST-HOC ANALISYS*

Snežana MATIĆ-KEKIĆ, Lazar SAVIN, Nebojša DEDOVIĆ¹

Summary: A formulated model of linear programming, which minimizes the total harvest and transport time for the observed farm company, is necessary for achieving the maximum profit from combine harvester rentals. Interdependent field operations have been mutually connected. The calculated optimal operating time showed that more human labour and machinery were used than necessary. This was later used for the post-hoc analysis which determined the profit from the combine harvester rentals for the observed period.

Key words: optimisation, maximum profit, minimum operating time, machinery pool.

INTRODUCTION

Linear programming is one of the methods that enable the fulfilment of criteria related to planning the capacities and structure of machinery pool, as well as the exploitation of the machinery. Fokkens and Puylaert (1981) developed a mathematical model as a tool for organisation of harvest operation at a large scale grain farm. They created three types of variables in order to determine combine and transport capacity, transfers of combine harvesters and number of unloading pits for each crop. In the paper of Camarena et al. (2004), a programme for making decisions on investing in the machinery utilised in a multifarm system was presented based on the mixed integer programming. Ouhimmou et al. (2009) presented a comparative study of traditional decision making versus optimal decision making.

If there are showers, the greatest losses occur due to grain dispersal and if it rains slightly and repeatedly for a longer period of time the losses occur due to physiological processes in the grain. In Serbia, oilseed rape, wheat and winter barley are harvested during June and July when the probability of rainy days was in some years even 70% in the observed region (Đurić et al., 2010). From the aspect of machinery exploitation, they are most often used in September and October during sunflower and corn harvesting. However, on small farms, corn is harvested by corn pickers and corncobs are stored in the barns so there is no special need for renting a combine harvester. Certainly, this is not the case with small grains which is harvested by combine harvesters only, and which harvesting period is short because of the climatic conditions and variety characteristics. Therefore, main goal of this study is to minimize

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the operating time of the combine harvesters and tractors in June-July in order to perform harvesting on “Pobeda” farm as fast as possible. Moreover, this will lead to a significant profit from combine harvester rentals. This research specifies which type of combine harvester would operate on a specific parcel and which type of a trailer would be used for unloading the combine harvester bunker. It is assumed that, after being loaded, combine harvester bunkers are emptied into two trailers with load bearing capacity of 7 or 8 tons and then towed by a tractor. Moreover, the determined number of tractors is sufficient to provide one free tractor with two trailers which will carry the harvested crops as soon as the combine harvester bunkers are loaded. It was further assumed that there was no possibility to change the machinery pool and that available mechanization and human resources should be used to minimize the total operating time. Post-hoc analysis was additionally conducted in order to determine the profit from renting combine harvesters to small and medium farms in the observed period. In comparison to the cited papers, integer variables were not used in our mathematical model which reduced the model complexity. Also, interdependent field operations were additionally connected in the constraints.

MATERIAL AND METHODS

Notation and characteristics of machinery pool and operations necessary for optimisation

Notation			
t_{vpki}	total operating time per combine harvester or tractor type v with trailer type p at parcel k in operation i (h)	sl_{vk}	speed of combine harvester or tractor type v on the parcel k (km h^{-1})
s_{vpki}	operation or transport productivity per combine harvester or tractor type v with trailer type p at parcel k in operation i (ha h^{-1}), or (t h^{-1})	s_p	tractor speed with loaded trailer type p , (km h^{-1})
		w_v	working width of combine harvester type v (m)
		s	tractor speed on asphalt with empty trailers, (km h^{-1})
a_k	surface area of parcel k (ha)	D	number of working days in the observed period
d_k	distance from parcel k to silos (km)	W	number of operators on the farm company
y_k	crop yields of parcel k (t ha^{-1})	tu_p	unload time of trailer type p (h)
tb_{vk}	time of bunker loading for combine harvester type v at parcel k (h)	Subscripts	
n_v	number of combine harvesters or tractors type v		
m_p	number of trailers type p	v	combine harvester or tractor
wh	working hours per day (h)	p	trailer
ct_p	capacity of trailer type p (t)	k	parcel
c_v	bunker capacity of combine harvester type v (t)	i	operation ($i=1$ harvesting, $i=2$ crop transport)

Operating characteristics of combine harvesters JD ($v=1$), C ($v=2$) and Z ($v=3$) are given in Table 1.

Table 1: Speed sl_{vk} and working width w_v of combine type v on the parcel k , $v \in \{1,2,3\}$, $k \in \{1, \dots, 7\}$ and transport speed s_p with loaded trailers type p , ($p=1$ and $p=2$ for two trailers of 8 t and 7 t, respectively)

Type of combine	JD	C	Z
Oilseed rape - field speed sl_{vk} , km h^{-1}	$sl_{11}=5.5$	$sl_{21}=5.2$	$sl_{31}=4.2$
Wheat - field speed sl_{vk} , km h^{-1}	$sl_{12}=6$	$sl_{22}=5.7$	$sl_{32}=4.5$
Winter barley - field speed sl_{vk} , km h^{-1}	$sl_{13}=6.5$	$sl_{23}=6.2$	$sl_{33}=4.8$
Working width w_v , m	$w_1=6$	$w_2=6$	$w_3=5$
Tractor speed s_p , km h^{-1}	$s_1=14$ and $s_2=16$		

The following will be valid in the sequel: when subscript v takes values 1, 2 or 3, then the only operation is harvest ($i \in \{1\}$); when $v \in \{4\}$, it will refer to tractor M performing the transport of the harvested crops ($i \in \{2\}$).

Condition for the number of tractors used for harvested crop transportation. Unloading the grain from combine bunker to the tractor trailers is performed simultaneously with the harvesting (drive-by procedure) until the trailers are full. In order to avoid the situations when there is no free two trailer tractor for crop transfer after the bunker is

loaded, it is necessary to fulfil the condition (1) which implies that the time necessary to load a bunker operating on the parcel k should be longer than the time necessary for a tractor with any type of trailer to leave the parcel, empty the load and return to the parcel k . Combine type v will always have the tractor with trailer type p available if the following is valid for every parcel k :

$$\min(tb_{vk}, v=1,2,3) > \max\left(\frac{d_k}{s_p} + \frac{d_k}{s} + tu_p, p=1,2\right) \quad k=1,2,\dots,7. \quad (1)$$

Here, s_p is the speed of the tractor with two loaded trailers type p (Table 1), tb_{vk} is the loading time of a bunker for combine type v on the parcel k (Table 2); tu_p is the unloading time and s is the tractor speed on asphalt with empty trailers (Table 3). Table 2 shows the time tb_{vk} of bunker loading for combine type v on the parcel k which is obtained by

$$tb_{vk} = \frac{c_v \cdot 10}{w_v \cdot sl_{vk} \cdot y_k}, v=1,2,3, k=1,2,\dots,7. \quad (2)$$

Here, c_v is the bunker capacity for combine type v (Table 2), sl_{vk} and w_v are speed and working width of combine type v on the parcel k (Table 2), and y_k stands for crop yields of the parcel k .

Table 2: Capacity c_v and loading time tb_{vk} of bunker for combine type v on the parcel $k, p \in \{1,2\}, k \in \{1,2,\dots,7\}$				Table 3: Two trailers of capacity ct_p , unloading time tu_p and tractor speed s on asphalt		
	c_v, t	Oilseed rape, h $k=1,2$	Wheat, h $k=3,4,5$	Winter barley, h $k=6,7$	capacity $ct_1 \in \{2,8\} t$	capacity $ct_2 \in \{2,7\} t$
JD	7	$tb_{1k}=0.65$	$tb_{1k}=0.65$	$tb_{1k}=0.65$	unloading time, □ h	
C	7	$tb_{2k}=0.71$	$tb_{2k}=0.71$	$tb_{2k}=0.71$	$tu_1 \in \{\square\square\square\} h$	$tu_2 \in \{\square\square\square\} h$
Z	5	$tb_{3k}=0.83$	$tb_{3k}=0.83$	$tb_{3k}=0.83$	tractor speed on asphalt $s \in \{20\} kmh^{-1}$	

If the inequation (1) is not valid, which usually happens for more distant parcels, parcels with lower yield or combine harvesters with high harvesting speed, it is necessary to use more tractors and one driver per tractor in order to ensure that there will always be an available tractor with two trailers. Therefore, the required number of tractors nt_{vpk} with two trailers type $p, p \in \{1,2\}$, that should be additionally employed and which would be used on every parcel $k, k \in \{1,2,\dots,7\}$, for the combine type v (if the combine operates on the parcel k), is:

$$\left\lceil \left(\frac{d_k}{s_p} + \frac{d_k}{s} + tu_p \right) \cdot c_v / (tb_{vk} \cdot ct_p) \right\rceil = nt_{vpk} \quad (3)$$

for every $v, v \in \{1,2,3\}$ and $p \in \{1,2\}$. Here, $\lceil x \rceil$ represents the smallest integer which is higher than x , while ct_p represents the capacity of trailer type p (Table 3), and c_v is the bunker capacity for combine type v (Table 2). One additional tractors are required on the parcel $k=1,3,6$, and 7 for all combine and both trailer type. On the 5-th parcel, there is no need for additional tractor in the case of combine Z with 7 t trailers, only.

Finally, Table 4 shows the productivity values s_{vpk1} of combine and s_{4pk2} of tractor needed for model (5-11).

Table 4: Combine productivity s_{vpk1} and tractor productivity $s_{4pk2}, v=1,2,3, p \in \{1,2\}, k \in \{1,2,\dots,7\}$

Combine or tractor type		Harvesting productivity on the parcels			
		Oilseed rape $k \in \{1,2\}$	Wheat $k \in \{3,4,5\}$	Winter barley $k \in \{6,7\}$	
JD	$s_{1pk2}, ha h^{-1}$	$s_{1pk1} \in \{3.3\}$	$s_{1pk1} \in \{3.6\}$	$s_{1pk1} \in \{3.9\}$	
KJ	$s_{2pk1}, ha h^{-1}$	$s_{2pk1} \in \{\square\square\} .12$	$s_{2pk1} \in \{\square\square\} .42$	$s_{2pk1} \in \{\square\square\} .72$	
Z	$s_{3pk1}, ha h^{-1}$	$s_{3pk1} \in \{2.1\}$	$s_{3pk1} \in \{2.25\}$	$s_{3pk1} \in \{2.4\}$	
Transport productivity from the parcels to silos					
		$p \in \{1\}$	$p \in \{2\}$	$p \in \{1\}$	$p \in \{2\}$

M	$s_{4pk2}, \text{ t h}^{-1}$	$s_{1pk1} \square 3.9$	$s_{1pk1} \square 3.9$	$s_{1pk1} \square 3.9$	$s_{1pk1} \square 3.9$	$s_{1pk1} \square 3.9$	$s_{1pk1} \square 3.9$	
		$s_{2pk1} \square 72$	$s_{2pk1} \square 72$	$s_{2pk1} \square 72$	$s_{2pk1} \square 72$	$s_{2pk1} \square 72$	$s_{2pk1} \square 72$	$s_{2pk1} \square 72$
		$s_{3pk1} \square 2.4$	$s_{3pk1} \square 2.4$	$s_{3pk1} \square 2.4$	$s_{3pk1} \square 2.4$	$s_{3pk1} \square 2.4$	$s_{3pk1} \square 2.4$	$s_{3pk1} \square 2.4$

Combine operating productivity s_{vpki} is calculated based on the equation (4) for combine type v and for tractor with trailers type p on the parcel k .

$$s_{vpki} = \frac{w_v \cdot s_{l_{vk}}}{10}, v = 1,2,3; k = 1,2,\dots,7 \quad \text{and} \quad s_{4pk2} = \frac{ct_p \cdot s_p}{d_k}, p = 1,2; k = 1,2,\dots,7 \quad (4)$$

Formulation of linear programme. The problem of linear programming was solved by using the software Mathematica (Wolfram, 2011). This software is applied on problems related to agriculture (Matić-Kekić et al., 2011) and optimisation (Savin et al., 2014). The advantage of this software is that one can easily handle the problems while programming if the problems include matrices, lists, numerical fitting, integral calculus, symbolic calculus, statistics, etc. The suggested model: 1) minimizes total harvesting time for a real situation in the farm company; 2) determines the type of combine and period during which it will be used on each parcel; 3) determines the type of trailer that will be used during the transport of crops from the parcel to silos.

Objective function. The objective function which minimizes the total operating time needed for harvest completion and transport operations during the harvesting agrotechnical period ($D=20$ days) is given in (5).

$$F = \min \left(\sum_{v=1}^3 \sum_{p=1}^2 \sum_{k=1}^7 t_{vpk1} + \sum_{p=1}^2 \sum_{k=1}^7 t_{4pk2} \right) \quad (5)$$

Here, t_{vpk1} represents the time that one combine type v , served by trailer type p on the parcel k , spends during the harvesting process. Time spent during the crop transport from parcel k to silos, using tractors with trailers type p , is denoted as t_{4pk2} . The objective function is subject to four types of constraints.

Constraint type Ia: Harvesting must be completed on all parcels. This type of constraint implies that all crops on the parcel k have to be harvested (operation $i \square 1$) Constraint for each parcel is needed

$$\sum_{v=1}^3 \sum_{p=1}^2 s_{vpk1} \cdot t_{vpk1} = a_k \quad \text{for } k \square 1,2,\dots,7 \quad (6)$$

where s_{vpk1} (see equation (4) and Table 4) is operating productivity per combine type v , served by trailer type p on the parcel k during the harvest ($i \square 1$), and a_k is the surface area of parcel k .

Constraint type Ib: Harvested crops must be transported to silos. This type of constraint implies that all harvested crops have to be transported by tractors ($v \square 4$). The number of loaded pairs of trailers with the capacity of 8t ($p=1$) or 7t ($p=2$) must be equal to the number of trailers with the same capacities transported to the silos:

$$\sum_{v=1}^3 y_k \cdot s_{vpk1} \cdot t_{vpk1} = s_{4pk2} \cdot t_{4pk2} \quad \text{for } p \square 1,2 \text{ and } k \square 1,2, \dots,7, \quad (7)$$

where y_k represents the crop yields of the parcel k and s_{4pk2} (Table 4) is the tractor productivity with loaded trailer type p on the parcel k during the transportation ($i \square 2$).

Constraint type IIa: Working hours of available combines and tractors must not be exceeded. Number of working days D in the observed period, multiplied by the number of working hours per day wh , gives maximum working hours for each combine or tractor. These constraints ensure that the total working hours of combines type v (first constraint in (8)) and tractors (second constraint in (8)) never exceed the limit. Restriction for each combine type v and for each tractor is represented by

$$\sum_{p=1}^2 \sum_{k=1}^7 t_{vpk1} \leq n_v \cdot D \cdot wh \quad \text{for } v \square 1,2,3 \text{ and} \quad \sum_{p=1}^2 \sum_{k=1}^7 \sum_{v=1}^3 (1 + nt_{vpk}) \cdot t_{vpk1} \leq n_4 \cdot D \cdot wh \quad (8)$$

where n_v is the number of combines type v ; n_4 is the number of tractors; D is the number of working days in the observed period; nt_{vpk} is the number of tractors that should be additionally employed for the combines type v with trailer p on the parcel k , and wh is the number of working hours per day. Values of n_1, n_2, n_3 and n_4 are 1, 1, 2 and 15, respectively. The second inequation (8) shows the operating time of one tractor expressed as total harvest time t_{vpk1} , since tractors are involved in either transport and reloading during the harvest, or wait on the parcel until their

trailers are loaded. The total operating time of a tractor should also include the operation times of additionally employed tractors which number is denoted by nt_{vpk} .

Constraint type IIb: Working hours of available trailers must not be exceeded. This constraint refers to the total working hours of trailers type p , on different parcels, serving the combines type v :

$$\sum_{v=1}^3 \sum_{k=1}^7 (1 + nt_{vpk}) \cdot t_{vpk1} \leq m_p \cdot D \cdot wh \quad \text{for } p=1,2 \quad (9)$$

where m_p is the number of pairs of trailers type p ($m_1=m_2=18$).

Constraint type III: Total working hours of manpower must not be exceeded. This constraint implies that the total working hours per combine and tractor operator should never be exceeded. Then, the following constraint must be met:

$$\sum_{v=1}^3 \sum_{p=1}^2 \sum_{k=1}^7 t_{vpk1} \cdot (2 + nt_{vpk}) \leq W \cdot D \cdot wh \quad (10)$$

where W is the number of needed combine and tractor operators. Since every combine needs to operate with at least one tractor for grain transport, in the brackets in inequation (10), is the sum of two operators (one combine and one tractor operator) with the number of additionally hired tractor operators (nt_{vpk}).

Constraint type IV: Unknown variables should be non-negative. This constraint is quite logical because the total time per combine type v , served by trailer type p , on the parcel k during the operation i cannot be negative:

$$t_{vpki} \geq 0 \quad \text{for } v \in \{1,2,3,4\}, p=1,2, k=1,2,\dots,7 \text{ and } i=1,2. \quad (11)$$

RESULTS AND DISCUSSION

Objective function (5) with constraints (6-11) reaches minimum $F_{min} = 252.5 \text{ h} = 217.5 \text{ h}$ (harvesting time) + 35 h (transport time from parcels to silos), with the involvement of all available combines at the farm (Table 5). Operation time for combines JD and C is 60 h, while two combines Z work together 97.5 h. For example, harvesting time for combine JD at parcel $k=3$ is 16.8 h. This combine is served by a tractor with two trailers capacity 8 tons which spends 3.4 h transporting grain from parcels to silos. Values of operating times t_{4pk2} for tractors M (Table 5), represent time spent during crops transport from parcel to silos, only. Tractors M, with two trailers of capacity 8 tons (7 tons), during transportation, spend 18.8 h (16.2 h).

Table 5. Total operating time required for harvesting and transport using all available resources at farm "Pobeda"

Parcel	Oilseed rape				Wheat						Winter barley				F_{min} (h) 252.5	
	k=1		k=2		k=3		k=4		k=5		k=6		k=7			
Trailers	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	Total (h)	
JD	t_{1pk1}	0.0	0.0	0.0	0.0	16.8	0.0	0.0	2.6	37.3	3.3	0.0	0.0	0.0	0.0	60.0
C	t_{2pk1}	0.0	0.0	0.0	0.0	0.0	0.0	12.1	0.0	0.0	0.0	0.0	7.8	0.0	40.1	60.0
Z	t_{3pk1}	50.5	0.0	9.5	0.0	0.0	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	97.5
M	t_{41k2}	8.0	-	0.3	-	3.4	-	1.1	-	6.0	-	0.0	-	0.0	-	18.8
	t_{42k2}	-	0.0	-	0.0	-	4.7	-	0.3	-	0.5	-	2.3	-	8.4	16.2

Since working time is 10 hours per day and harvest on the farm is done for 60 working hours (the harvest organisation is given in Table 5), it follows that theoretically 6 working days is needed for harvest. However, it is not realistic because of the possibility of bad weather and malfunctions. It is important to consider the fact that not every working day can be used during the specified agrotechnical period. The number of working days is a function of climate conditions, soil type, terrain inclination and performed operation (ASAE D497.6 JUN2009).

Probability of working days spent during an agrotechnical period, represents a ratio between the numbers of working days during which the harvesting is performed and total number of available days in the specified agrotechnical period for a given region. The influence of climatic factors on the number of available working days in different regions was investigated by a considerable number of authors, such as Dyer (1980), Rotz et al. (1983), while in the Republic of Serbia important research was conducted by Nikolić (1983), Savin (2004) and Mileusnić et al. (2010). The real number of working days also depends on the *operational reliability* of combine harvester. Operational reliability is defined as the statistical probability that machine will function under specified conditions at any given time (ASAE D497.6 JUN2009). Probability of real working days spent during an agrotechnical period for

the harvesting operation in the observed region was calculated to be 0.81. The operational reliability is high and it is 0.94 for considered type of combines. Harvest of oilseed rape, wheat and barley is the first one to be performed, since the soybean, sunflower and corn harvests are conducted later in autumn. Operational reliability is expected to be high since prior to the harvest combines are technically prepared. According to this, the product of probability of working days and operational reliability (here: *meteo-technical coefficient*) is $0.81 \cdot 0.94 \approx 0.76$. It increases theoretical number of working days (D^{opt}) to the real number of working days (D^{real}) needed for harvesting to, i.e. $D^{real} \approx D^{opt}/0.76$. Under the considered conditions (meteorological and technical), maximum real number of working days spent on combine harvesting, during an agrotechnical period, is $D \cdot 0.76 = 20 \cdot 0.76 \approx 15$ days.

Post-hoc analysis. Post-hoc analysis should give an answer to the question which organisation of the harvest and renting provides the highest profit. Organisation of the harvest and renting includes the following rules: 1. if the combine harvests in the considered farm, it cannot be rented until the harvest of the farm is completed; 2. if the combine is rented at the beginning of harvest, then it remains rented till the end of the harvest. In the example $l=3$ (Table 6), $n_2^u = 0$ means that combine C will be rented from the beginning of harvest for $D_2^{rent} = 15$ working days, while $n_1^u = 1$ and $n_3^u = 2$ mean that combine JD and two combines Z will harvest on parcels "Pobeda" for $D^{opt}=8$ working days (11 real days), firstly, and then will be rented for 7 working days ($D_1^{rent} = 7, D_3^{rent} = 2 \cdot 7 = 14$), while maximum theoretically number of days for renting are $D_1^{rent,max} = 12, D_2^{rent,max} = 20$ and $D_3^{rent} = 2 \cdot 12 = 28$. Suggested model (5-11) gave output values D^{opt} (Table 6) for all eleven values of input parameters n_1^u, n_2^u, n_3^u .

If no malfunctions occur in the period of 20 days, and if every day is suitable for the field work, then the maximum number of renting days, marked as $D_v^{rent,max}$, is calculated for every combine type v . Maximum profit gained in this way is marked as $T_l^{rent,max}$. The real profit from combine renting is marked as T_l^{rent} .

Table 6. Inputs: n_1^u, n_2^u, n_3^u are the number of combines type JD, C and Z operating in the parcels, respectively; Outputs: D^{opt} and D^{real} are the minimum number and real number of working days needed in order to complete field and transport operations on the farm, respectively; $D_v^{rent,max}$ and D_v^{rent} are the maximum and real number of days for combine type v renting, respectively; $T_l^{rent,max}$ and T_l^{rent} are the maximum and real total profit from combines renting for l -th example. Subscripts 1, 2 and 3 correspond to combines type JD, C and Z, respectively.

l	l -th example					$D_v^{rent,max}$			D_v^{rent}			$T_l^{rent,max}$		T_l^{rent}
	n_1^u	n_2^u	n_3^u	D^{opt}	D^{real}	$v=1$	$v=2$	$v=3$	$v=1$	$v=2$	$v=3$	dinars ^a	€	€
1	1	1	2	6	8	14	14	28	9	9	18	4,298,842	41,736	26,830
2	0	0	2	15	20	20	20	10	15	15	0	4,727,062	45,894	30,988
3	1	0	2	8	11	12	20	24	7	15	14	4,506,075	43,748	28,842
4	0	1	2	9	12	20	11	22	15	6	12	4,368,696	42,415	27,509
5	1	1	1	7	9	13	13	33	8	8	23	4,321,748	41,959	27,053
6	1	1	0	10	13	10	10	40	5	5	30	4,013,362	38,965	24,059
7	1	0	1	11	14	9	20	29	4	15	19	4,411,420	42,829	27,923
8	0	1	1	12	16	20	8	28	15	3	18	4,343,516	42,170	27,264
9	1	0	0	18	24	Harvest not completed in the agrotechnical period								
10	0	1	0	19	25	Harvest not completed in the agrotechnical period								
11	0	0	1	29	38	Harvest not completed in the agrotechnical period								

^aOne euro was equal to 103 dinars during the period June – July 2011.

Renting profit. In order to calculate the profit gained from renting, total expenses e_v^{rent} and renting price p_v^{rent} per combine type v per hour (dinars h^{-1}) must be taken into account. Therefore, the following factors were taken: renting price of 6000 dinars per hectare for one combine, agrotechnical period of 20 days for harvesting (from 1st July to 20th July), number of working days when harvesting is performed on the parcel (in the example $l \square 1$, there are 6 working days, Table 6). Other data needed for the calculation are: number of working hours per day (10), fuel consumption per combine (JD - $54 \text{ l } h^{-1}$, C - $49.5 \text{ l } h^{-1}$ and Z - $32 \text{ l } h^{-1}$), considering that one litre of euro-diesel fuel costs 130 dinars, while one litre of diesel fuel costs 117 dinars. Sum of total (variable and fixed) costs per combine type v , which are the costs of fuel and lubricants, personal income of combine operator, amortization costs, costs for the maintenance of combine technical validity, insurance and loan costs are all included in e_v^{rent} , Savin (2004). Total

expenses e_v^{rent} and renting price p_v^{rent} per combine type v per hour (dinars h⁻¹) are equal 10,588.51 and 21,600.00, 10,253.08 and 20,520, 8786.20 and 13,500.00 per combine JD, C and Z, respectively. The real profit from combine renting is calculated by $T_l^{rent} = \sum_{v=1}^3 (p_v^{rent} - e_v^{rent}) \cdot D_v^{rent} \cdot wh$, for l-th example in Table 6. The least profit from combine

renting is in the example l=6 (Table 6), when harvest can be completed in 13 real days (100 working hours) with combines JD and C, while the renting is performed during the remaining 7 real days (50 working hours), in comparison to the two combines Z which can be rented for 20 real days (2·15·10=300 working hours).

The highest profit can be achieved with the types of combines that have the greatest engine power and which are used for renting only, like in the example l=2. In that case, harvesting period on the farm company is prolonged 2.5 times. This example can hardly be considered as a global optimal solution due to the increased risk of bad weather in the period of 20 days during which harvest is performed.

Global optimal solution. Two conflicting requests: (a) to harvest crops on the “Pobeda” Farm Company as fast as possible and (b) to achieve the maximum profit from combine harvester rentals, can be met by three organisations of the harvest (basic parameters are given in Table 7) of all possible organisations of the harvest (Table 6). If it requires only (a), the optimal solution is given in Table 5. In the case that requires only (b), the optimal solution is given in Table 8. If both requests (a,b) need to be fulfilled, then global optimal solution is given in Table 9.

Table 7. Number of engaged combines JD^e, C^e, Z^e, tractors M^e, number of trailers, number of hired operators W^e, working hours wh^a per day and minimum days D^{opt} needed for the harvest completion at considered farm household

	JD ^e	C ^e	Z ^e	M ^e	8 t	7 t	wh (h)	W ^e	D ^{opt}
	n ₁ ^u	n ₂ ^u	n ₃ ^u	n ₄ ^u	m ₁ ^u	m ₂ ^u			
Request (a)	1	1	2	7	8	6	10	11	6
Request (b)	0	0	2	4	8	0	10	6	15
Requests (a,b)	1	0	2	6	6	6	10	9	8

^aworking shift is from 7am to 8pm

Optimal solution for the observed problem (5-11), with request (a), is given in Table 7. This solution suggests engagement of all available combines so that the harvest can be completed in the shortest period possible. However, this engagement still does not provide the maximum profit. Maximum profit (Table 6, l=2) can be achieved by the organisation of the harvest given in Table 8, but with harvesting period prolonged from 6 to 15 days.

Table 8. Total operating time required for harvesting and transport for the input parameters given in Table 7 at farm “Pobeda”, request (b)

Parcel	Oilseed rape				Wheat				Winter barley				F _{min} (h)		
	k=1	k=2	k=3	k=4	k=1	k=2	k=3	k=4	k=1	k=2	k=3	k=4	321.1		
Trailers	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	Total (h)
JD	t _{1pk1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	t _{2pk1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Z	t _{3pk1}	50.5	0.0	9.5	0.0	64.4	0.0	22.7	0.0	64.9	0.0	12.1	0.0	62.1	286.2
M	t _{41k2}	8.0	-	0.3	-	8.1	-	1.4	-	6.5	-	2.3	-	8.4	35.0
	t _{42k2}	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Example from Table 9 could be considered as the most favourable organisation of the harvest, considering the two opposite goals (a,b): the fastest harvest on the parcel and the highest profit from renting.

Table 9. Total operating time required for harvesting and transport for the input parameters given in Table 7 at farm “Pobeda”, requests (a,b)

Parcel	Oilseed rape				Wheat					Winter barley				F _{min} (h)	
	k=1	k=2	k=3	k=4	k=3	k=4	k=5	k=6	k=7	k=6	k=7	k=6	k=7	272.1	
Trailers	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	16 t	14 t	Total (h)
JD	t _{1pk}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.4	0.0	7.4	0.0	38.2	80.0
C	t _{2pk}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Z	t_{3pk}	0.0	50.5	9.3	0.2	0.0	64.4	22.7	0.0	0.0	9.9	0.0	0.0	0.0	157.0
M	t_{41k}	0.0	-	0.3	-	0.0	-	1.4	-	5.5	-	2.3	-	8.4	17.9
	t_{42k}	-	8.0	-	0.1	-	8.1	-	0.0	-	1.0	-	0.0	-	17.2

CONCLUSION

Linear programme for minimising total harvesting time was applied to “Pobeda” Farm Company from Vojvodina, Serbia, located in North Bačka district. Out of 1380 ha (soil type: clay loam), “Pobeda” Farm Company has: wheat on 342 ha (25%), winter barley on 178 ha (13%) and oilseed rape on 126 ha (9%). The developed programme is flexible since it can be easily modified for the examinations which include more parcels, combines, tractors, implements and operators.

One of the problem solutions shows that renting only the combines with good characteristics provides the highest profit, but then the number of days for harvesting increases 2.5 times in comparison to the minimum time needed for harvesting. For optimal solution can be taken the case when one JD and two Z combines harvest crops on the observed farm for 11 real days (80 working hours) and after that 9 real days (70 working hours) are rented. Similiar problem was solved by Savin et al. (2014) where the objective function included the risk of yield reduction due to bad weather conditions and the deliberate extension of the harvest on the primary farm. They created a general LP model and a profit maximization algorithm for harvesting during an agrotechnical period.

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OPTIMIZACIJA ŽETVE I POST-HOC ANALIZA

Snežana MATIĆ-KEKIĆ, Lazar SAVIN, Nebojša DEDOVIĆ

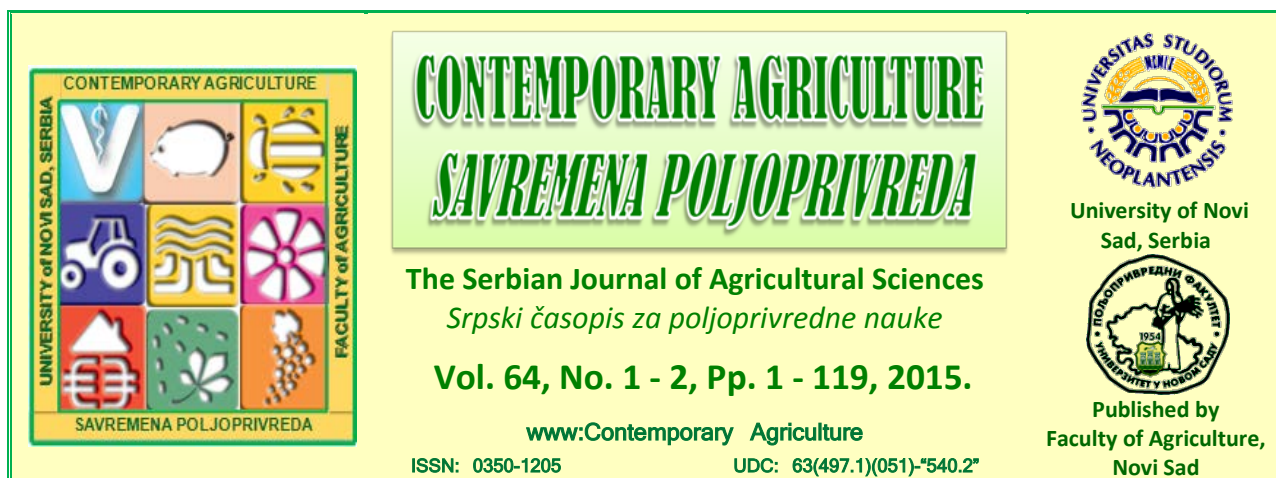
Izvod: Formiran je model koji minimizira ukupno vreme žetve i vreme transporta useva do silosa u cilju dobijanja maksimalnog profita od iznajmljivanja kombajna i prodaje useva. Nezavisne operacije u polju su međusobno povezane. Dobijeno optimalno vreme rada potvrđuje da se koristi više ljudske snage i više

poljoprivrednih mašina nego što je potrebno. Ovo je kasnije korišćeno u post-hoc analizi koja određuje profit od iznajmljivanja kombajna.

Ključne reči: optimizacija, maksimalan profit, optimalno vreme rada , mašinski park.

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YIELDING OF DIFFERENT MATURITY GROUPS POTATOES AT THREE SITES AND TUBER QUALITY DURING STORAGE*

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Summary: Two-year field trials (2001-2002) were conducted with aim to investigate the effect of growing site and maturity group of ten potato cultivars on yielding and changing of tuber quality during long-term storage. The biggest number of tubers per plant and highest mean tuber weight were obtained in Čačak, what gave the highest yields in both seasons; the lowest values were obtained in Guča. Favourable soil characteristics had more important role than weather conditions on potato field performance. Early cultivars gave the smallest number of tubers per plant and highest mean tuber weight, while opposite to this was recorded in mid-late to late cultivars (ML-L). The share of tubers >55 mm was consistently high in early cultivars. ML-L maturity group gave the highest yields at all sites and the highest initial tubers dry matter (DM), >20% in 2001 and >21% in 2002, while it was <20% in early cultivars overall experiment. Constant increase in DM content and decrease in starch content in tubers DM was recorded after two-month and seven-month storage under conditions with ventilation system without control of humidity. The effect of site on tuber DM was significant at mid-early to mid-late (ME-ML) and ML-L in 2002, when it was the highest in tubers originated from Sombre; there was no effect on starch content. Presented study showed that high yields can be achieved by growing potato cultivars of different maturity groups and Čačak has been shown to be the site with good agro ecological conditions; ME-ML (with exemption of cv. Condor) and ML-L groups gave the highest yields of tubers with DM content sufficient for processing for French fry and chips.

Key words: potato cultivars, maturity groups, storage, dry matter, starch.

INTRODUCTION

Storage capacities for both seed and ware potato are limited in Serbia, still less than 10% of total potato production (Bročić and Stefanović 2012). In comparison with developed countries, storage losses for both ware and seed potato are much higher due to lack of control of air temperature and humidity. Storage conditions should

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provide good quality potato suitable for processing. Various properties of tubers change during storage, causing losses (for review see Wustman and Struik, 2007). During storage starch is converted into sugars and temperatures below 4°C have been shown to enhance this process. Also, losses are affected by duration of storage.

In Serbia, large number of potato cultivars of different origin and quality are grown in regions differing in agroecological conditions. Potato breeding is very intensive and continuous, so newer cultivars are present in Serbia, too. Due to limited and inadequate storage capacities there is lack of results showing the effect of storage on the post harvest quality of potato grown in Serbia. The origin of tubers was mainly investigated as a potential source of biological viability in seed potato Poštić et al. (2012), while Milošević et al. (2012) indicated that production site might have significant impact on seed potato quality.

The aim of presented study was to investigate productivity of potato cultivars of different maturity groups grown at three sites, as well as changing in tubers dry matter (DM) and starch content during 7-month storage.

MATERIAL AND METHODS

Ten potato cultivars of different maturity groups: early (Jaerla, Cleopatra); mid-early to mid-late – ME-ML (Frisia, Red Star, Kondor); mid-late to late – ML-L (Bintje, Desiree, Van Gogh, Asterix, Agria) were grown at three experimental sites in Serbia: Sombor (85 m asl), Čačak (240 m asl) and Guča (370 m asl) in 2001 and 2002. Crops were grown on loam soil (chernozem) in Sombor, sandy loam (alluvium) in Čačak and clay loam (pseudogley) in Guča. Seed material was pre-sprouted; planting dates were April 10th at Sombor and Čačak and April 20th at Guča in both seasons. Planting pattern was 70 x 35 cm and size of the plots was 15 m², with two border rows were. Experiment was established as randomized completely block design with three replications. There was no irrigation and basic soil properties at 0-20 cm depth were: in Sombor (pH_{KCl}–7.5, P₂O₅– 23.5 mg 100 g⁻¹, K₂O–27.9 mg 100 g⁻¹, CaCO₃–7.6%, organic matter 3.4%, total N–0.25%); in Čačak (pH_{KCl}– 5.9, P₂O₅–30.2 mg 100 g⁻¹, K₂O –19.6mg 100 g⁻¹, CaCO₃–1.5%, organic matter 2.9%, total N–0.2%); in Guča (pH_{KCl}– 4.3, P₂O₅–3.5 mg 100 g⁻¹, K₂O – 23.7mg 100 g⁻¹, CaCO₃–0.1%, organic matter 2.0%, total N – 0.3%). All plots were fertilized with NPK mineral fertilizer (15:15:15): 150 kg ha⁻¹ N, 150 kg ha⁻¹ P₂O₅ and 150 kg ha⁻¹ K₂O. In Sombor and Čačak early and ME-ML cultivars were harvested during the first week of September and at third week of September in Guča in both seasons. ML-L cultivars were harvested 7-10 days later with aim their tubers skin to be fully developed. Number of tubers, mean tuber weight per plant and tuber size were determined at the harvest using ten plants.

Data on weather conditions recorded at three sites during two potato growing seasons are given in Table 1. In 2001, at Sombor and Čačak rainfall sum was lower in comparison to 30-year average, due to lower rainfall recorded in May, June and September. In 2002, in Sombor due to high rainfall in July (108 mm) higher rainfall sum was recorded in comparison 30-year average. In Guča amount of rainfall recorded in both seasons was similar but still lower than 30-year average. In 2002, slightly higher temperatures were recorded at all sites in June, July and August and the rest of data for the growing season was consistent with long-term data.

After harvest the tubers originating from the three sites were stored in the facility situated in Guča. The store was equipped with ventilation system with air recirculated through floor, without control of humidity. Air temperature was maintained at 3-4°C. The samples for dry matter (DM) content in tubers and starch content in DM determination were made by mixing tubers of different sizes. Measurements were determined at harvest, two-month (2-m) and seven-month (7-m) after storage, with three replications. Tuber DM content was determined by drying tubers at 105°C. Starch in tuber DM was determined by Ewers' polarimetric method based on the partial HCl hydrolysis of starch, followed by measurement of the optical rotation of the gelatinized starch (specific rotation factor of 185.7°) (Thybo et al., 2006).

One-way ANOVA was performed to evaluate the effect of cultivar within each site and the effect of site within each cultivar. Means were compared by LSD test (p<0.05). Costat software package was used for processing obtained data.

Table 1. Weather conditions during potato growing season in Sombor (S.), Čačak (Č.) and Guča (G.) for 2001 - 2002 and 30 years mean data

Month	Monthly sum of rainfall (mm)									Mean monthly temperature (°C)								
	S.	Č.	G.	S.	Č.	G.	S.	Č.	G.	S.	Č.	G.	S.	Č.	G.	S.	Č.	G.
	2001			2002			30 years mean			2001			2002			30 years mean		
IV	55	44	10	45	23	32	45	58	77	7.7	7.2	5.7	12.6	13.0	11.2	11.6	10.2	9.5
V	24	42	60	58	50	96	60	75	60	17.2	16.0	16.0	16.0	14.2	14.9	17.1	15.2	14.0
VI	81	37	27	69	11	51	82	88	13	20.4	19.4	19.0	19.0	20.1	19.5	20.2	18.3	18.5
VII	79	50	98	10	39	50	66	76	10	20.2	19.6	20.5	20.5	21.2	21.9	21.0	20.0	20.2
VIII	45	98	93	74	11	42	53	60	56	20.6	19.6	20.5	20.5	21.2	21.4	21.3	19.5	19.5
IX	46	2	45	82	96	13	54	66	69	16.9	15.2	14.4	14.4	14.9	15.0	16.5	15.1	11.7
Mean/Sum	330	272	427	434	329	408	360	423	500	16.9	16.0	15.7	15.7	17.4	17.0	18.1	16.4	15.6

RESULTS

Number of tubers was significantly higher in ME-ML and ML-L in comparison to early cultivars overall experiment ($p < 0.05$) (Table 2). In early cultivars there were 9.2 tubers over three sites in both seasons, while ML-L gave the biggest number per plant (12.3 and 11.9, respectively). Only at Sombor in 2002, ME-ML cultivars gave bigger number of tubers than ML-L (11.7 and 10.8, respectively). In both seasons, cultivars of all maturity groups grown in Čačak had the biggest number of tubers. The effect of site within maturity groups was significant in both seasons ($p < 0.05$). Early cultivars gave the biggest tubers over all sites in both seasons (on average >90 g), while the smallest mean tuber weight was recorded in ML-L cultivars (average over three sites in both seasons around 75 g) (Table 3). The smallest mean tuber weight over three maturity groups was recorded in Guča in both seasons (81.1 g and 74.7 g, respectively), while it was the highest in Čačak (97.3 g and 92.6g, respectively). The effects of maturity group and site within each maturity group were significant ($p < 0.05$). Mean two-year results showed that early cultivar Jaerla had consistent share $>50\%$ of >55 mm tubers, mid-early to mid-late Kondor had $>60\%$ when grown in Sombor and Guča (Figure 3). Mid-late to late cultivar Agria had the highest share of >55 mm tubers, while tubers of 35-55 mm were dominating in this maturity group. The share of <35 mm tubers was less than 10% overall experiment; it was the lowest in early cultivars (1-3%). The highest tuber yields in all maturity groups were recorded in Čačak in both seasons, on average 38.8 t ha^{-1} and 40.4 t ha^{-1} (Table 4). Yields obtained in Sombor and Guča were similar in both seasons and significantly lower in comparison to Čačak within all maturity groups. Over three sites, ML-L group gave slightly higher yields than early and ME-ML ones in both seasons (34.6 t ha^{-1} and 34.3 t ha^{-1} , respectively). The effect of maturity group on tubers yield was significant overall experiment ($p < 0.05$), with exemption of Sombor in 2001. In Guča in 2001 the highest yield was obtained in early cultivars, while for the rest of the experiment this was the case with ML-L.

Table 2. Number of tubers per plant of potato cultivars of different maturity groups grown in Sombor, Čačak and Guča in 2001 and 2002

Maturity group (A)	Site (B)							
	2001				2002			
	Sombor	Čačak	Guča	Mean (A)	Sombor	Čačak	Guča	Mean (A)
Early	8.5a ^b	9.5b	9.5b	9.2	7.5a	11.0b	9.0a	9.2
ME-ML	12.3a	12.3a	10.7b	11.8	11.7a	12.3a	10.7a	11.6
ML-L	12.4a	12.6a	11.8a	12.3	10.8a	12.8b	12.2b	11.9
Mean (B)	11.1	11.5	10.7	11.1	10.0	12.0	10.6	10.9
LSD _{0.05} ^a	0.6	1.0	0.8		1.0	1.3	1.0	

^a LSD test – comparison between individual means of cultivars within each site; ^b – different letters in a row indicate significant difference between sites at $p < 0.05$; LSD test.

Table 3. Mean tuber weight of potato cultivars of different maturity groups grown in Sombor, Čačak and Guča in 2001 and 2002

Maturity group (A)	Site (B)							
	2001				2002			
	Sombor	Čačak	Guča	Mean (A)	Sombor	Čačak	Guča	Mean (A)
Early	89a ^b	102b	94c	95	92a	104b	81c	91.8
ME-ML	73a	99b	77 a	83	98a	89b	76c	87.7
ML-L	73a	91b	72a	78	70a	85b	68a	74.6
Mean (B)	97.3	97.3	81.1	85.6	86.7	92.6	74.7	84.7
LSD _{0.05} ^a	11.1	8.2	10.3		7.1	16.1	ns	

^a LSD test – comparison between individual means of cultivars within each site; ^b– different letters in a row indicate significant difference between sites at $p < 0.05$; LSD test.

Table 4. Tuber yield (t ha⁻¹) of potato cultivars of different maturity groups grown in Sombor, Čačak and Guča, 2001 and 2002

Maturity group (A)	Site (B)							
	2001				2002			
	Sombor	Čačak	Guča	Mean (A)	Sombor	Čačak	Guča	Mean (A)
Early	30.4a ^b	36.2b	35.4b	33.9	27.3a	40.8b	28.9a	32.3
ME-ML	30.8a	37.5b	31.5a	33.3	31.0a	39.0b	29.7a	33.3
ML-L	31.9a	42.8b	29.0a	34.6	30.5a	41.3b	31.2a	34.3
Mean (B)	31.0	38.8	31.9	33.9	29.6a	40.4b	29.9a	33.3
LSD _{0.05} ^a	ns	1.2	1.3		1.3	1.2	1.7	

^a LSD test – comparison between individual means of cultivars within each site; ^b– different letters in a row indicate significant difference between sites at $p < 0.05$; LSD test.

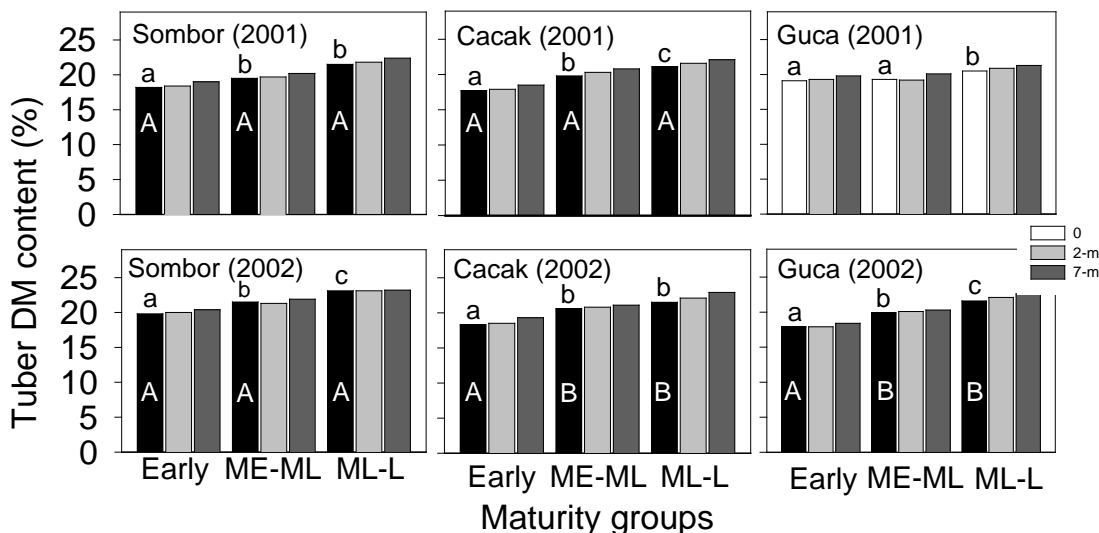


Figure 1. Tuber DM content (%) at harvest and during storage of potato cultivars of different maturity groups grown in Sombor, Čačak and Guča in 2001 and 2002. Measurements were performed at harvest (0), two months (2-m) and seven months (7-m) after storage. ME-ML (mid-late to late cultivars), ML-L (mid-late to late cultivars). Significant differences in initial tuber DM content between different maturity groups are indicated by different low case letters and between sites by different capital letters ($p < 0.05$).

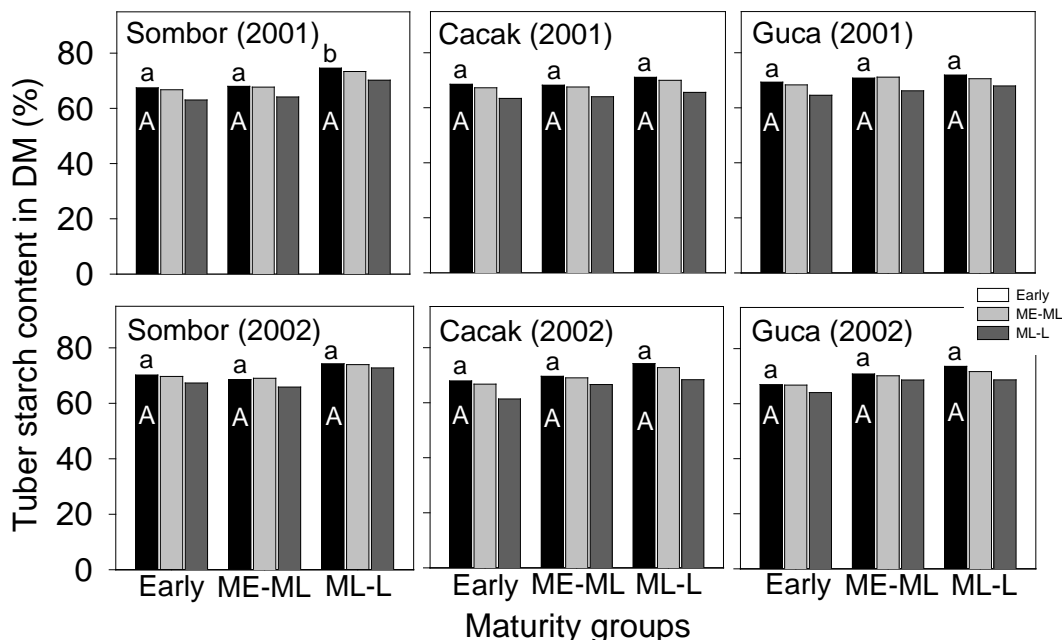


Figure 2. Tuber starch content in DM (%) at harvest and during storage of potato cultivars of different maturity groups grown in Sombor, Čačak and Guča in 2001 and 2002. Measurements were performed at harvest (0), two months (2-m) and seven months (7-m) after storage. ME-ML (mid-late to late cultivars), ML-L (mid-late to late cultivars). Significant differences in initial tuber DM content between different maturity groups are indicated by different low case letters and between sites by different capital letters ($p < 0.05$).

Initial tuber DM content of ML-L cultivars originated from three sites was $>20\%$ in 2001 and $>21\%$ in 2002, while it was $<20\%$ in early cultivars overall experiment; ME-ML cultivars gave tubers with DM content $\geq 20\%$ in 2002 (the exemption was cv. Kondor, with DM $< 20\%$, data not shown) (Figure 1). The effect of maturity group on initial DM content was significant overall experiment ($p < 0.05$). Constant increase in DM content was recorded overall experiment during storage. After 7-month storage DM content was increased 0.7 – 1.5% overall experiment. On average overall maturity groups, the lowest initial and final DM content were recorded at tubers originating from Guča in both seasons. In 2002, slightly higher values were recorded for Sombor in comparison to Čačak at all measurements in all maturity groups. The effect of site was significant at ME-ML and ML-L in 2002.

There was no significant effect of maturity group and site on initial starch content in DM in both seasons ($p < 0.05$) (Figure 2). Initial starch content $< 70\%$ was recorded in early cultivars overall experiment. In ME-ML cultivars values $> 70\%$ were obtained at plants grown in Guča in 2002, while in ML-L this was the case at all sites and both seasons. After 2-month and again after 7-month storage starch content was decreased in all groups and sites. During 7-m storage, starch content was decreased overall experiment for 2-6% without clear pattern. Values obtained for three sites were similar, showing the same trend of decrease of starch content in DM.

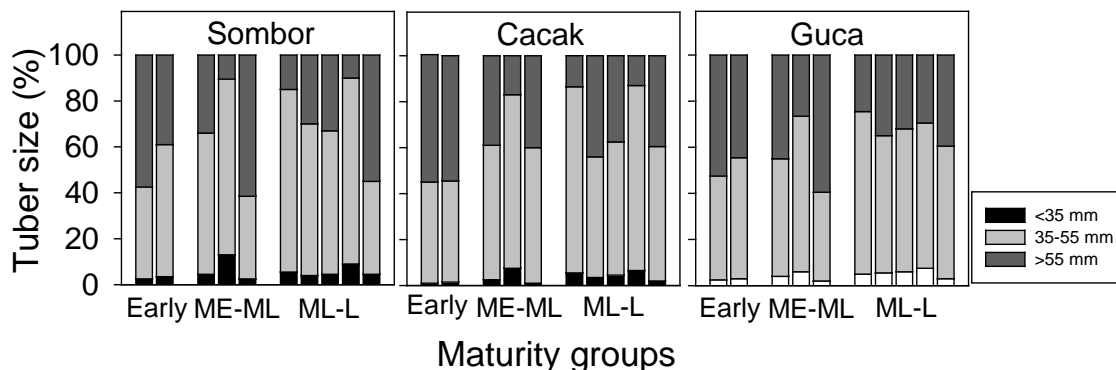


Figure 3. Size of tubers of ten potato cultivars of different maturity groups grown in Sombor, Čačak and Guča. Shown results are mean values for 2001 and 2002. ME-ML (mid-late to late), ML-L (mid-late to late). Each column represents a cultivar; the order of cultivars is as listed in Materials and methods.

DISCUSSION

Presented results showed that high tuber yields, much higher than 14.2 t ha^{-1} , which is average potato tuber yield for Serbia (Statistical Year Book of the Republic of Serbia, 2014) could be achieved by growing early, ME-ML and ML-L cultivars (Table 4). At the same time, the highest yields in all maturity groups were recorded in Čačak. Rainfall sum recorded in Čačak was much lower than in Sombor and Guča in both seasons, indicating that weather conditions did not have strong effect on yielding over two-year study. As suggested by Ilin et al. (2000), rainfall sum of 460-480 mm for potato growth season is considered as sufficient. During our study, lower rainfall sum than proposed was recorded at all sites in 2001 and in 2002 it was higher only in Sombor (Table 1). It could be taken under consideration that high rainfall recorded in Čačak in June 2002 contributed to obtaining higher yields than in 2001. Although some authors highlighted that number of tubers per plant is determined by cultivar (Bročić et al., 2000; Đorđević, 2000) seems that soil characteristics played an important role for obtaining the biggest number of tubers per plant of all tested maturity groups in Čačak in both seasons (Table 2); sandy loam soil had good texture and high level of ground water.

In Sombor, loam soil also had good texture, but due to its dark colour it warms up quickly. It is known that optimal temperature for tuber formation is 20°C (Bročić and Stefanović 2012). The effect of temperature depends upon cultivar and part of the plant affected. In presented study, in Guča, poor soil conditions such as texture might be associated with small number of tubers in some cases and lower mean tuber weight in comparison to other sites (Table 2, Table 3). According to Firman (2008) number of tubers per stem is generally lower on clay soils than average number on coarse loams. Haverkort and Anisimov (2007) also stated that even in clay soils the majority of tubers were of 40-80 mm size class at soils with better texture and 40-60 mm size class was dominating at soils with less favourable physical properties, but this was not the case in all tested cultivars in presented study (Figure 3). Cultivars such as Kondor and Red Star which gave the smallest and biggest number of tuber, respectively (data not shown) had slightly higher share of $>55 \text{ mm}$ tubers in Guča in comparison to other sites (Figure 3). Tubers size is one of the main characteristics of potato cultivar, but it could be also affected by growing practices, number of tubers per plant, length of stolons, etc; this is why data on share of different tubers size is presented for each cultivar.

Quality of potato tubers, such as DM content is of great importance for processing industry. For French fries processing 19-23% DM is needed, while for crisps it is 22-24% (Kabira and Berga 2003). Presented results clearly showed that ME-ML and ML-L cultivars give the best quality tubers at harvest while tested early cultivars are not suitable for processing (Figure 1). Our results are in agreement with findings by Zhang et al. (2002) who stated that DM content differs between potato cultivars, while Alva et al. (2007) found similar. In presented study, the lowest DM content at harvest overall sites was recorded in Guča in both seasons. This could be a result of the highest rainfall sum recorded in Guča among all sites in 2001 (Table 1) and also the highest altitude. Long shape tubers and oval-long shape tubers, with diameter $>55 \text{ mm}$ are used for French fries processing and oval tubers with size 40-60 mm are used for chips. Our results showed that ML-L high quality cultivar Agria which is used for French fries processing gave $>50\%$ of tubers with $>55 \text{ mm}$ diameter over two seasons when grown in Sombor and 40% in Čačak

and Guča (Figure 3). Also, ML-L cultivars Van Gogh and Asterix which are suitable for chips processing gave good quality tubers under different agroecological conditions, which is confirmed by our study (Fig 1).

Asmamaw et al. (2010) reported significant interaction of cultivar and growing environment with respect to DM content. In contrast to this, our results showed that effect of site was not significant (Figure 1). Constant increase in DM content was observed during long-term storage overall experiment (Figure 1).

In presented study initial starch concentration in DM was around 70% overall experiment. During storage starch content decreased in all maturity groups, but it was maintained over 60% after 7-m storage. Content of the starch is determined by cultivar (Jansen et al., 2001) but various environmental factors also affect it (Morrison et al., 2000). In our study, the highest initial starch content in tubers DM was recorded in ML-L cultivars in both seasons, with significant effect recorded only in Sombor in 2001 (Figure 2). On the other hand, it has been shown that potato cultivars might differ significantly in starch content (Casañas Rivero et al., 2003).

CONCLUSIONS

Growing potato cultivars of different maturity groups under different agroecological conditions can give high yields of good quality tubers. Among three tested sites Čačak has been shown to be the most suitable for growing of tested cultivars. Early cultivars had the biggest mean tuber weight and the highest share tubers >55 mm diameter. ME-ML (with exemption of cv. Kondor) and ML-L gave the highest yields of tubers with DM content sufficient for processing for French fry and chips. High quality cultivars which are used for processing also had high share of tubers of desirable size. As it was expected, DM content was increased and starch content in tuber DM was decreased while during long-term storage.

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PRINOS SORTI KROMPIRA RAZLIČITIH GRUPA ZRENJA GAJENIH NA TRI LOKACIJE I KVALITET KRTOLA TOKOM SKLADIŠTENJA

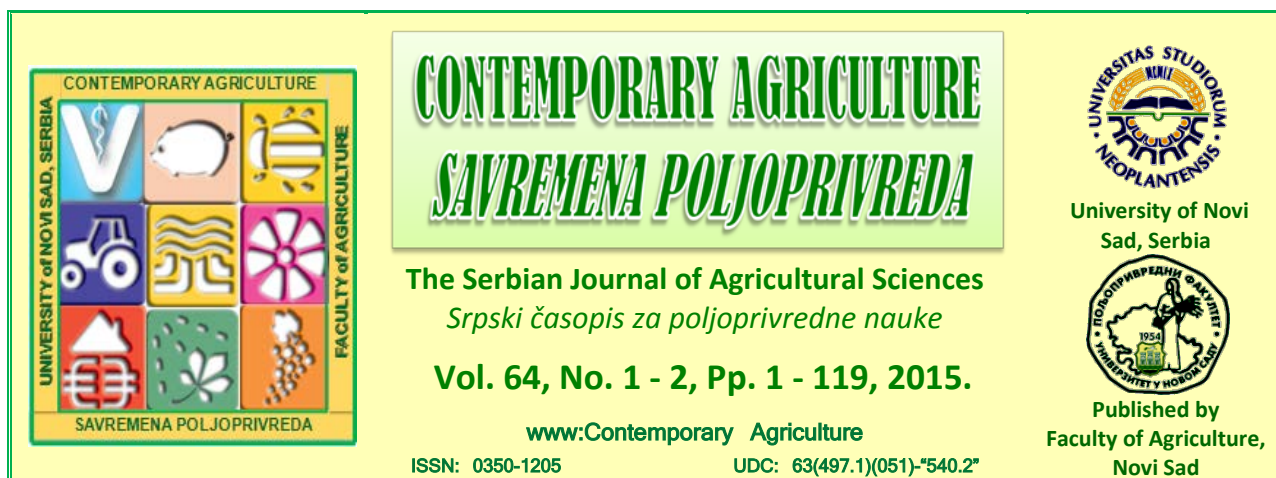
ZORAN BROČIĆ, DRAGO MILOŠEVIĆ, JASNA SAVIĆ

Izvod: Dvogodišnja istraživanja (2001-2002) su izvođena sa ciljem da se prouči uticaj lokacija (Sombor, Čačak, Guča) na kojima je gajen krompir i grupe ranostasnosti deset sorti na prinos i promene kvaliteta krtola tokom višemesečnog skladištenja. Najveći broj krtola po biljci i najveća prosečna masa krtola zabeleženi su u Čačku, što je dalo i najviše prinose u obe godine istraživanja, dok su najmanje vrednosti zabeležene u Guči. Povoljne osobine zemljišta u Čačku imale su značajniju ulogu nego klimatski uslovi na porast i prinosnost krompira. Ranostasne sorte su dale najmanji broj krtola po biljci i najveću prosečnu masu krtola, dok su suprotni rezultati zabeleženi kod srednje-kasnih do kasnih sorti. Najveći udeo krtola kod kojih je najveća dužina >55 mm bio je konzistentno visok kod ranostasnih sorti. Na sve tri lokacije najveći prinos zabeležen je kod srednje-kasnih do kasnih sorti, kao i sadržaj suve materije (SM) na početku skladištenja (>20% u 2001, >21% u 2002), dok je kod ranostasnih bila <20% na nivou celog eksperimenta. Nakon dva i sedam meseci skladištenja sa ventilacijom i bez kontrolisane vlažnosti, zabeleženo je kontinuirano povećanje SM i smanjenje sadržaja skroba u suvoj materiji krtole. Uticaj lokacije na kojoj je gajen krompir bio je značajan 2002. godine, u okviru srednje-ranih do srednje-kasnih i srednje-kasnih do kasnih sorti, kada je najveći sadržaj SM zabeležen kod krtola biljaka gajenih u Somboru; uticaj na sadržaj skroba nije bio značajan. Rezultati prikazanih istraživanja pokazali su da se gajenjem sorti sa različitim dužinom vegetacionog perioda na različitim lokacijama mogu postići visoki prinosi, a među njima Čačak ima agroekološke uslove koji najviše pogoduju proizvodnji krompira. Gajenjem srednje-ranih do srednje kasnih sorti (sa izuzetkom sorte Kondor) i srednje-kasnih do kasnih sorti postižu se najveći prinosi sa odgovarajućim sadržajem SM potrebnom za preradu u pomfrit i čips.

Ključne reči: sorte krompira, grupa zrenja, skladištenje, suva masa, skrob.

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EVALUATION OF COW'S ENERGY STATUS CHANGES DURING EARLY LACTATION BASED ON THE CONCENTRATIONS OF ORGANIC MILK INGREDIENTS

Đorđe SAVIĆ[♦], Stoja JOTANOVIĆ, Ivan STANČIĆ, Nermin PRAČIĆ, Marinko VEKIĆ¹

Summary: The aim of the study was to evaluate the energy status of cows ($n=132$) based on the concentration of organic milk constituents during early lactation. Cows were divided into three groups according to the stage of lactation (group A, $n=43$, 15-30 DIM; group B, $n=33$, 31-45 DIM; group C, $n=56$, 46 to 60 DIM). Concentrations of milk fat and protein and the milk fat: protein ratio tended to decrease, and the concentration of urea had increased towards to the end of the evaluated period. Statistically significant differences were found in concentrations of milk fat (41.58 ± 37.15 vs. 37.15 ± 3.63 and 36.11 ± 4.57 g/L, respectively), urea (2.86 ± 0.50 vs. 3.48 ± 0.69 and 3.61 ± 0.56 mmol/L, respectively) in all three groups, and milk fat : protein ratio in the group A compared to the other two groups, and also in lactose concentration between groups A and B (47.14 ± 0.29 vs. 47.71 ± 1.4 g/L). The ratio of the concentration of milk fat and protein, as well as relations between urea and protein showed a strong energy deficit in all tested cows, with more or less pronounced deficit or relative surplus of protein. Such condition negatively affects health status and milk production of evaluated cows, as well as their reproductive performance.

Key words: cows, lactation, energy status.

INTRODUCTION

Disruptions in energy status are one of the most important group of health disorders of dairy cows in a narrow period of transition from the dry to an early lactation period. This group of disorders has a particular importance for high-yielding cows, which had an extremely large load of metabolism during early lactation (Drackley, 1999, Šamanc et al., 2005a, Horvat et al., 2007). Due to the growing milk production and inability to enter sufficient amount of energy through diet negative energy balance (NEB) regularly appears during early lactation. Sinovec (2003) and Šamanc et al. (2005, 2006) suggest that, although glycogen body proteins participate in compensation of

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energy deficit, the main source of energy in this period is body fat reserves, i.e. lipomobilisation. Lipomobilisation is particularly pronounced in animals that were not adequately prepared to initiation of lactation or those animals that start the lactation in obese condition (Kampl, 2005; Goff, 2006). Fats mobilized from body depots are passing through the liver, thus limiting its synthetic and detoxifying capacity, which, together with NEB, can adversely affect the further cow's productive and reproductive characteristics (Sinovec, 2003, Šamanc et al., 2005a, Horvat et al., 2009).

Given the above mentioned, the period of early lactation represents the most critical period in the production and reproductive cycle of high lactating dairy cows, so special attention to should be given the diagnosis of health disorders in this period. This applies particularly to subclinical health disorders. Valid indicators of the metabolic status of cows in early lactation are body condition score, concentration of biochemical blood parameters and metabolically active hormones, and the concentration and ratio of organic milk ingredients (Jovičičin et al., 2005, Kampl, 2005, Šamanc et al., 2006, Savić et al., 2010, 2011).

Estimation of energy status of cows on the basis of determination of the concentration and the ratios of organic milk ingredients is a method which, has the advantage over these other because of its simplicity, reliability and economy (Jonker and Kohn, 2001, Marenjak et al., 2004, Kampl, 2005 Šamanc et al., 2006, Horvat et al., 2007, 2009) and is widely used in practice. The parameters for the evaluation in this method are the concentrations of fat, protein and urea in milk, as well as the ratio of urea and protein, and fat and protein (fat to protein ratio, FPR). A more detailed description of this method, as well as the characteristics of the physiological processes that underlie are given in our previous papers (Savić et al., 2010, 2011), and review paper by Kirovski et al. (2012). The target group for the assessment of energy status by using this method are cows between 15 and 60 days of lactation, which is considered as a critical period for future productive and reproductive performance of the cows.

The results of our previous study (Savić et al., 2010, 2011), as well as research by other authors (Kampl, 2005, Šamanc et al., 2006, Horvat et al., 2007, 2009) suggest significant deviation values of these parameters in the individual animals compared to the average group results. These deviations can be, inter alia, associated with the stage of lactation, i.e. current day of lactation in each tested animal and its distance from the upper or lower limit of the interval from the 15th to 60th day of lactation, as well as the individual characteristics of the each animal. So, the aim of this paper is to form groups of cows as accurately as possible in the stage of lactation within a given interval, and to assess the differences in the energy status and milk yield between the groups.

MATERIAL AND METHODS

The study was conducted at the industrial type dairy farm, during the period June-July 2009. Tested Holstein cow (n=132) were in the first (n=84) and second (n=48) lactation. All 132 cows were in early lactation (15-60 days in milk, DIM), and were divided into three groups based on lactational stage. The first group (group A, n=43) were cows with 15-30 DIM, second (group B, n=33) 31-45 DIM, and the third (group C, n=56) cows with 46-60 DIM. All cows were kept under usual farm conditions (free-stall system) and fed a diet adapted to the given productive category and period of the year.

Milk samples were taken during regular milking. In all samples, the concentrations of milk fat, protein, lactose and urea were determined, while data on daily milk yield were taken from the farm records. Concentrations of fat, protein and lactose were determined on Bentley 150 Infrared Milk Analyzer, and urea concentration on Bentley Chemspec150 Urea Analyzer for Milk. Based on the obtained data, the ratios of urea and protein and FPR were calculated. The results were analyzed by descriptive statistics and presented in tables. Statistical significance of differences between the studied parameters was determined by t-test at the level of $p < 0.05$ and $p < 0.01$. Ratios of urea and protein, and milk fat and protein are shown graphically.

RESULTS AND DISCUSSION

The values of the concentration of organic milk ingredients and milk yield of tested groups of cows are shown in Table 1.

Table 1. Values of the concentration of organic milk ingredients and milk yield of tested groups of cows

Parameter	Group	M±SD	CV	IV	Statistical significance of differences
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					p<0.05	p<0.01
Milk fat (g/L)	A	41.58±5.35	12.86	30.40-52.70		A:B, A:C
	B	37.15±3.63	9.77	29.50-47.30		
	C	36.11±4.57	12.65	24.30-45.10		
Proteins (g/L)	A	30.58±2.04	6.69	26.40-36.30		
	B	30.27±2.08	6.87	25.60-33.60		
	C	29.91±2.46	8.21	23.00-35.30		
FPR	A	1.36±0.19	13.74	0.97-1.82		A:B, A:C
	B	1.23±0.11	9.15	1.04-1.45		
	C	1.21±0.15	12.02	0.82-1.70		
Urea (mmol/L)	A	2.86±0.5	17.53	1.73-4.76		A:B, A:C
	B	3.48±0.69	19.66	1.95-4.74		
	C	3.61±0.56	15.52	2.26-4.58		
Lactose (g/L)	A	47.14±1.29	2.74	41.60-49.20	A:B	
	B	47.71±1.04	2.18	45.80-49.40		
	C	47.59±1.34	2.81	43.00-50.80		
Milk yield (L)	A	27.89±8.4	30.10	8.40-55.70		
	B	31.19±7.92	25.37	14.20-45.90		
	C	30.90±7.04	22.79	14.90-43.10		

The data presented in Table 1 indicate significant discrepancies of investigated parameters within all three groups, which could be interpreted to individual variations. The exception in terms of deviations is found only in the lactose concentration, which is in a relatively narrow range in all groups, as evidenced by the very low coefficient of variation.

Kirovski et al. (2012) reported that average physiological concentrations of organic milk ingredients in Holstein cows are between 32 and 36 g/L for milk fat, about 30.6 g/L for protein, and between 2.0 and 6.0 mmol/L for urea, respectively. According to these authors, urea concentrations above 4 mmol/L, protein below 32 g/L and fat content over 45 g/L, respectively, are taken as the limit value indicating the presence of metabolic disorders. Mulligan et al. (2006) reported that the large percentage of cows in early lactation with a protein concentration below 3.05%, and those with FPR above 1.50 indicates the presence of a serious health disorders at the herd level. The average concentration of fat in all the examined group of cows was within the physiological range and consistent with the breed characteristics. Average concentration of fat had a declining trend, which could be interpreted as a consequence of decrease of lipomobilisation in the later stages of surveyed period, i.e. improving the energy status of the examined cows (Drackley, 1999, Goff, 2006). The percentage of cows with milk fat concentration above the critical threshold of 45 g/L was the highest in the group A (27.91%), while in the other groups was negligible.

The average protein concentration in all three groups of cows was on the lower limit of the physiological range. In all groups of cows large individual variations are noticeable, as well as a large number of cows (from 51.52% in group B, up to 58.14% in group A) with values below the critical limit of 3.05%, reported by Mulligan et al. (2006). Similarly to milk fat, protein concentration also decreases with the progress of lactation, indicating a lack of supply of protein through the ration, as well as their reduced utilisation due to prolonged NEB. The protein concentration in all three groups of cows is consistent with the values found in our previous studies (Savić et al., 2010, 2011). The present data on the average values of the FPR indicate its declining trend with the progress of lactation, which is in line with the expected trend of the energy balance (Šamanc et al., 2006). Only within the group A we found a certain number cows (25.58%) with the values above the critical threshold of 1.50, which suggests a strong lipomobilisation (Mulligan et al., 2006). This finding is in line with the expected most unfavorable energy balance in this group of cows, so its adversely effect on their health and milk production in the later stages of lactation, and reproductive performance can be expected.

The concentration of urea in all groups of cows was within the physiological range. Urea concentration above the physiological limit indicates an imbalance of energy and protein in the diet, which is particularly pronounced in the groups B and C (30.30% and 30.36%, respectively). There is a noticeable trend of increasing concentrations of

urea with the progress of lactation, which may be explained by increased ration intake, but with insufficient energy density, so the rumen microflora is not able to bind the liberated ammonia (Kampl, 2005; Savic et al., 2010).

Stability of lactose concentration among all groups of cows and its maintenance within narrow limits indicate a strong homeorrhetic control of its synthesis. Metabolic priority of the mammary glands and milk synthesis in the early lactation was indicated by Stamatović et al. (1983), who found significantly higher levels of glucose in blood samples from v. subcutanea abdominis in relation to ones from v. auricularis magna during early lactation.

The average milk yield had trend of a slight increase towards the middle of the surveyed period, and then stagnation, rather than the upward trend continues. Such trend can be attributed to the inadequate formulation of ration, which do not meet needs of animals for a given stage of lactation, so the peak of milk production was achieved significantly earlier than usual. Extremely large individual variations in milk yield within each group of cows were observed, which, when taken into account that all cows were fed identical regardless of production, certainly affects the degree of satisfying their individual needs and individual energy status of each cow. A similar trend was observed in our previous studies and by other authors (Šamanc et al., 2006, Horvat et al., 2009; Savić et al., 2010, 2011).

The present data on concentrations of milk fat and urea, as well as the FPR, together with the previously set forth interpretation, indicate a trend of energy status stabilisation with the progress of lactation. In all three parameters statistically significant differences between the first and the other two groups were found, which can be associated with intensive lipomobilisation and extremely NEB in the first group of cows.

Decline of the milk fat concentration with the progress of the lactation may be, in addition to a restoration of impaired energy balance and decreasing of lipomobilisation intensity, also linked to the ration composition, based on the use of large amounts of green forage (Horvat et al., 2009). Additional factor, especially pronounced in decreasing of protein concentration with the progress of lactation, can be emptying of body reserves, which culminates at the end of the surveyed period, and leads to exhaustion of the organism.

The statistical significance of differences in the lactose concentration between groups A and B can be seen as a result of hypoglycemia, which is usually present in early lactation cows (Šamanc et al., 2005), causing mammary gland does not get enough precursors for the synthesis of lactose. Also, impact of individual variations in the concentration of lactose, which is the highest for cows first group, should be taken into account. The results are also shown graphically. The ratio of the concentrations of urea and protein in three groups of cows is shown in Figures 1, 2 and 3.

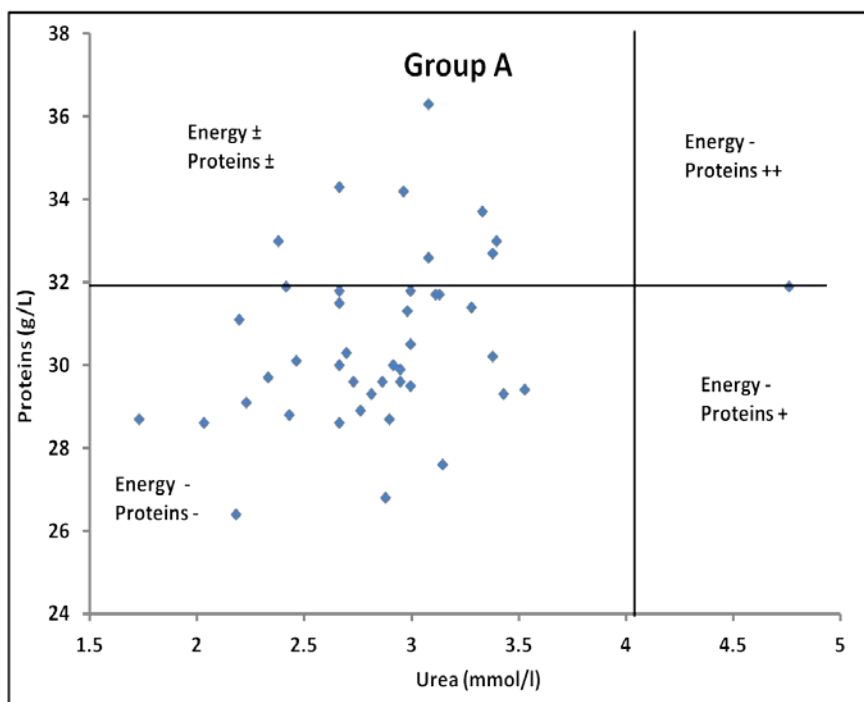


Figure 1. Urea to protein ratio in milk of cows from group A

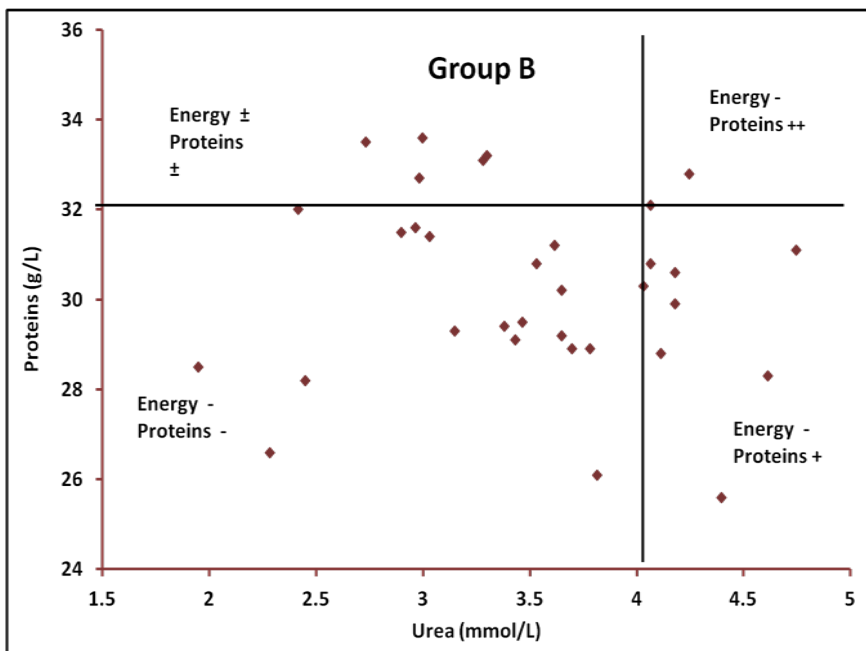


Figure 2. Urea to protein ratio in milk of cows from group B

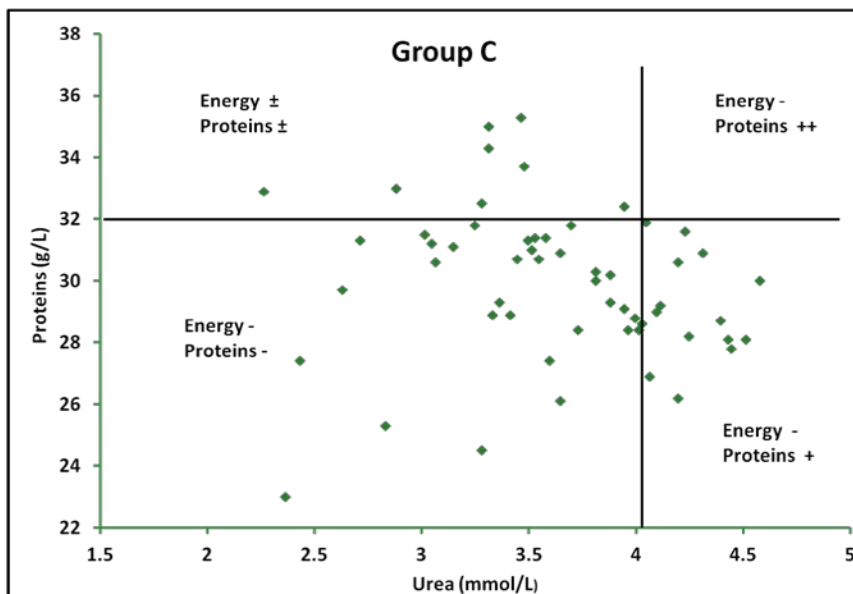


Figure 3. Urea to protein ratio in milk of cows from group C

The data presented in Figures 1, 2 and 3 indicate the existence of a combined deficit of energy and protein in the majority of cows from all three surveyed groups. Deficit of energy and protein was particularly pronounced in the group A, while in groups B and C situation was somewhat better, which can be attributed to the increased food intake, as well as adapting to the increasing energy needs. Low energy content in the diet, with easily digestible protein and a small amount of cellulose leads to development of energy deficit and relative surplus of protein in small number of cows from groups B and C, indicated by their position within a chart. Only a few cows from all three groups were adequately supplied with energy and protein (upper left quadrant), which can be interpreted by individual variation in milk production and utilization of available nutrients from ration, i.e. their better adaptability to present conditions. The ratio of the concentrations of milk fat and protein in three groups of cows is shown in Figures 4, 5 and 6

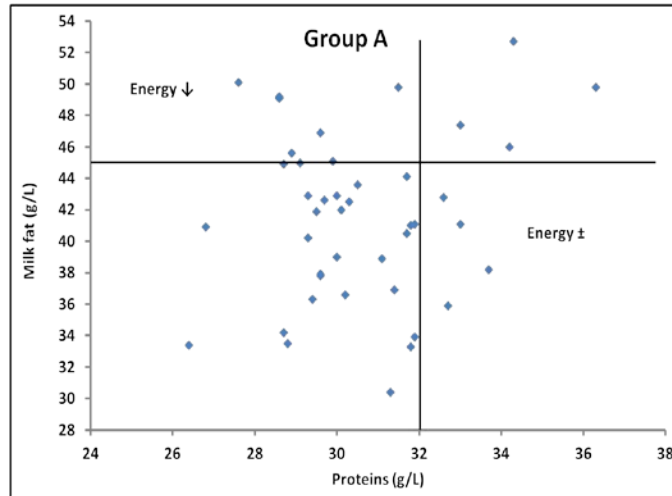


Figure 4. Milk fat:protein ratio in milk of cows from group A

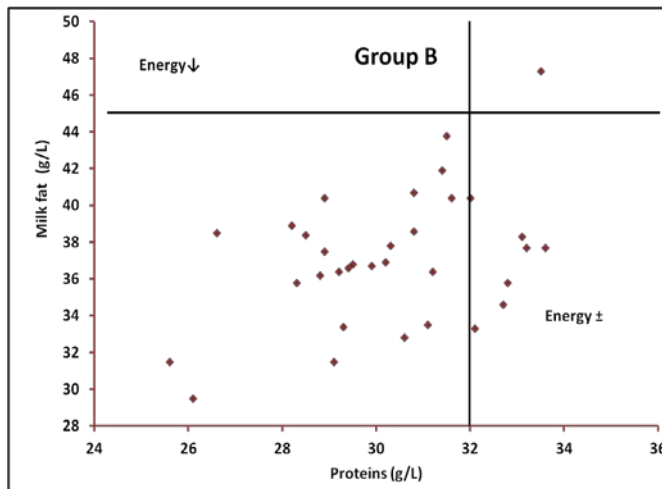


Figure 5. Milk fat:protein ratio in milk of cows from group B

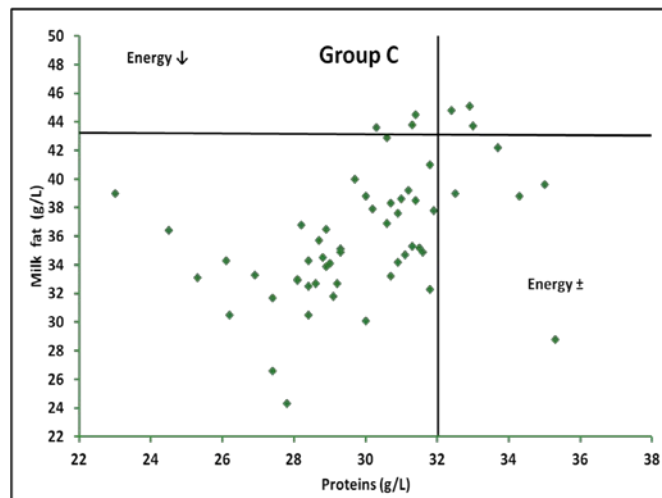


Figure 6. Milk fat:protein ratio in milk of cows from group

Displayed concentration ratio of milk fat and protein also indicates the energy deficit in the ration. In cows of the group A share of those with milk fat concentration above 45 g/L was the highest, indicating also intensive lipomobilisation. In the other two groups, the number of these cows was significantly lower. In all three groups of we found cows with milk fat concentrations below 30 g/L. This phenomenon was most likely formed as a result of ration containing low cellulose content, so a smaller amount of acetic acid as a precursor of milk fat was absorbed from the rumen. Also, the energy deficit in the ration adversely affects the abundance of cellulolytic microflora, which further inhibits the synthesis of lower fatty acids, primarily acetic. Reduced synthesis of acetic acid, besides its effect on milk fat content, will adversely affect the synthesis of estrogen, and so the emergence of the first postpartum estrus and ovulation.

CONCLUSION

The data presented in this paper indicate serious shortcomings in preparing of ration for cows, ranging from the dry period and preparation for the upcoming lactation, which is why cows during early lactation are prone to occurrence of metabolic disorders. Large variations in milk yield within the surveyed groups of cows, with the data presented about their energy status indicate inadequate management of nutrition, which leads to insufficient utilization of genetic potential for milk production. Exhaustion of organism by high milk production, combined with the unbalanced ration, will inevitably result in the clinical and subclinical health disorders, decline in milk production, extension of service period and low reproductive performances, which will all together adversely affect the replacement rate and economic feasibility of production.

In this regard, it is necessary to perform essential corrections of ration, and to form a more narrow group of cows according to stage of lactation and milk yield, better adjusted to their needs. Determination of the relationship of organic milk constituents, if routinely conducted over a period of time, could be used as a simple and reliable method for monitoring the effects of the ration correction.

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PROCJENA KRETANJA ENERGETSKOG STATUSA KRAVA TOKOM RANE LAKTACIJE NA OSNOVU KONCENTRACIJE ORGANSKIH SASTOJAKA MLIJEKA

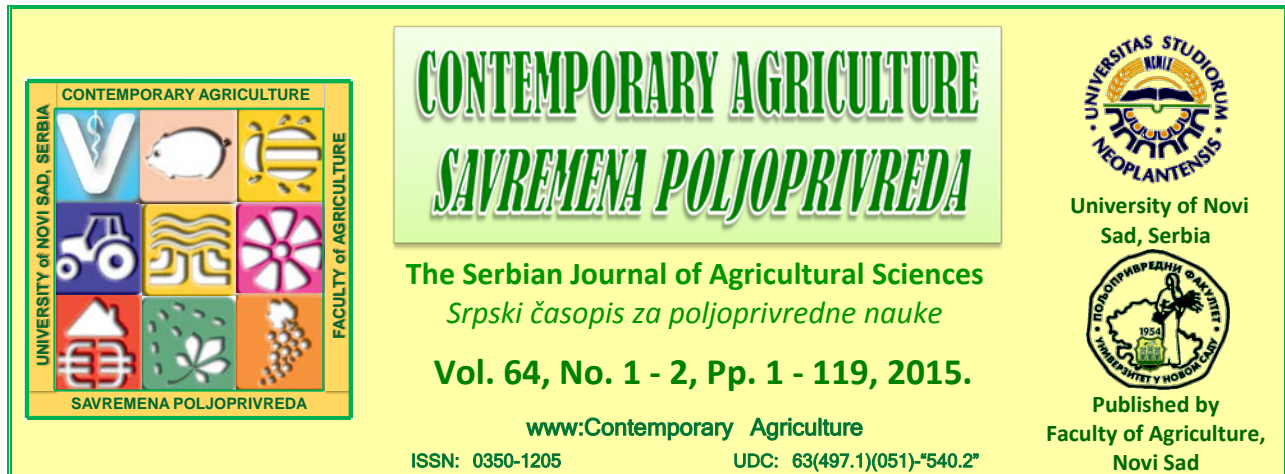
Dorđe SAVIĆ, Stoja JOTANOVIĆ, Ivan STANČIĆ, Nermin PRAČIĆ, Marinko VEKIĆ

Izvod: Cilj istraživanja bio da se na osnovu koncentracije organskih sastojaka mlijeka procijeni energetske status krava (n=132) tokom perioda rane laktacije, podijeljenih u tri grupe prema stadijumu laktacije (grupa A, n=43, 15-30 dana; grupa B, n=33, 31-45 dana; grupa C, n=56, 46-60 dana laktacije). Koncentracije mliječne masti i proteina i odnos mliječna mast:proteini imali su tendenciju opadanja, a koncentracija uree tendenciju porasta prema kraju ispitivanog perioda. Statistički značajne razlike ustanovljene su u koncentracijama mliječne masti (41.58 ± 5.35 naprema 37.15 ± 3.63 , odnosno 36.11 ± 4.57 g/L), uree (2.86 ± 0.50 naprema 3.48 ± 0.69 , odnosno 3.61 ± 0.56 mmol/L) i odnosu mliječna mast:proteini između prve u odnosu na druge dvije grupe, kao i u koncentraciji laktoze između prve i druge grupe krava (47.14 ± 1.29 naprema 47.71 ± 1.04). Na osnovu odnosa koncentracije mliječne masti i proteina, kao odnosa uree i proteina ustanovljeno je da kod svih ispitanih krava postoji jak deficit energije, uz više ili manje izražen deficit ili relativni suficit proteina. ovakvo stanje se nepovoljno odražava na zdravlje i proizvodnju mlijeka ispitanih krava, kao i na njihove reproduktivne karakteristike.

Ključne riječi: krave, laktacija, energetske status.

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MORPHOMETRIC CHARACTERISTICS OF LIVER TISSUE IN COWS DURING EARLY LACTATION*

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Ivana LAKIĆ, Nenad STOJANAC, Ognjen STEVANČEVIĆ¹

Summary: Early lactation cows usually express negative energy balance (NEB), hypoglycemia and greater lipolysis in in adipose tissue. This factors are causing higher degree of lipogenesis and accumulation of triacylglycerol in fatty vacuoles in hepatocytes. The aim of this work is determination of dimensions and optical density of fatty vacuoles and nuclei in liver tissue during early lactation. Experiment was performed on 10 Holstein-Friesian cows. Hepatic tissue samples were collected by biopsy, 3-15 days after calving. Sampels were carefully processed, stained with HE and Sudan and examined microscopically. According to results of previously analysed metabolic profile two group of cows were determined and confirmed by microscope evaluation of liver tissue. First group showed medical report which indicate fatty liver existence. Second one showed physiological report. Comparing dimensions and optical density of fatty vacuoles of both group, we have noted that first group have significantly greater dimensions of fatty vacuoles, reduced dimensions of nuclei, reduced level of optical density of nuclei and higher level of optical density of fatty vacuoles. Relationship between fatty vacuole and nuclei diameters was negative. This can be related with fatty degeneration, necrosis or apoptosis in fatty infiltrated hepatocytes.

Keys words: morphometry, liver, early lactation, cows.

INTRODUCTION

In early lactation leads to large metabolic changes in cattle, a major change is a negative energy balance. Then it happens an increased lipid mobilization (Cincović et al., 2012; Đokovic, et al., 2014) in order to use fat for energy purposes, but the cows become prone to developing ketosis and fatty liver. As a consequence of lipolysis, increases concentration of the non esterified fatty acids (NEFA) in the bloodstream. This condition occurs as a result of endocrine and metabolic changes, primarily due to the presence of insulin resistance (Cincović et al, 2014). They are deposited in hepatocytes in the form of triglycerides (TGC), forming fatty vacuoles, leading to fatty liver hepatocytes

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(Mihajlovic and Jovanovic, 2008), which changed its histological, morphometric and metabolic characteristics (Đokovic et al, 2011).

Size and dimensions of fat vacuoles, as well as their optical density indicates the degree of fatty liver. The flow of NEFA and fatty degeneration of hepatocytes caused a series of microscopically visible changes that are manifest in all organelles, and they can be seen in the nucleus in the form of changes in size, shape and optical density (Đoković i Šamanc, 2004). The basic structures of the liver unit are hepatocytes. Hepatocytes are liver lobules arranged radially, and in the spaces between them are hepatic sinusoid. The diameter of each of hepatocytes is 20-30 microns, eosinophilic cytoplasm, contains one and sometimes two nuclei, lysosomes, has abundant agranulirani and granular endoplasmic reticulum, which creates clusters of basophil-corpules. An important component of the hepatocyte of glycogen granules, involved in maintaining normoglycemia as well as the mitochondria. Lipid droplets are volatile component of the cytoplasm of hepatocytes, and their quantity is changed in accordance with the physiological state of the organism. Lipids in liver hepatocytes deposited in the form of triglyceride-TGC (Bobe et al, 2004).

The aim of our study was to examine the morphometric properties of liver tissue in early lactation as a function of fatty infiltration of hepatocytes.

MATERIAL AND METHODS

The experiment included 10 Holstein-Friesian cows: 5 cows healthy control group and 5 cows with a high concentration of ketones and bilirubin in the metabolic profile and a low concentration of triglycerides. The cows were in early lactation period of 3 to 15 days after calving.

To identify the site at which to insert the needle, first identify the 10th intercostal space. Remember there are 13 ribs and 12 intercostal spaces, so the 10th intercostal space is the third from the last intercostal space. Then draw an imaginary line from the tuber coxae (hook) to the elbow. The point at which this line intersects the 10th intercostal space is the point at which to insert the needle.

The tissue was processed and stained with HE stain and Sudan, according to standard procedures. From each sample of the liver is made two histological blocks and from each blocks 2 micrograms preparations. At each micrograms was analyzed 100 hepatocytes, selected randomly. Calibration was performed by microscopic measurement. All samples were photographed, and the photographs processed in ImageJ software. Stereological procedures were analyst according to basic principles described by Reid (1980).

The optical density and the dimension of the nucleus and of fatty vacuoles were calculated by the software. We examined the correlation between measured parameters.

RESULTS AND DISCUSSION

Cows were grouped into two main groups based on histological findings. The physiological findings with the presence of small fat droplets in the cell, which visually does not exceed the size of the sail and make up less than 10-15% of the cells. Another finding is the finding of typical fatty liver, which is histologically characterized by infiltration of centrolobular fatty vacuoles, while in one case there was infiltration through the entire lobulus, it is dominated by vacuoles filled with grease that completely alter cell histology. These two characteristic findings are shown in Figure 1 and 2. The optical density of nuclei was 401477.2 at physiological findings, ie 171361.3 in preparations with fatty liver ($p < 0.001$). In cows with fatty liver found significantly lower optical density of the nucleus. The diameter of the nucleus was also significantly lower in samples with fatty liver disease compared to healthy (7353.3: 5194.8 nm) ($p < 0.001$). The results are shown in Table 1. The optical density of the fatty vacuoles was 2258360.2 with fatty liver, or in the physiological findings 708578.1. The diameter of fat vacuoles with physiological findings amounted to 4658.7 nm, while the mixture with a fatty liver amounted to 19901.9 nm. The results are shown in Table 2. The correlation between the diameter of the core and of fatty vacuoles is negative: $r = -0.85$ ($p < 0.01$). Regression analysis is shown in Figure 3 and Table 3 and 4. It was also found negative correlation between the optical density of nucleus and fat droplets, and this relationship towards to linearity ($p < 0.1$) (results not shown).

Liver biopsy is the only reliable method to determine severity of fatty liver in dairy cattle in the transitional period. Blood, urine and milk metabolites or blood enzyme activity have been proposed as diagnostic tools. Our previously investigation demonstrated that in healthy transitional cows a mild fatty infiltration occurred in liver during the late pregnancy and early lactation. The histopathological examination showed a moderate to severe degree of fatty liver in ketotic cows. The lipomobilisation markers, serum BHB and NEFA concentrations were markedly enhanced in puerperal ketotic cows. However, liver steatosis compromised hepatocyte metabolism, leading to

significantly weaker circulating concentrations of glucose, TG and total cholesterol, and induced some cellular lesions as evidenced by significant increases in the serum bilirubin concentrations and in the AST enzyme activities in puerperal ketotic cows. All these biochemical metabolites may be used as important biochemical indicators in the determination of the functional status of the liver in high-yielding dairy cows during the transition period (González ET AL, 2011; Djokovic et al, 2013; Đoković et al., 2014).

The negative relation between the diameter and the optical density of nucleus with the same characteristics of fat vacuoles indicates that hepatocytes due to fatty infiltration may enter into a process of degeneration, apoptosis and necrosis. These processes are shown by Hübscher (2006), Wang et al. (2008) and Alkhouri et al. (2011). It is known that in tissues that are subject to necrosis, can simultaneously take place and apoptosis in cells with minor damage. Cells during hepatocyte fatty infiltration and / or degeneration may die (cell death) and necrosis and apoptosis depending on how much damage cells. Regardless of the type of cell death, reducing the dimension of of nucleus-karyopyknosis, is characteristic of cells whose viability is compromised (Knezevic and Jovanovic, 2008).

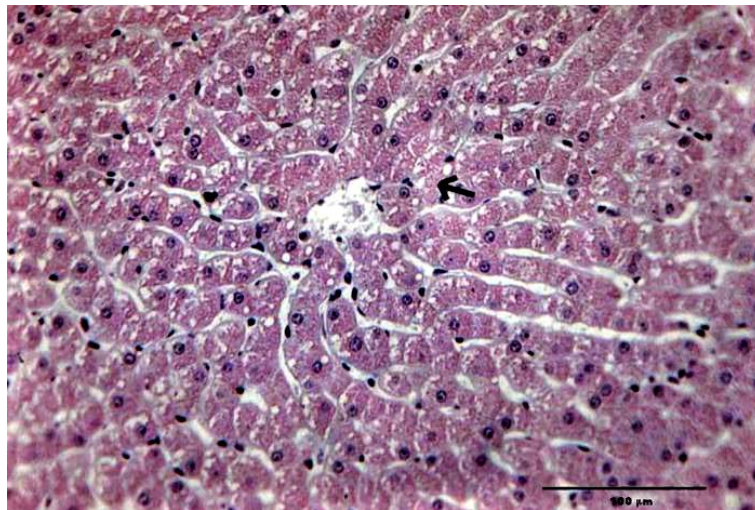


Figure 1. Histological finding of normal liver tissue

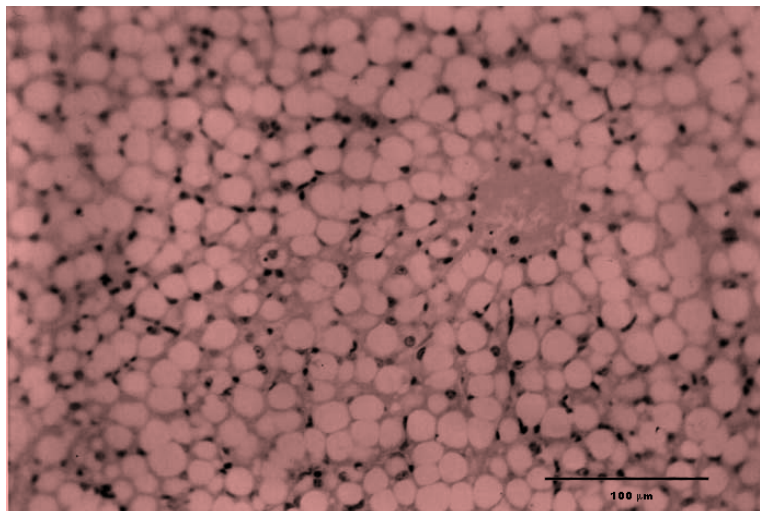


Figure 2. Histological finding of fatty liver tissue

Table 1. Optical density of nucleus and nucleus diameter in cows with normal liver tissue (control) and fatty liver infiltration

	Optical density of nucleus		Nucleus diameter (nm)	
	Control	Fatty liver	Control	Fatty liver
Average	401477.2	171361.3	7335.3	5194.8
SD	64484.1	51201.9	999.9	1219.8
CV	16.06%	29.88%	13.63%	23.48%
p	P <0.001		P <0.001	

Table 2. Optical density and diameter of fatty vacuoles in cows with normal liver tissue (control) and fatty liver infiltration

	Optical density of fatty vacuoles		Diameter of fatty vacuoles (nm)	
	Control	Fatty liver	Control	Fatty liver
Average	708578.1	2258360.2	4658.7	19901.9
SD	155922.2	309406.3	1116.4	3165.3
CV	22%	13.7%	23.9%	15.9%
P	P <0.001		P <0.001	

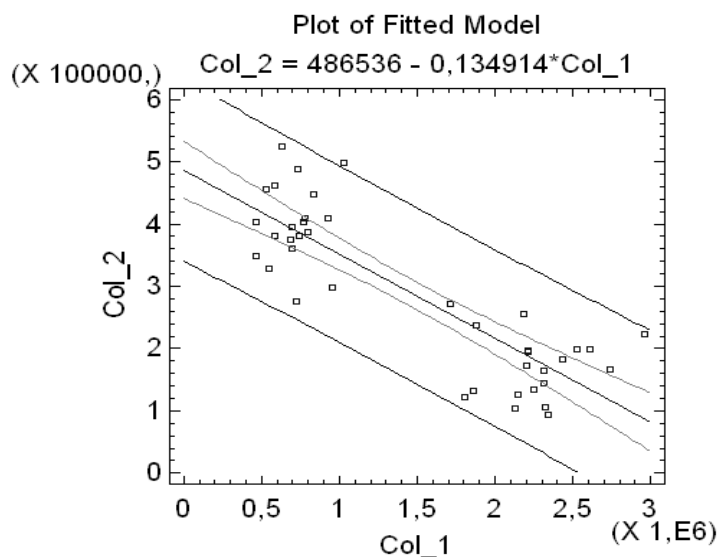


Figure 3. Correlation between diameter of nucleus and fatty vacuoles

Table 3 and 4. Regression analysis of relation between diameter of nucleus and fatty vacuoles

Coefficients				
	<i>Least Squares</i>	<i>Standard</i>	<i>T</i>	
<i>Parameter</i>	<i>Estimate</i>	<i>Error</i>	<i>Statistic</i>	<i>P-Value</i>
Intercept	486536,	22681,8	21,4505	0,0000
Slope	-0,134914	0,0134183	-10,0545	0,0000

Analysis of Variance					
<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	4,78488E11	1	4,78488E11	101,09	0,0000
Residual	1,79859E11	38	4,73314E9		
Total (Corr.)	6,58348E11	39			

Correlation Coefficient = -0,852527
R-squared = 72,6802 percent
R-squared (adjusted for d.f.) = 71,9612 percent

CONCLUSION

Morphometric characteristics of liver tissue comprising diameter and optical density cores and fatty vacuoles in hepatocytes were significantly different in histologic finding derived from cows with fatty liver compared to control. In the fatty liver tissue there was founded fat vacuoles with large diameter, and the dimensions of the nucleus was significantly lower. Also, there is a higher optical density of fat globulesvacuoles and the lower the optical density of the nucleus in relation with normal histological findings. The negative correlation between the dimensions of fat vacuoles and size of nucleus confirm that fatty infiltration significantly endanger the viability of hepatocytes.

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MORFOMETRIJSKE KARAKTERISTIKE TKIVA JETRE KOD KRAVA U RANOJ LAKTACIJI


*Radojica ĐOKOVIĆ, Marko R. CINCOVIĆ, Branislava BELIĆ, Miloš PETROVIĆ,
Ivana LAKIĆ, Nenad STOJANAC, Ognjen STEVANČEVIĆ*

Izvod: Krave u ranoj laktaciji su podložne nastanku negativnog energetskeg bilansa, hipoglikemiji i pojačanoj lipolizi u masnom tkivu, što dovodi do višeg stepena lipogeneze i akumulacije triglicerida u masnim vakuolama hepatocita. Cilj ovog rada je određivanje dimenzija i optičke gustine masnih vakuola i jedra krava u ranoj laktaciji. U eksperiment je uključeno 10 krava Holštajn-frizijske rase, čiji su uzorci jetrinog tkiva uzeti biopsijom 3-15 dana nakon teljenja (5 krava kontrolne grupe i 5 krava sa metaboličkim profilom koji ukazuje na masnu jetru). Uzorci su pažljivo obrađeni, obojeni hematoksilin-eozin i Sudan tehnikom i mikroskopski pregledani. Upoređivanjem veličina i optičkih gustina masnih vakuola kod ove dve grupe krava zaključili smo da grupa krava sa masnom jetrom ima značajno veće dimenzije masnih vakuola, manje dimenzije jedra, znatno manju volumetrijsku gustinu jedra i veću volumetrijsku gustinu masnih vakuola. Korelacija između promera masnih vakuola i jedra je bila negativna. Ovo možemo povezati sa masnom degeneracijom i procesima nekroze i apoptoze koji otpočinju u hepatocitima krava sa masnom jetrom.

Ključne reči: morfometrija, jetra, rana laktacija, krave.

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RELATIONSHIP BETWEEN HEMATOLOGICAL AND METABOLIC PARAMETERS IN COWS DURING EARLY LACTATION*

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Summary: Energy metabolism in cows was significantly altered in early lactation, there is an increased concentration of BHB and bilirubin, a decreased concentration of glucose. Metabolism of protein is also altered so that the concentration of total protein, albumin and urea lowest in the first week after parturition, but then slowly grew. In the peripartum period, especially on the day of calving and the first days of lactation cows have fewer red blood cells and a lower concentration of hemoglobin. There is a decline in the total number of leukocytes. The aim of this study is to determine link between blood count and parameters of metabolic status of cows in early lactation. The research was conducted on 40 Holstein-Friesian cows. Blood samples were taken in the first week after calving. According to the statistical analysis, it was noticed that there was not relation between the total number of leukocytes and the metabolic status of cows in early lactation. Also, it was noticed that cows with higher values of N:L ratio, lower hemoglobin concentration and smaller number of red blood cells had higher concentration of BHB, AST and TBIL and lower concentrations of ALB. Changes in the values of metabolic parameters in a function of hematological parameters were statistically significant. Based on the above, the link between blood count parameters that indicate inflammation and metabolic adaptations in cows in early lactation is demonstrated. In further research should examine the relationship between inflammatory and metabolic parameters.

Key words: blood count, metabolic status, cows.

INTRODUCTION

In lactating, dairy cows does not significantly affect the parameters of the white blood cells, or endocrine changes, peripartum stress and the development of infections significantly alter blood to the image of cows in the peripartum period. Of particular interest is the determination of total leukocytes, neutrophils, lymphocytes and neutrophils relation to the number of lymphocyte (N: L ratio). There is a decline in the total number of leukocytes in the peripartum period (Klinkon at al., 1999; Sattar and Mirza, 2009; Mirzadeh et al. 2010; Belic et al., 2011).

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The ratio of neutrophils and lymphocytes (N: L ratio) is an important indicator of the health burden and stress of animals (Davis et al., 2008). This ratio in adult cattle is about 0.5: 1, while the value of the ratio N: L over 1 indicates that cows are loaded inflammation or other stressors (Latimer and Prasse, 2003). In the period around calving, acute stress and hypercortisolemia lead to an increase in the concentration of neutrophils, which leads to the increase in the neutrophil and lymphocyte (N: L) ratio is often more than 1 (Tornquist and Rigas, 2010). After adaptation to stressful conditions N: L ratio returns to the physiological level in the period 2-7 days (Lynch et al., 2010).

In the peripartum period, especially on the day of calving and the first days of lactation cows have fewer red blood cells and a lower concentration of hemoglobin (Klinkon et al., 1999; Sattar and Mirza, 2009; Mirzadeh et al. 2010; Belic et al., 2011). The emergence of anemia and decreased hemoglobin concentration with metabolic load and elevated concentrations of NEFA may predispose cows to the emergence of perinatal inflammation of the uterus (Belic et al., 2012). Ketosis in cows in the peripartum period has an impact on erythrocyte lineage that can show signs of anemia (Belic et al., 2010).

Energy metabolism in cows was significantly altered in early lactation, there is an increased concentration of BHB and bilirubin, a decreased concentration of glucose. This finding indicates the presence of negative energy balance and expressed ketogenesis. Metabolism of protein is also altered so that the concentration of total protein, albumin and urea lowest in the first week after parturition, but then slowly grew. This situation arises as a result of insufficient food consumption, but also loads of liver function and reduction of its synthetic capacity. This is confirmed by the hepatic profile, because the enzyme activity of ALT and AST and bilirubin, or significantly higher in the first or in the second week after parturition (Wathes et al., 2007). Negative energy balance and metabolic adaptation in early lactation are in relation with inflammation profile, blood profile and healthy status of cows (Sordillo et al, 2009; Cincović et al., 2012; Djokovic et al, 2014)

The aim of this study is to determine link between blood parameters as indicators of inflammation and parameters of metabolic status of cows in early lactation.

MATERIAL AND METHODS

The experiment was performed on 40 Holstein-Friesian cows. Cows were kept in equal environmental and technology condition. Blood samples were taken in the first week after calving.

We determined following hematological parameters: the total number of leukocytes, neutrophils:lymphocyte ratio (N:L), hemoglobin concentration and red blood cell count by using a Hemavet analyzer. Also, we determined following parameters of the metabolic profile in blood serum: beta-hydroxybutyrate (BHB), aspartate aminotransferase (AST), total bilirubin (TBIL) and albumin (ALB) by standard biochemical kits (Randox) using a Rayto spectrophotometer.

For statistical analysis cows were classified according to the hematological parameters into quartiles (four groups). We examined the differences in metabolite concentrations between the four mentioned groups by using ANOVA model with post-hoc LSD test. We examined the significance of changes in the trend function of the concentration of metabolites in the blood values by using the Cochran-Armitage test.

RESULTS AND DISCUSSION

The results show that there is no significant relation ($P>0.05$) between the total number of leukocytes and the metabolic status of cows in early lactation. This results are presented in Table 1. Cows with higher values of neutrophils:lymphocyte ratio (N: L), lower hemoglobin concentration and smaller number of red blood cells have a higher concentration of BHB, AST and TBIL and lower concentrations of ALB. Changes in the values of metabolic parameters in a function of hematological parameters was statistically significant ($P<0.05$) and it is presented in Table 2, 3 and 4. Wathes et al. (2007) reported that in early lactation, there is an increased concentration of BHB, bilirubin, albumin and AST what is similar with our results. Also, our results are matched with group of authors Klinkon et al. (1999), Sattar and Mirza (2009), Mirzadeh et al. (2010), Belic et al. (2011), who claimed that in the peripartum period, cows have fewer red blood cells, lower concentration of hemoglobin and decline in the total number of leukocytes.

That fact was noticed in our examination according to the statistical analysis is that there was not relation between the total number of leukocytes and the metabolic status of cows in early lactation. Also, it was noticed that

cows with higher values of N:L ratio, lower hemoglobin concentration and smaller number of red blood cells had higher concentration of BHB, AST and TBIL and lower concentrations of ALB.

Table 1. Influence of total leukocytes number to metabolic status of cows

Total number of leukocytes	BHB mmol/l	AST IU/l	TBIL μ mol/l	ALB g/l
<25	0,8 \pm 0,12 ^a	111,5 \pm 12,14 ^a	7,5 \pm 2,4 ^a	63,5 \pm 2,1 ^a
25-50	0,7 \pm 0,14 ^a	108,8 \pm 13,11 ^a	6,9 \pm 2,9 ^a	59,9 \pm 2,6 ^b
51-75	0,9 \pm 0,11 ^b	105,6 \pm 15,1 ^a	8,1 \pm 2,5 ^a	63,4 \pm 2,4 ^a
>75	0,7 \pm 0,14 ^a	114,4 \pm 12,18 ^a	7,8 \pm 3,01 ^a	61,7 \pm 2,2 ^a
Trend	NS	NS	NS	NS

^{a,b} Values with different superscripts significantly differ (P<0.05).

Table 2. Influence of the N:L ratio to metabolic status of cows

N:L	BHB mmol/l	AST IU/l	TBIL μ mol/l	ALB g/l
<25	0,81 \pm 0,08 ^a	98,5 \pm 9,9 ^a	5,8 \pm 2,6 ^a	62,1 \pm 2,15 ^a
25-50	0,88 \pm 0,1 ^b	104,4 \pm 8,9 ^b	6,5 \pm 2 ^a	61,5 \pm 2,2 ^a
51-75	0,95 \pm 0,09 ^b	111,1 \pm 9,9 ^b	7,7 \pm 2,01 ^a	59,8 \pm 2,1 ^b
>75	1,14 \pm 0,11 ^c	118,8 \pm 11,14 ^c	8,99 \pm 1,8 ^b	57,6 \pm 2,5 ^b
Trend	<0,01	<0,01	<0,01	<0,01

^{a,b} Values with different superscripts significantly differ (P<0.05).

Table 3. Influence of the hemoglobin concentration to metabolic status of cows

HGB	BHB mmol/l	AST IU/l	TBIL μ mol/l	ALB g/l
<25	1,11 \pm 0,12 ^a	116,7 \pm 10,1 ^a	7,9 \pm 1,9 ^a	59,1 \pm 2,2 ^a
25-50	0,8 \pm 0,11 ^b	103,6 \pm 9,5 ^b	5,6 \pm 2,05 ^b	62,5 \pm 2,1 ^b
51-75	0,6 \pm 0,09 ^c	107,4 \pm 9,3 ^b	5,8 \pm 2,1 ^b	63,3 \pm 2,5 ^b
>75	0,7 \pm 0,12 ^c	102,5 \pm 9,5 ^b	5,7 \pm 2,17 ^b	63,5 \pm 2,4 ^b
Trend	<0,01	<0,05	<0,05	NS

Table 4. Influence of the number of red blood cells to metabolic status of cows

Number of erythrocytes	BHB mmol/l	AST IU/l	TBIL μ mol/l	ALB g/l
<25	0,99 \pm 0,09 ^a	115,5 \pm 10,3 ^a	7,5 \pm 1,8 ^a	59,2 \pm 2,01 ^a
25-50	0,75 \pm 0,1 ^b	111,1 \pm 11,1 ^a	5,8 \pm 1,6 ^b	60,5 \pm 2,1 ^b
51-75	0,8 \pm 0,12 ^b	99,9 \pm 12,2 ^b	5,1 \pm 2,1 ^b	62,5 \pm 2,05 ^b
>75	0,73 \pm 0,11 ^b	102,3 \pm 9,9 ^b	5,2 \pm 2,2 ^b	63,1 \pm 2,2 ^b
Trend	<0,05	<0,05	<0,05	<0,05

^{a,b} Values with different superscripts significantly differ (P<0.05).

CONCLUSION

According to the statistical analysis, it was noticed that there was not relation between the total number of leukocytes and the metabolic status of cows in early lactation. Also, it was noticed that cows with higher values of N:L ratio, lower hemoglobin concentration and smaller number of red blood cells had higher concentration of BHB, AST and TBIL and lower concentrations of ALB. Changes in the values of metabolic parameters in a function of

hematological parameters were statistically significant. Based on the above, the link between blood count parameters that indicate inflammation and metabolic adaptations in cows in early lactation is demonstrated. In further research should examine the relationship between inflammatory and metabolic parameters.

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ISPITIVANJE VEZE IZMEĐU KRVNE SLIKE I PARAMETARA METABOLIČKOG STATUSA KRAVA U RANOJ LAKTACIJI

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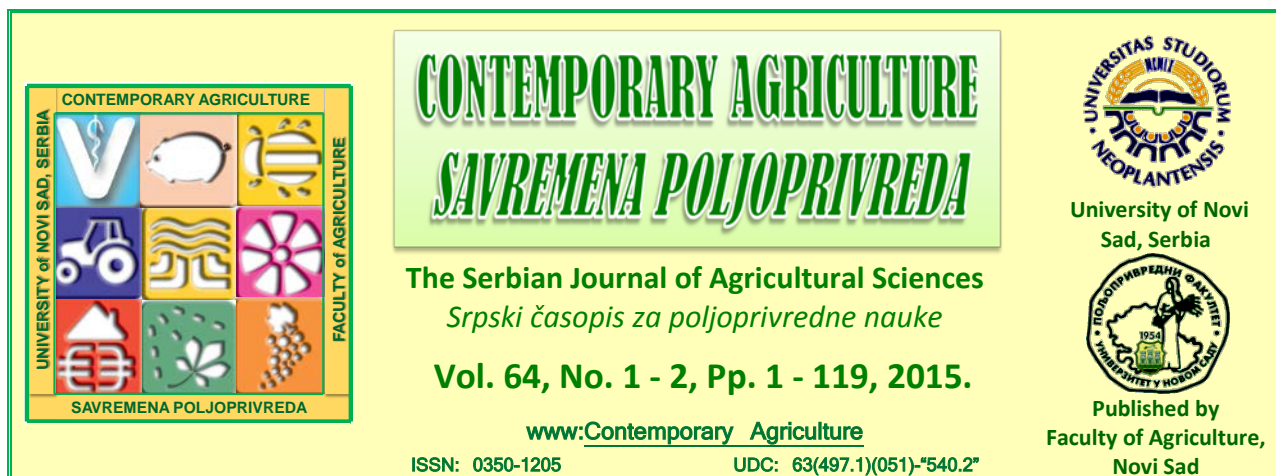
Izvod: Energetski metabolizam kod krava je značajno izmenjen u ranoj laktaciji, postoji povećana koncentracija BHB i bilirubina, a snižena koncentracija glukoze. Metabolizam protein je takođe izmenjen tako da je koncentracija ukupnih proteina, albumina i uree najniža u prvoj nedelji posle partusa, da bi potom lagano rasla. U peripartalnom periodu, posebno na sam dan porođaja i u prvim danima laktacije krave imaju manji broj eritrocita i nižu koncentraciju hemoglobin. Dolazi do opadanja ukupnog broja leukocita. Israživanje je izvedeno na 40 krava Holštajn-frizijske rase. Uzorci krvi su uzeti u prvoj nedelji nakon teljenja. Cilj ove studije je bio da se utvrdi veza između krvne slike i parametara metaboličkog statusa krava u ranoj laktaciji. Na osnovu statističke analize primećeno je da ne postoji veza između ukupnog broja leukocita i metaboličkog statusa krava u ranoj laktaciji. Takođe, primećeno je da krave sa višim vrednostima N:L odnosa, nižom koncentracijom hemoglobina i manjim brojem eritrocita imaju višu koncentraciju BHB, AST i TBIL i nižu koncentraciju ALB. Trend promene u vrednostima metaboličkih parametara u funkciji hematoloških parametara je statistički značajan. Navedeni parametri pokazuju da postoji veza između parametara krvne slike koji ukazuju na inflamaciju i parametara koji ukazuju na

metaboličku adaptaciju krava u ranoj laktaciji. U budućim istraživanjima treba ispitati vezu između inflamatornih i metaboličkih parametara kod krava u ranoj laktaciji.

Ključne reči: krvna slika, metabolički status, krave.

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INFLUENCE OF METABOLIC PROFILE IN EARLY LACTATION AND WELFARE SCORE OF FARM TO MILK PRODUCTION AND SERVICE PERIOD IN DAIRY COWS*

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Summary: Assessment of welfare and stress of cows at farms is a priority task for all involved in the milk production. The aim of this study was to investigate the effect of the level of welfare on the farm and metabolic adaptations in cows in early lactation on milk production and length of service period, the risk for the occurrence of below-average productivity of cows and benefits while eliminating stressful on the farm. Milk production and service period as most important productive characteristics of cows are dependent on welfare score of farm (principle of good health and good feeding) and metabolic adaptation of cows. Low welfare score and poor metabolic adaptation in early lactation showed additive effect in reducing of productive characteristics. Risk for decrease of productivity in cows exposed to low welfare score on farm and metabolic stress is higher then 1.9 to 2.8 time compared to cows that did not suffer from same stressors. Reduction of stressors by preventive inspection of locomotion system and hoof corection, determination of metabolic status using a metabolic profile and supplementation with energetics and antioxidants give the best balance of cost and benefit when used together.

Key words: cows, welfare score, metabolic profile, milk production, service period.

INTRODUCTION

Assessment of stress and welfare of cows at farms is a priority task for all involved in the production of milk. Environmental factors and metabolic adaptability of animals affect all aspects of health and productivity of cows. Animal welfare is a degree of its adaptation to environmental conditions (Vučinić, 2006). The most important

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indicators of the health and productivity of dairy cows: milk production and length of service period. Using the protocol Welfare Quality® scoring system (2009) includes assessment of the 4 main principles of welfare on farms cows: good health, good housing, good feeding and appropriate behavior of animals. It also gives the overall assessment of well-being on a farm. Results from previous studies have shown that there is significant between cow productivity and value assessment criteria for good health on the farm, then the criterion of good nutrition and eventually criterion of good accommodation (Čincović et al, 2012). Productive traits such as milk production and length of service period significantly correlated with factors of health, food and accommodation. It is shown that there are differences in the metabolic adaptation of cows originating from farms in which the principles of welfare are met to varying degrees. In cows with lower marks for the principle of health and nutrition there is a higher concentration of stress hormone cortisol, NEFA and BHB. These changes are expressed as the cows in early lactation (when they are most vulnerable to stress), and in the cows in middle of lactation (Belić et al., 2013).

The aim of this study was to investigate the effect of the level of welfare on the farm and metabolic adaptations in cows in early lactation on milk production and length of service period, the risk for the occurrence of below-average productivity of cows and benefits while eliminating stressful on the farm.

MATERIAL AND METHODS

At nine dairy farm welfare assessment was performed according to the protocol Welfare scoring system (Welfare Quality®, 2009). At the same time, on the same farm was designated a metabolic profile with 15 cows in early lactation (1-15 days after calving). The following metabolic parameters were determined in the metabolic profile: nonesterified fatty acid (NEFA), beta-hydroxybutyrate (BHB), glucose, total bilirubin (TBIL) and Ca (Randox kit and Rayto biochemistry device). At the same cow was monitored daily milk production in early lactation (first 60 days), and length of service period (days). Farms are classified into those with a value of well-being scores were below 50 and with a score greater than 50. The classification of a cow to metabolic profile was performed according to the following critical, risk values: glucose <2 mmol / l, NEFA> 0.6; BHB> 0.9 mmol / l; TBIL> 9 mmol / l and Ca <2 mmol / l. The influence of the metabolic status of cows in early lactation and evaluated scores of well-being on milk production (l / day) and length of service period (in days) was determined using ANOVA analysis with posthoc LSD test within the framework of generalized linear models. The model included the effect of the individual, the impact of recent well-being, the impact of metabolite concentrations and the combined impact of the welfare × metabolites. Then logistic regression was performed to determine the extent to which environmental factors and metabolic factors affecting milk production and reproductive efficiency by testing of regression parameter. The relative risk was calculated, and it shows how many times more frequently reduced milk production below average farm with cows burdened stress compared to cows with absent of the working stressors or stress reduction is performed. To examine the feasibility of reducing stress factors on the farm was determined percentage ratio between the resources to invest in removing the most common stress-inducing (hoof trimming, metabolic profile assessment, energetic and mineral supplement application, climatisation, balancing of exist meal, induction of reproductive function) and benefits that are realized on this occasion. It was used Statgraphic and SPSS statistical software.

RESULTS AND DISCUSSION

Results showed that the principle of good health and the principle of good feeding are important factors that affect the productivity of cows. Classification of cows according to the principles of good housing and appropriate behavior did not lead to significant differences in milk production and length of service period, so these results are not shown. Service period was significantly longer in cows originating from farms with lower marks of principles of good nutrition and health. In cows with higher concentrations of NEFA, BHB and TBIL there is also a longer service period. Poor metabolic findings are shown as a intensifying factor. Cows from the farm with a low score of welfare that had a high concentration of NEFA, BHB and TBIL in early lactation show further longer service period. Milk production was significantly lower in cows originating from farms with a low score of the principles of nutrition and health. In cows with a lower concentration of glucose and Ca and higher concentrations of BHB there was a lower milk production. The interaction of environmental factors and metabolic adaptations were not statistically significant. The results are shown in Tables 1 and 2.

All tested parameters are placed in a single logistic model. This model gives correct detection of cows with service period over 120 days in 81% of cases and correct detection of cows with below-average production of milk in 56% of cases. Service period depends primarily on the concentration of BHB and NEFA as well as the assessment of

health on farms. Milk production is determined by the concentration of calcium, glucose and BHB and assessment of quality food. The results are shown in Table 3.

The cows that have inadequate metabolic adaptation in early lactation and live in less favorable environmental factors have an increased relative risk that their milk production is below the expected average, and that the service is longer than 120 days. The greatest risk is at hyper cows on farms where there are lower estimates of the principles of good health and nutrition with increased concentrations of NEFA and BHB. Risk for decrease of productivity in cows exposed to low scores welfare on farm and metabolic stress is higher from 1.9 to 2.8 time compare to cows that did not suffer from the same stressors. The results are shown at the Figure 1.

Elimination of stress factors leads to an increase in milk production and a reduction in calving interval. When comparing the cost of introducing measures against stressors with the benefits that they generate, we conclude that regular inspection and correction hoof metabolic profile and the application supplements allow realizing of 83% of benefit from 47% of total cost of introducing these procedures. The results are shown in Table 4.

Table 1. Influence of welfare score of good feeding and metabolic adaptation to milk production and service period in cows

		Score for principle of good feeding <50		Score for principle of good feeding ≥50		Welfare score	Metabolism	Welfare score × Metabolism
		Metabol ok	Metabol risk	Metabol ok	Metabol risk			
NEFA	Service (day)	135±5	149±5	121±7	130±6	<0.05	<0.01	<0.05
	Milk ()	23±2,5	21±3	26±3,5	24±1,5	<0.05	NS	NS
BHB	Service (day)	141±6	155±6	119±7	133±6	<0.05	<0.01	<0.01
	Milk (L)	24±3	22±2,5	28±2	26±3	<0.01	<0.01	NS
Glucose	Service (day)	132±5	142±8	125±5	122±4	<0.05	NS	NS
	Milk (L)	22±2,5	19±2	26±2	24±3	<0.05	<0.05	NS
Total bilirubin	Service(day)	129±7	139±6	107±5	133±5	<0.01	<0.05	<0.05
	Milk (L)	23±3	25±2,5	24±2	26±3	NS	NS	NS
Ca	Service (day)	129±6	133±5	101±5	106± 6	<0.05	NS	NS
	Milk (L)	22±1,5	20±2	24±2	23±1,5	<0.05	<0.05	NS

Table 2. Influence of welfare score of good health and metabolic adaptation to milk production and service period in cows

		Score for principle of good health <50		Score for principle of good health ≥50		Welfare score	Metabolism	Welfare score × Metabolism
		Metabol ok	Metabol risk	Metabol ok	Metabol risk			
NEFA	Service (day)	133±4	148±6	121±7	130±6	<0.05	<0.01	<0.05
	Milk (L)	24±2	22±3	26±1,5	25±2	<0.05	NS	NS
BHB	Service (day)	139±5	151±6	119±7	133±6	<0.05	<0.01	<0.01
	Milk (L)	25±2	22±3	27±3	25±2,5	<0.01	<0.05	NS
Glucose	Service (day)	133±6	144±8	125±5	122±4	<0.05	NS	NS
	Milk (L)	23±1,5	20±2,5	25±1,5	23±2	<0.05	<0.05	NS
Total bilirubin	Service(day)	130±5	145±4	107±5	133±5	<0.01	<0.01	<0.05
	Milk (L)	22±3	24±3	24±3	25±2,5	NS	NS	NS
Ca	Service (day)	131±7	137±5	101±5	106± 6	<0.05	NS	NS
	Milk (L)	23±2	21±2	24±1,5	22±1,5	<0.05	<0.05	NS

Table 3. Influence of welfare score and metabolic adaptation to prediction of cows with above average milk

production and service period greater than 120 days

Parameter	Regression coefficient for milk	p	Regression coefficient for service period	p
NEFA	-0,06	NS	4,5	<0.05
BHB	-0,42	<0.01	3,2	<0.01
Glucose	0,51	<0.05	-0,5	NS
Total bilirubin	-0,08	NS	0,4	NS
Ca	0,44	<0.01	-0,5	NS
Score for principle of good health	0,06	NS	1,1	<0.05
Score for principle of good feeding	0,11	<0.05	0,6	NS
General score of welfare on farm	0,02	NS	0,5	NS
% of correct detection of cows with low productivity	56%		81%	

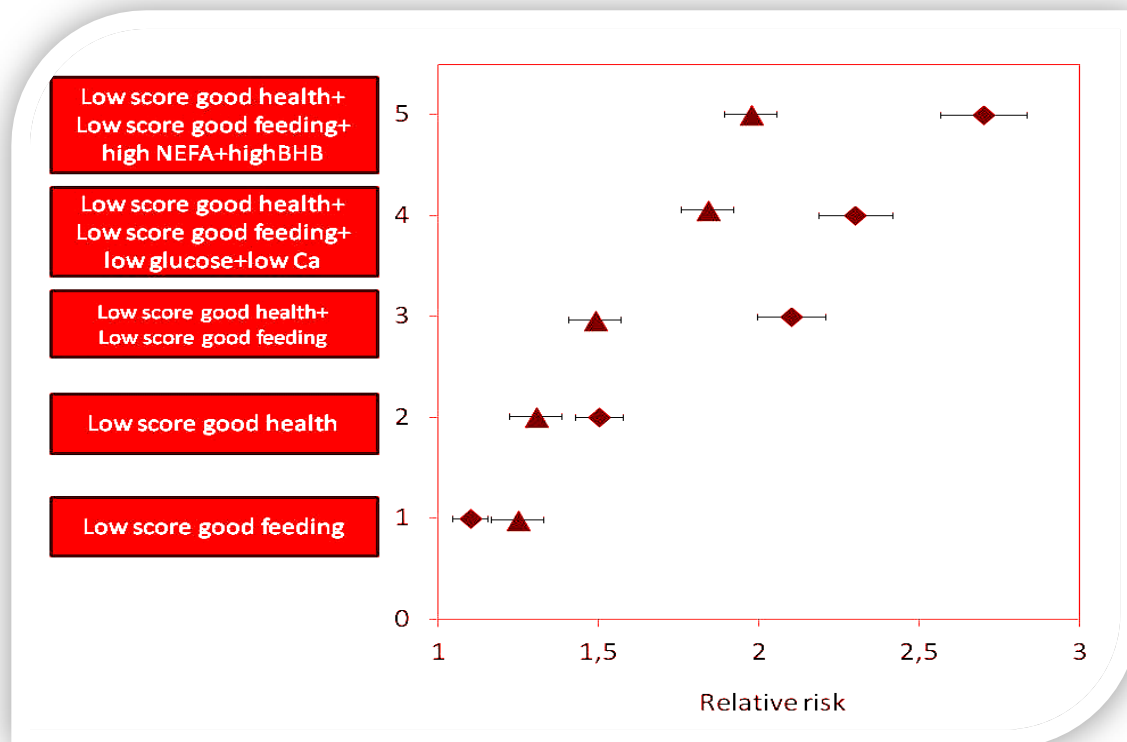


Figure 1. Relative risk for low milk production (triangle) and long service period (rhombus) in cows exposed to low score of welfare and risk metabolic status

Table 4. Cost-benefit analysis in implementation of procedures for the reduction of stress

Procedures for reduction of stress	Cost (%)	Benefit (%)
<i>Preventive inspection of locomotion system and hoof corection*</i>	12	30
<i>Determination of metabolic status using a metabolic profile*</i>	28	64
<i>Suplementation with energetics and antioxidans*</i>	47	83
Climatisation	80	89
Additional balancing of meals	86	95
Hormonal induction of reproductive cycle	100	100

*Procedures marked with an asterisk give the best balance of cost and benefit when used together

Our previous results indicate that the metabolic adaptation cows depends on the level of well-being (Belić et al., 2013): Cows from farm with lower welfare score according to principle of good housing showed a tendency to lower glucose and calcium concentration in mid lactation period. Cortisol concentration was higher in early and mid lactation. Metabolic adaptation of cows in relation to welfare principle of good feeding showed that cows from farm with low scores showed: higher NEFA, BHB, bilirubine and cortisol concentration in early lactation. Metabolic changes in mid lactation showed tendency to higher BHB, and lower glucose and urea concentration. Cows from farm with lower welfare score according to principle of good health showed higher NEFA, BHB and cortisol and lower glucose concentration. The metabolic characteristics of cows depend on providing the principle of welfare on farms. Cows from farm with lower welfare scores showed higher cortisol concentration and sings of metabolic stres, such as higher NEFA, BHB and bilirubin concentration and lower concentration of glucose and urea. The most important principles, which significantly affects these characteristics, are the principles of good health and good feeding. These results show that it is possible to estimate the welfare of cows based on metabolic status, which requires further investigation.

Cincović et al. (2012) showed that on farms with lower level of welfare as well as the poorer implementation of principles of good nutrition, significantly lower milk production was found, as well as a greater percentage of cows with a long service period, lameness and a higher percentage of very thin cows. Providing good accommodation principles will significantly improve milk production, reduce the number of cows with a long service period as well as the ones with lameness, and it is particularly interesting that obliging this principle will provide less subclinical mastitis on a farm and less cows with dystocia and skin lesions. The most important principle, which has demonstrated a significant effect on the values of all parameters, is the principle of good health. Providing the implementation of this principle on farms will result in significant improvement of all parameters of production and health. In addition to medical syndromes such as lameness or dystocia, the score of good health depends on the occurrence of ocular, nasal and vaginal discharge, which supports the infection of the corresponding organs. Productive characteristics such as milk production and length of service period significantly correlate with the factors of health, food and accommodation. Ensuring good health of farm animals is the most important task in the process of securing the welfare and sustainable production on cow farms.

Elimination of stress factors on the farm leads to the improvement of the productive characteristics of cows. Eliminating stress factors included: analyzing the metabolic status and its relation with milk production, health and reproductive efficiency; the application of energetic or antioxidants supplements; regular inspection of hooves and the locomotion system, correction meals and air conditioning facilities. The positive effects of these processes are characterized by improved production and metabolic adaptations.

Milk production is depend of metabolic adaptation in dairy cows (Belić et al, 2014). The results show that cows with lower values of insulin and higher values of cortisol and STH have higher milk production in the first weeks of lactation. However, these relationships are not easy and apparently are caused by other factors, and depend on the week in which we investigated the concentrations of the hormones themselves. When cows classified according to the metabolic status, so to compare milk production between cows with unfavorable and favorable metabolic status at the result showing that cows who have an unfavorable metabolic status (lower concentrations of glucose and calcium and higher BHB and bilirubin concentration) showed lower milk production. Impact of endocrine and metabolic status on milk production has been determined. A relationship between hormones, metabolites, and milk production is complex and must be interpreted within the concept of homeostasis and homeoresis or biological capacity of cows to adjust to lactation but also to maintain the continuity of the body.

Cincović et al (2012a) showed that metabolic adaptation in early lactation is depend of lipide mobilization and ketogenesis. According to NEFA and BHB concentration it is possible to predict metabolic adaptation of protein, lipide and carbohydrate metabolism. Belić et al. (2012) showed that in cows suffering from metritis had a significantly lower number of erythrocytes in the period before calving, and week after calving, while the hemoglobin concentration was significantly lower in the week after calving compared to control healthy cows. Metritic cows had higher leukocyte number in week before and week after calving. Leukocyte profile of these cows was characterized by increased neutrophile percent in week after calving and decreased monocyte percent in week after and week before calving compared to control group. Cows with mastitis showed higher neutrophile percent in week before and week after calving and lower percent of eosinophils in same period compared to healthy cows. NEFA concentration was significantly higher in diseased cows in week after calving. Prediction of metritis is possible in function of monocyte percent and level of anemia. Percent of eosinophil may indicate the mastitis in dairy cows. Cincović et al (2014) demonstrated that reproductive efficiency of cows is in relation with concentration of insulin, IGF-I, NEFA and BHB.

Cows that received propylene glycol are less burdened with metabolic stress (Cincović et al, 2013). Thus, cows treated with propylene glycol have higher concentrations of glucose, insulin, IGF-I and lower concentrations of NEFA, BHB and bilirubine. Post-hock analysis it can be concluded that cows in early lactation show these differences, but the differences in metabolite concentrations before calving were not significant. Cows in which was applied propylene glycol are less burdened by metabolic stress, which is reflected in lower proportion of cows with signs of metabolic stress. Efficacy of propylene glycol may be seen in the fact that propylene glycol is changing relationships and strength of links between metabolites. Davidov et al (2014) showed that selenium and zinc supplementation had significant positive effect on udeer health. In cows supplemented with selenium there was significantly lower number of somatic cells during the following lactation period. In the parenchyma of the udder there was found less pronounced infiltration of leukocytes, notably thicker keratin layer of *ductus papillaris* and less expressed repairing processes that indicate a chronic inflammation of the udder in the samples after exclusion of the cows from production. Zinc from blood and udder had a negative correlation with the number of somatic cells, had a positive correlation with the thickness of *ductus papillaris* keratin layer and had no influence on the level of leukocyte infiltration of udder parenchyma.

Toholj et al. (2012) showed that etiopathogenesis of musculoskeletal disease tract or acropodium, is complex and includes some classic, like many emerging factors in intensive production. It was found that in a free way of keeping the percentage of patients suffering from digital dermatitis significantly higher. The emergence of the hoof Rusterholzu ulcer was significantly higher in cows tied in the way of keeping in relation to the cows in the free housing. Digital dermatitis, also known as digital or interdigital papillomatosis is an infectious disease of cattle, which is considered one of the main causes of lameness. Its causation is multifactorial and complex pathology and it is described in this paper. Ultrasound examination of hoof could provide early detection of sole ulcer in dairy cows (Toholj et al., 2014).

Cincović et al (2013a) demonstrated that air conditioning during heat stress improves milk production with important metabolic adaptation. Exposure of cow to fan leads to an increase in milk production and an increase in food consumption, but it does not reach the values that existed in the optimum temperature action. Metabolic characteristics of cows after exposure to fans were as follows: lower pH levels, higher blood glucose and insulin value, the tendency of increase of the concentration of NEFA and reduced sensitivity to insulin, while the concentration of T3 and T4, cortisol and growth hormone showed a tendency to increase. The parameters that indicate the degree of hydration of cows (hematocrit, total protein and creatinine) were not significantly different.

CONCLUSION

Milk production and service period as most important productive characteristics of cows are dependent on welfare score of farm (principle of good health and good feeding) and metabolic adaptation of cows. Low welfare score and poor metabolic adaptation in early lactation showed additive effect in reducing of productive characteristics. Risk for decrease of productivity in cows exposed to low welfare score on farm and metabolic stress is higher then 1.9 to 2.8 time comparet to cows that did not suffer from same stressors. Reduction of stressors by preventive inspection of locomotion system and hoof corection, determination of metabolic status using a metabolic profile and supplementation with energetics and antioxidans give the best balance of cost and benefit when used together.

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UTICAJ METABOLIČKOG PROFILA U RANOJ LAKTACIJI I OCENE DOBROBITI NA FARMI NA PROIZVODNJU MLEKA I DUŽINU SERVIS PERIODA KOD MLEČNIH KRAVA

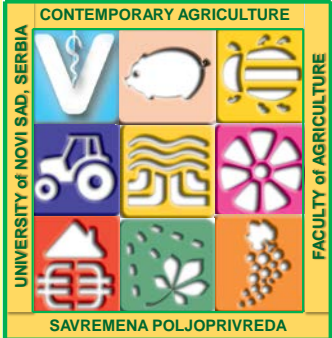
Branislava BELIĆ, Marko R. CINCOVIĆ, Milenko STEVANČEVIĆ, Bojan TOHOLJ, Anka POPOVIĆ VRANJEŠ, Radovan PEJANOVIĆ, Ivana DAVIDOV, Aleksandar POTKONJAK, Ivan STANČIĆ

Izvod: Ocena dobrobiti i stresa na farmama krava predstavlja veoma značajan postupak za sve ljude uključene u proizvodnju mleka. Cilj ovog rada je da se ispita uticaj nivoa dobrobiti na farmi i metaboličkog statusa krava u ranoj laktaciji na proizvodnju mleka i dužinu servis perioda kod krava, da se oceni rizik za nastanak ispodprosečne proizvodnje krava i uradi analiza ulaganja i profita koji postoje prilikom uvođenja mera za otklanjanje stresnih faktora na farmama. Proizvodnja mleka i dužina servis perioda kao najvažnije produktivne osobine krava zavise od ocene dobrobiti na farmi (principi dobrog zdravlja i dobre ishrane) i metaboličke adaptacije krava. Niska ocena dobrobiti i loša metabolička adaptacija pokazuju aditivni efekat dovodeći do pada u produktivnosti krava. Rizik za pad produktivnosti kod krava koji borave na farmi sa lošim skorom dobrobiti i koje pokazuju lošu metaboličku adaptaciju je od 1,9 do 2,8 puta veći u odnosu na krave koje nisu izložene ovim spoljašnjim i unutrašnjim stresorima. Redukovanje stresora redovnim pregledom i korekcijom papaka, izradom metaboličkog profila i procenom metaboličkog statusa te aplikacijom različitih suplemenata u hrani omogućuje najbolji odnos između uloženi sredstava i dobiti koja se tom prilikom ostvaruje.

Ključne reči: krave, ocena dobrobiti, metabolički profil, proizvodnja mleka, servis period.

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INFLUENCE OF NIACIN ON LIPID METABOLISM IN DAIRY COWS DURING EARLY LACTATION*

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Summary: The aim of our study was to examine the influence of niacin administration on lipid metabolism in cows during early lactation. Thirty Holstein-Friesian cows were included in experiment and were divided into two groups: cows supplemented with niacin (15) and negative control nonsupplemented cows (15). Blood samples were taken by venepuncture of v.coccigea in vacutainers for serum separation. Sampling was performed at calving and in first and second weeks of lactation. Concentration of blood NEFA, BHB, cholesterol and triglycerides was determined. NEFA:cholesterol ratio was calculated as index of liver lipidosis. Cows that received niacin showed lower concentrations of NEFA and BHB and a higher concentration of triglycerides and cholesterol, also there is a significantly higher concentration of cholesterol per unit NEFA. Application of niacin in cows during periparturient period showed positive effects on lipid metabolism such as decreased lipid mobilization (decreased NEFA), ketogenesis (decreased BHB) and liver lipidosis (higher triglycerides and cholesterol in blood and higher concentration of cholesterol per NEFA unit) in early lactation.

Key words: cows, niacin, lipid metabolism, periparturient period.

INTRODUCTION

Metabolic changes in periparturient period sets metabolism of dairy cows to start with lactation. The most of important changes could be detected in lipid metabolism. In early lactation exists negative energy balance with insulin resistance and consequently lipid mobilization (with higher NEFA in blood) increases in order to use fat for energy purposes. This mechanism provides sparing of glucose for milk production. High lipid mobilization leads animals to higher ketogenesis in liver with increase of BHB. They are deposited in hepatocytes in the form of

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triglycerides (TGC), forming fatty liver. Fat accumulation in liver decreased transport form of cholesterol. Consequently concentration of TGC and CHOL decrease in early lactation (Đoković et al., 2015; Cincović, 2014; Đoković et al., 2014).

Niacin is a water-soluble vitamin, also known as vitamin B3. Niacin is the generic term for nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (nicotinic acid amide) and the coenzyme forms of the vitamin. Nicotinamide is the active form, which functions as a constituent of two coenzymes, namely, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NAD is included in next metabolic pathway: glycolysis, gluconeogenesis, fatty acid degradation and ketogenesis. NADP is included in fatty acid synthesis and cholesterol synthesis (Kutas, 1994). Niacin stabilizes and balances lipid metabolism through different way in liver, lipid tissue and inflammatory response (Kamanna and Kashyap, 2008).

The aim of our study was to examine the influence of niacin administration on lipid metabolism in cows during early lactation.

MATERIAL AND METHODS

Thirty Holstein-Friesian cows were included in experiment and were divided into two groups: cows supplemented with niacin (15) and negative control nonsupplemented cows (15). Niacin was administered in a dose of 60-80 g / day in the period before parturition (2 weeks) and early lactation (1 week).

Blood samples were taken by venepuncture of *v.coccigea* in vacutainers for serum separation. Sampling was performed at calving and in first and second weeks of lactation. Concentration of blood NEFA, BHB, cholesterol and triglycerides was determined by standard colorimetric kit (Randox, UK) on Rayto spectrophotometric device. NEFA:cholesterol ratio was calculated as index of liver lipodosis.

Statistic: Difference between concentration of lipid metabolites and NEFA:cholesterol ratio were calculated for each week by t-test.

RESULTS AND DISCUSSION

Effect of niacin on lipid metabolism in cows in early lactation is multifaceted. Cows that received niacin showed lower concentrations of NEFA (graphic 1) and BHB (graphic 2) and a higher concentration of triglycerides (graphic 3) and cholesterol (graphic 4), there is also a significantly higher concentration of cholesterol per unit NEFA (graphic 5), so the risk of occurrence of fatty liver hepatocytes was lower. The concentration of the above parameters agrees with our previous studies of metabolism in cows during early lactation (Cincović et al., 2012)

Nicotinic acid in a very high dosage has the ability to suppress the release of fat. The pharmacological dose of nicotinic acid inhibits lipolysis in cattle. The concentration of NEFA in plasma was reduced in only one study of eleven studies when small dose of free niacin is given as a supplement in dairy cows. So nicotinic acid is a powerful antilipolytic agent in cattle that have a negative energy balance during food shortages and Sustainable decrease NEFA can be achieved as long as there is a supply of nicotinic acid in the lower part of the gastrointestinal tract where it is absorbed. Where it was used niacin protected in degradation in the rumen, the dose of 24 g encapsulated niacin (which provides bioavailability of 9.6 g per day) has been successful in the inhibition of lipolysis in postpartum cows. A dose of 12 g per day of encapsulated niacin provides bioavailability source of niacin, which modifies lipid metabolism (NRC, 2001; Pires et al., 2007a, 2007b; Niehoff et al., 2009; Morey et al., 2011; Yuan et al., 2012).

Studies have shown that niacin supplements reduce the concentration of BHB and NEFA in the blood plasma with increasing glucose concentration. Results were obtained with significant effects of niacin on BHB where the level of BHB concentration in plasma was lower in cows fed with niacin compared with the control group. Significant reduction of the BHB was obtained in cows fed with 12 grams of niacin per day, and reduction was lesser in cows that received 6 grams per cow per day compared with the control-group (Karkoodi and Tamizrad, 2009; Al-Abbasy, 2013; Pescara et al., 2010). There was a decreased concentration of triglycerides in the liver of cows fed with niacin. This occurs because the accumulation of triglycerides in liver directly related to the concentration of NEFA in the blood, and reduction of NEFA and lessens accumulation of triglycerides (Yuan et al., 2012)

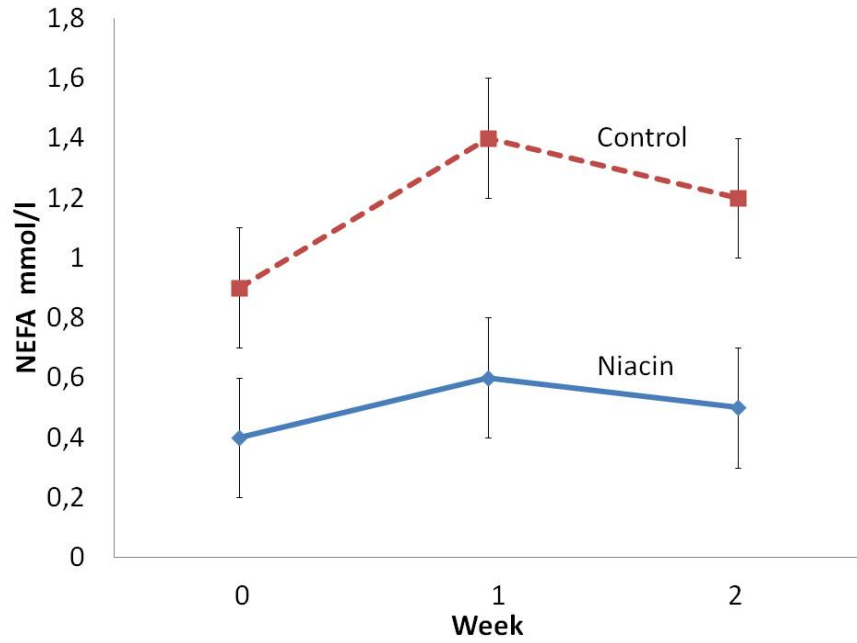


Figure 1. Concentration of NEFA in cows supplemented and non-supplemented (control) with niacin

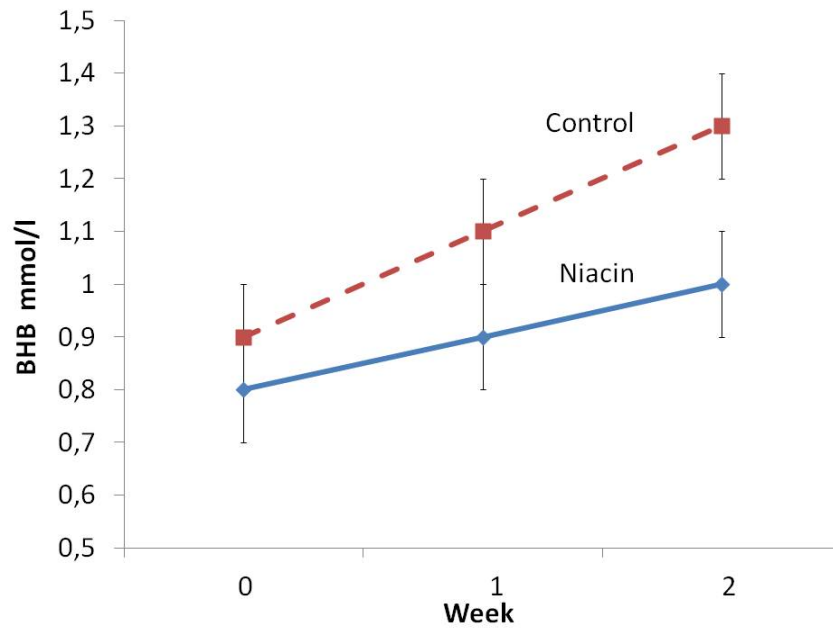


Figure 2. Concentration of BHB in cows supplemented and non-supplemented (control) with niacin

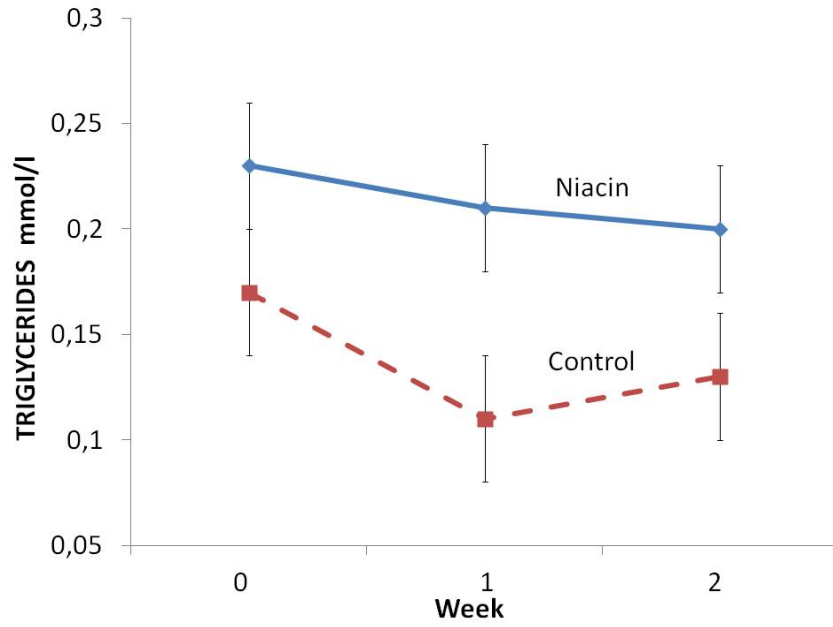


Figure 3. Concentration of triglycerides in cows supplemented and non-supplemented (control) with niacin

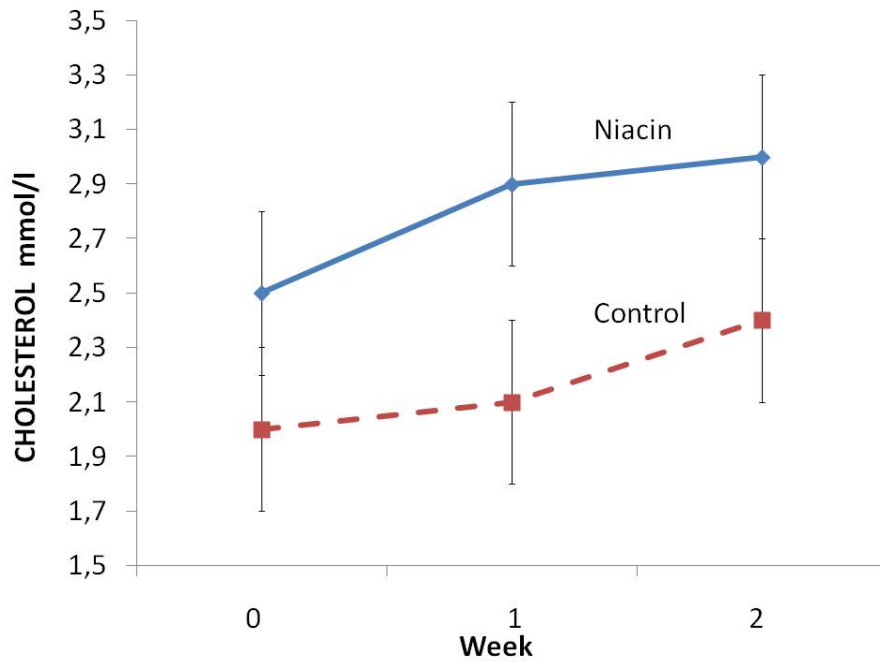


Figure 4. Concentration of cholesterol in cows supplemented and non-supplemented (control) with niacin

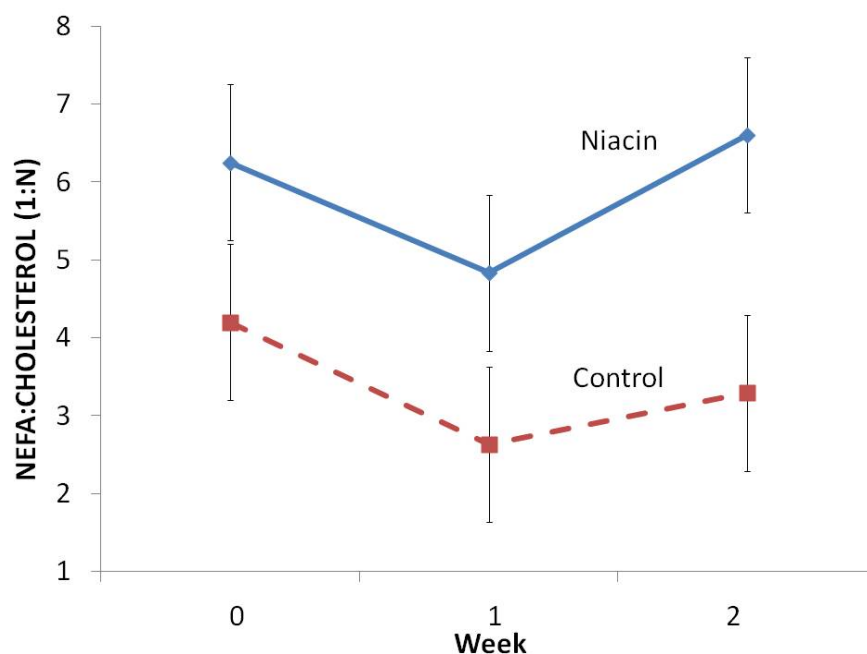


Figure 5. NEFA:cholesterol (1:n) ratio in cows supplemented and non-supplemented (control) with niacin

CONCLUSION

Application of niacin in cows during periparturient period showed positive effects on lipid metabolism such as decreased lipid mobilization (decreased NEFA), ketogenesis (decreased BHB) and liver lipidosis (higher triglycerides and cholesterol in blood and higher concentration of cholesterol per NEFA unit) in early lactation.

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UTICAJ NIACINA NA METABOLIZAM MASTI KOD KRAVA U RANOJ LAKTACIJI

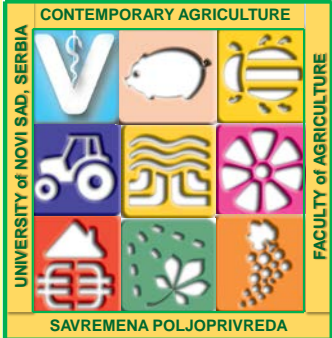
Marko R. CINCOVIĆ, Talija HRISTOVSKA, Branislava BELIĆ, Radojica ĐOKOVIĆ,
Zorana KOVAČEVIĆ, Miloš PETROVIĆ, Maja DOŠENOVIĆ, Biljana DELIĆ

Izvod: Najvažnije metaboličke promene u ranoj laktaciji kod mlečnih krava nalaze se u okviru metabolizma masti. U ovom periodu postoji negativni energetska bilans krava sa insulinskom rezistencijom i posledičnim povećanom mobilizacijom lipida, ketogenezom i zamašćenjem jetre. Cilj ovog rada je da se ispita uticaj aplikacije niacina na metabolizam lipida kod krava u ranoj laktaciji. U ogled je uključeno 30 krava koje su podeljene u dve grupe: krave koje su primale niacin (15) i krave koje nisu primale niacin (15). Krv je uzeta venepunkcijom v.coccigea u vakutajnere za separaciju seruma u momentu teljenja i u prvoj i drugoj nedelji posle teljenja. Određena je koncentracija NEFA, BHB, holesterola i triglicerida, a izračunat je i odnos NEFA:holesterol. Aplikacija niacina pokazuje pozitivne efekte na metabolizam masti kod krava u ranoj laktaciji dovodeći do smanjene lipidne mobilizacije (niža koncentracija NEFA), smanjenje ketogenze (niža koncentracija BHB) i smanjenog stepena zamašćenja jetre (viša koncentracija holesterola i triglicerida u krvi i veća koncentracija holesterola po jedinici NEFA).

Ključne reči: krave, niacin, metabolizam lipida, peripartalni period.

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MITIGATING LIPID PEROXIDATION IN LEAVES OF *PRUNUS SP.* ROOTSTOCKS USING EXOGENOUSLY APPLIED AUXINS*

Dušica DORIĆ[♦], Biljana KIPROVSKI, Đorđe MALENCIĆ, Vladislav OGNJANOV,
Mirjana LJUBOJEVIĆ, Goran BARAĆ¹

Summary: The effect of exogenously applied auxins on mechanical injury induced oxidative stress was studied in 6 promising rootstock selections of *Prunus cerasus* L., *P. mahaleb* L., and *P. fruticosa* Pall. Investigated selections were included in low-vigorous rootstock breeding programme for sweet and sour cherries. The standard rootstock PHL-A was used as a control, due to successful rooting. Leaves of investigated rootstocks were collected on: 0, 1st, 3rd and 7th day of inserting softwood cuttings into the rooting substrate under a fogging system with 95-99% average relative humidity. Exogenously applied auxin mixture consisted of 0.8% α -naphthylacetic acid (NAA) and 0.5% indolebutyric acid (IBA). Mechanical injuries during rooting period represent one of the factors that induce stress in softwood cuttings. Intensity of LP is used as a valuable biomarker of plant response to various abiotic factors. Differences in intensity of LP between auxin-treated and -untreated cuttings were examined. Almost all of investigated selections had lower LP intensity after auxin application (11.4-47.2%) between 1st and 3rd day. However, the most prominent change was in leaves of PHL-A (49.2%). The best LP-lowering effect were recorded in leaves of *P. fruticosa*, SV4 selection (56.9%) on 7th day and in *P. mahaleb*, M4 selection, on 1st and 3rd day (5.9, 5.8%), in comparison to untreated softwood cuttings. As for OV21 selection, LP intensity significantly increased in both treated and untreated cuttings on the 1st, 3rd and 7th day, but auxin-treated cuttings showed lower LP values, except on 7th day, reaching 221.3 nmol MDA equivalents g⁻¹ fresh weight. Intensity of LP during vegetative propagation of selected genotypes could be used as one of the biochemical parameters in further rootstock selection for sweet and sour cherries.

Key words: softwood cuttings, auxins, lipid peroxidation, oxidative stress.

INTRODUCTION

Fruit rootstock breeding and selection programmes have a number of goals. Above all, rootstocks should be adapted to environmental conditions, expected to be easily propagated, to result in uniform fruit tree behavior in the orchard. Selection success in breeding of vegetative rootstocks for sweet and sour cherry is dependent not only on

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genetic variability, but also on expediting propagation processes as a precondition for investigation of special combining abilities between rootstock and scion (Bošnjaković et al., 2012; Bošnjaković et al., 2013). To expedite propagation of rootstock selections, propagation by softwood cuttings is preferable.

Oxidative metabolism of normal cells and different stress situations generate highly reactive oxygen species (ROS). The ROS, such as, superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen (1O_2) have been implicated in a number of physiological disorders in plants (Scandalios, 1993; Blomster et al., 2011), which in turn leads to a decrease in plant productivity (Smirnoff, 1998; Pasternak et al., 2005). To prevent oxidative stress, plants have evolved a complex antioxidant system composed of non-enzymatic and enzymatic mechanisms that scavenge ROS (Casano et al., 2004; Malenčić et al., 2010). The formation of auxins conjugates may serve as a protection against oxidative degradation (Taiz and Zeiger, 2006). Antioxidant defense systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS. A ROS signal can be generated in a group of cells in the plant in response to wounding, pathogen attack or a local abiotic stress, and be transferred to the entire plant. The integration of ROS with auxin signaling networks, triggered by environmental factors, is known as the stress-induced morphogenic response. In this response, ROS and auxin metabolism interfere and lead to morphological changes that help avoid deleterious effects of environmental stress (Mittler et al., 2011).

The exogenous supply of auxins to leaf cuttings readily induces the new root formation, which ensures vegetative propagation. Adventitious root formation in some plant species initiates without any specific treatment, while in others, a medium supplied with growth regulators is required. In horticulture, the stimulatory effect of auxin on the formation of adventitious roots has been very useful for the vegetative propagation of plants by cuttings (Taiz and Zeiger, 2006). Lipid peroxidation (LP) is a natural metabolic process occurring in aerobic conditions and presents the most investigated effect of ROS on structure and function of cell membrane. Autocatalytic peroxidation of membrane lipids by ROS leads to loss of membrane semipermeability (Xu et al., 2006). ROS can cause cellular and molecular damage, protein modification and LP (Ali et al., 2005). Thus, the aim of this study was to measure the intensity of LP in the leaves of six cherry rootstocks selections in order to investigate the effect of exogenously applied auxins on the mechanical injury induced oxidative stress during rooting of softwood cuttings.

MATERIAL AND METHODS

Intensity of LP was determined in leaves of six promising rootstock selections of *Prunus cerasus* L., (OV21, OV22), *P. mahaleb* L., (M4, M6) and *P. fruticosa* Pall. (SV2st, SV4). Standard vegetative rootstock-PHL-A was used as a control, due to successful rooting. Softwood cuttings of investigated selections were collected from *ex situ* mother trees from the experimental field of the Faculty of Agriculture at Rimski Šančevi, near Novi Sad. The experiment was carried out in a plastic house under a fogging system with 95-98% relative humidity in average, where intervals lasted 90 s with 720 s pause. Fogging wasn't carried out during the night. The rooting substrate was a blend of white sphagnum and perlite. The 15-20 cm long terminal cuttings were treated with exogenously applied auxin mixture consisted of 0.8% α -naphthylacetic acid (NAA) and 0.5% indolebutyric acid (IBA). Leaves of investigated rootstocks were collected on 0, 1st, 3rd and 7th day after cutting and inserting softwood cuttings into the rooting substrate. Experiment was carried out with total 60 softwood cuttings per selection (30 auxin-treated and 30 auxin-untreated cuttings).

As a measure of LP intensity, the amount of malondialdehyde (MDA) was determined spectrophotometrically by the MDA or thiobarbituric acid-reactive-substances (TBARS) assay. MDA is formed through autooxidation and enzymatic degradation of polyunsaturated fatty acids in cells. This secondary end product of the oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) *via* an acid-catalyzed nucleophilic-addition reaction yielding a pinkish-red chromagen with an absorbance maximum at 532 nm (Hodges, 1999). Leaves of softwood cuttings were first homogenized and then extracted in 10% trichloroacetic acid (TCA) in ratio 1:5 (w/v) and centrifuged at 12000 x g for 30 min at 4 °C. One cm³ of supernatant was incubated with 4 cm³ 20% TCA containing 0.5% TBA for 30 min at 95 °C. The reaction was stopped by cooling on ice for 10 min and the product was centrifuged at 10000 x g for 15 min. The absorbance of the TBARS was measured at 532 nm and 600 nm and their concentration was determined using the MDA extinction coefficient of 155 mM cm⁻¹ and expressed as nmol MDA g⁻¹ fresh weight.

All determinations were made in triplicates, and values were expressed as the means \pm standard deviation. Statistical significance was tested by ANOVA followed by comparisons of means by Duncan's multiple range test ($P < 0.05$). The results were presented as a % of control.

RESULTS AND DISCUSSION

Relatively little is known about how ROS regulate plant growth and development under stress conditions, and how they interact with other signaling molecules, including phytohormones (Pasternak et al., 2005). Measurement of MDA levels is routinely used as an index of LP under stress conditions. The present investigation showed that mechanical injury induced oxidative stress in the leaves of rootstock selection for sweet and sour cherry, characterized by an accumulation of MDA, may be attributed to the genotypic differences in wounding tolerance. According to Ljubojević et al. (2011), differences in rooting capability among investigated rootstock selections were presented. Satisfying percentage of rooting was achieved in *P. cerasus* selections, while for *P. fruticosa*, method for vegetative propagation should be improved. Propagation protocol within and between species could not be uniformed, because it is strongly influenced by genotype (Bošnjaković et al., 2012). Results of Malenčić et al. (2012) showed a positive effect of exogenously applied NAA on LP-lowering effect in standard vegetative rootstock Gisela 5, as well as in four investigated sweet and sour cherry rootstocks selections. Similar was recorded for standard rootstock PHL-A in our experiment where a positive auxin effect on lowering LP intensity was noticed between 1st and 3rd collecting day, but on 7th day, an increase of LP was 65% compared to control (Fig. 1). In *P. mahaleb*, selection M4, MDA production was significantly lower in auxin-treated cuttings on 1st and 3rd collecting day. On 7th day LP intensity was similar in auxin-treated cuttings of both *P. mahaleb* selections, M4 and M6 (24.2% and 2.5%, respectively), comparing to untreated cuttings (23.9%, 5.5%) (Fig. 2 and Fig. 3). The enhancement of MDA accumulation, a cytotoxic product of lipid peroxidation, was recorded in *P. fruticosa* selection SV2st. on 3rd collecting day in both treated and untreated cuttings, contrary to 7th collecting day where auxin application showed lowering effect on LP intensity (Fig. 4). Lowering effect on LP intensity was noticed on 1st and 7th day in SV4 selection, also. Higher degree of membrane damage was noticed in untreated cuttings on 7th collecting day (154.5% of control) (Fig. 5).

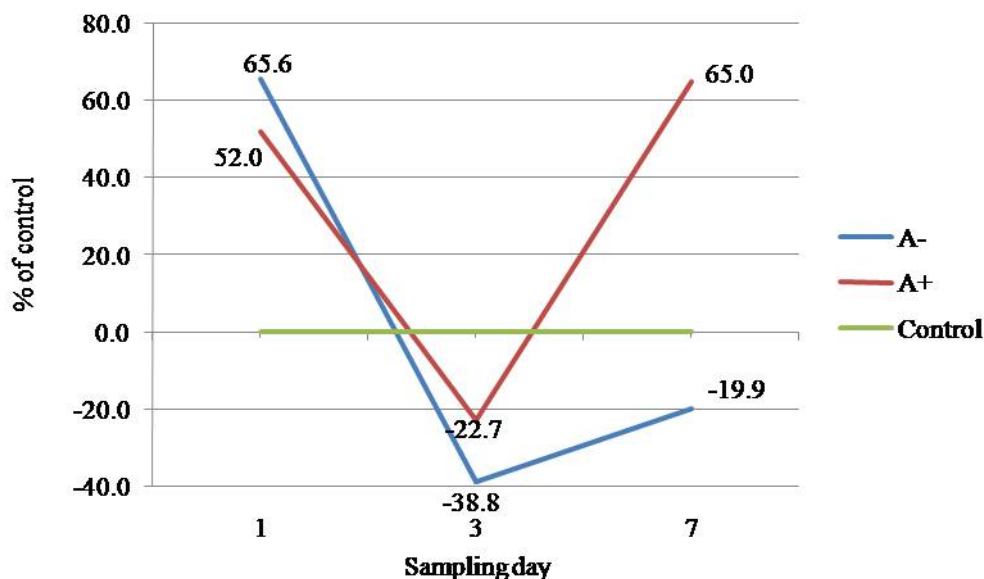


Figure 1. LP intensity in auxin treated and untreated standard vegetative rootstock PHL-A

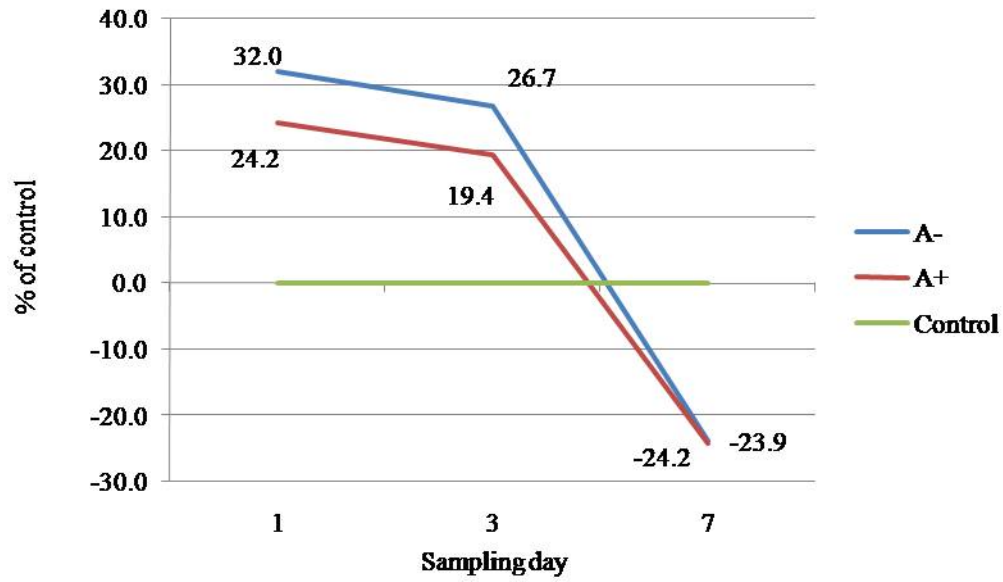


Figure 2. LP intensity in auxin treated and untreated rootstock selection *P. mahaleb*-M4

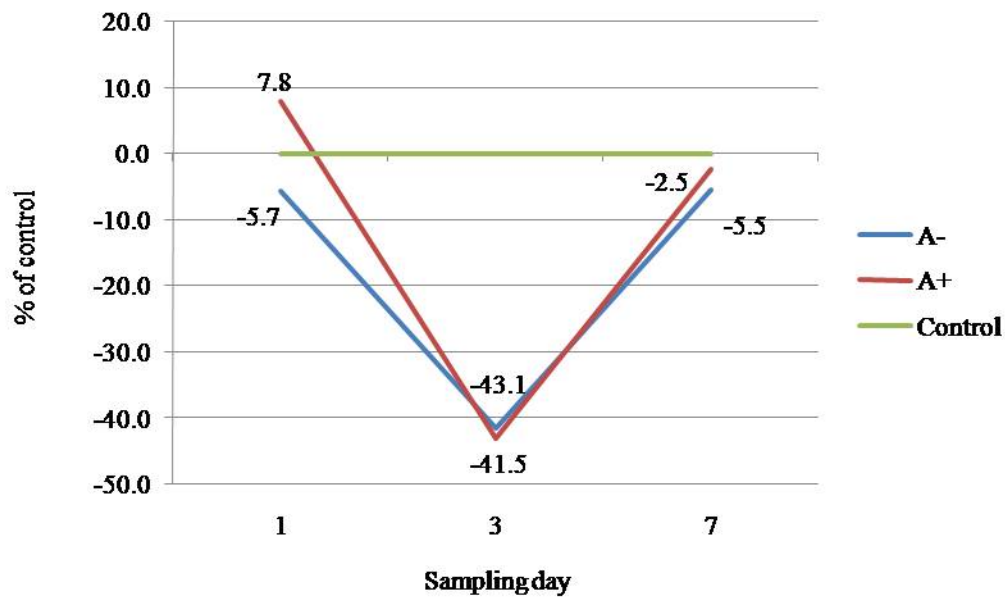


Figure 3. LP intensity in auxin treated and untreated rootstock selection *P. mahaleb*- M6

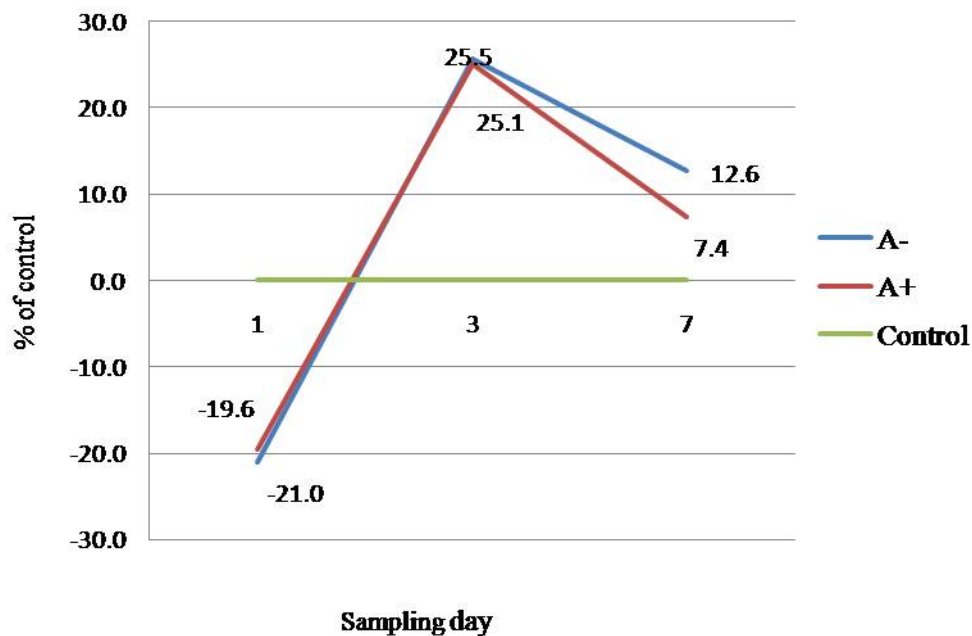


Figure 4. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa*-SV2st.

Figure 5. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa*-SV4

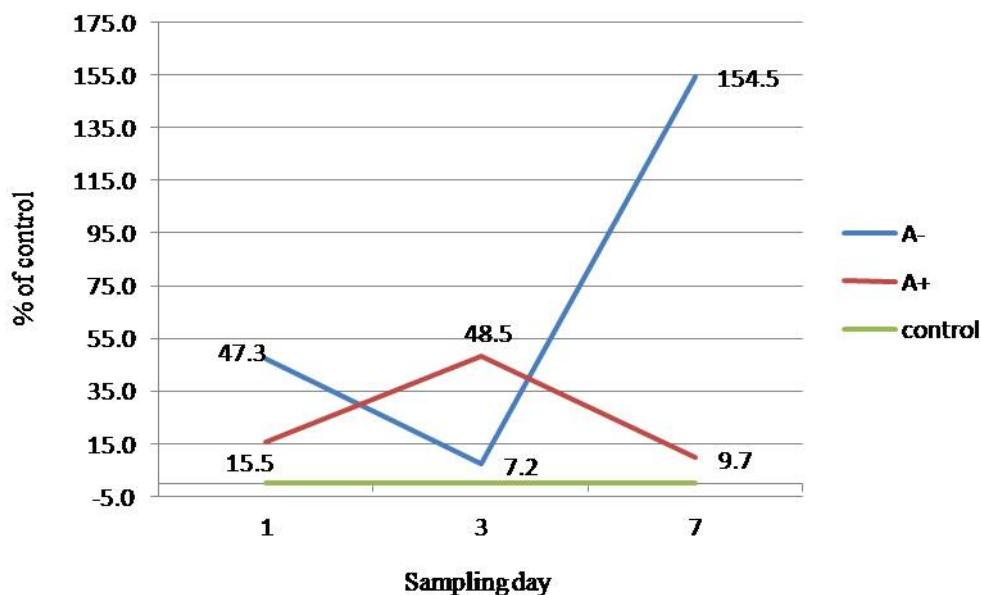


Figure 5. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa*-SV4

On 1st and 3rd collecting day auxin-treated cuttings of OV21 selection had lower LP intensity, but damage in membrane structure after auxin application was presented on 7th collecting day, reaching 221.3 nmol MDA equivalents g⁻¹ fresh weight (Fig. 6). As for OV22 selection (Fig. 7), higher LP intensity resulted from enhanced ROS production, indicate that this selection was under higher oxidative stress conditions. Positive effect of auxin application was recorded on 3rd collecting day, where LP intensity was significantly lower compared to untreated

cuttings and control. Plants respond to wounding by activating self-defense systems to restore damaged tissue (Castro-Mercado et al., 2009). According to Cheong et al. (2002), wounding negatively regulates IAA responsive genes, revealing a new level of crosstalk between wounding and auxin response in plants. Studies of expression patterns of genes regulated by wounding provided new information on the interactions between wounding and other signals, such as pathogen attack, abiotic stress factors, and plant hormones (Cheong et al., 2002). Different abiotic factors induce oxidative stress in cell, which increase MDA production in different plant species (Shalata and Tal, 1998; Sudhakar et al., 2001; Bor et al., 2003; Wu et al., 2003). Possibly, due to the fact that wounding inhibits auxin level in plants, exogenously applied auxin had a positive effect on rooting in our experiment, however, LP-lowering effect of applied hormone, had no uniform effect on all investigated selections.

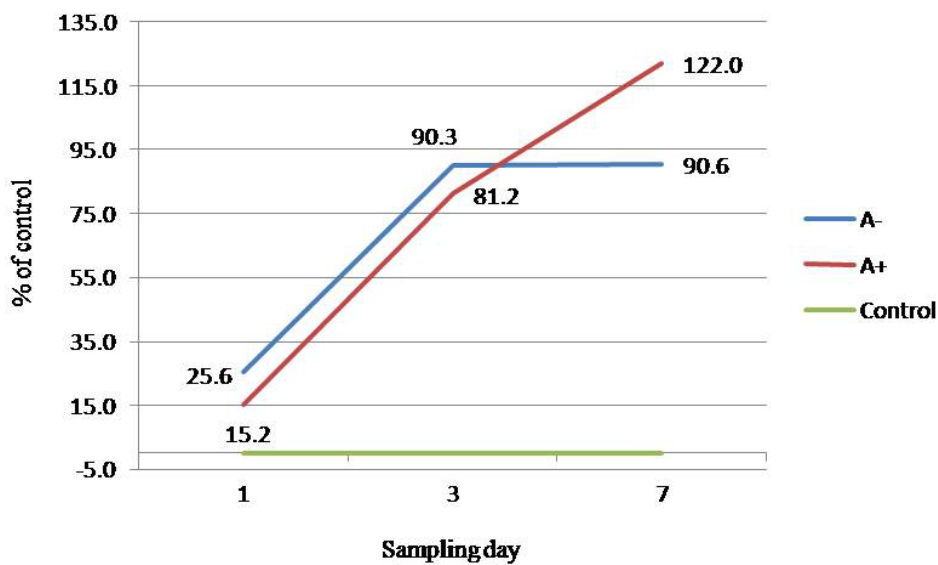


Figure 6. LP intensity in auxin treated and untreated rootstock selection *P. cerasus*-OV21

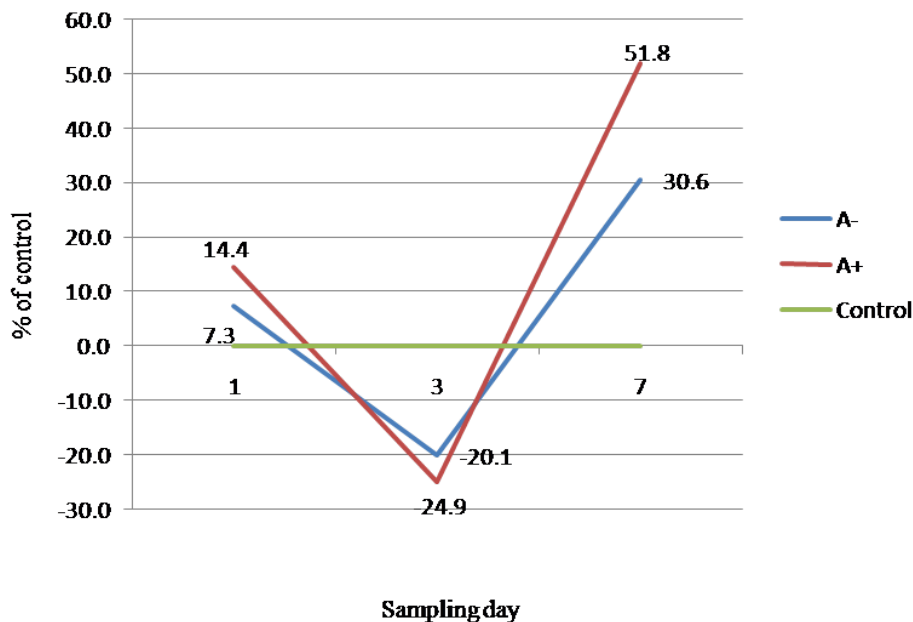


Figure 7. LP intensity in auxin treated and untreated rootstock selection *P. cerasus*-OV22

CONCLUSION

There is a noticeable difference in the alternation of MDA content among 7 investigated genotypes of *Prunus cerasus* L., *P. mahaleb* L. and *P. fruticosa* Pall., which implies to a great genetic variability in response of these rootstocks toward wounding and oxidative stress induced by investigated abiotic factor. Despite the fact that auxin has a positive effect on rooting and LP-lowering effect in most of treated selections, its application seems to have no universal LP-mitigating effect for treated rootstocks. Among all investigated selections, the best auxin LP-lowering effect was in *P. mahaleb*, M6 selection during the entire experiment. Also, the results gained from LP assay proved to be invaluable marker for resistance to mechanical injury induced oxidative stress and impact of propagation in sweet and sour rootstock selection.

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SMANJENJE INTENZITETA LIPIDNE PEROKSIDACIJE U LISTOVIMA SELEKCIJA VEGETATIVNIH PODLOGA ZA VIŠNJU I TREŠNJU UPOTREBOM EGZOGENO PRIMENJENIH AUKSINA

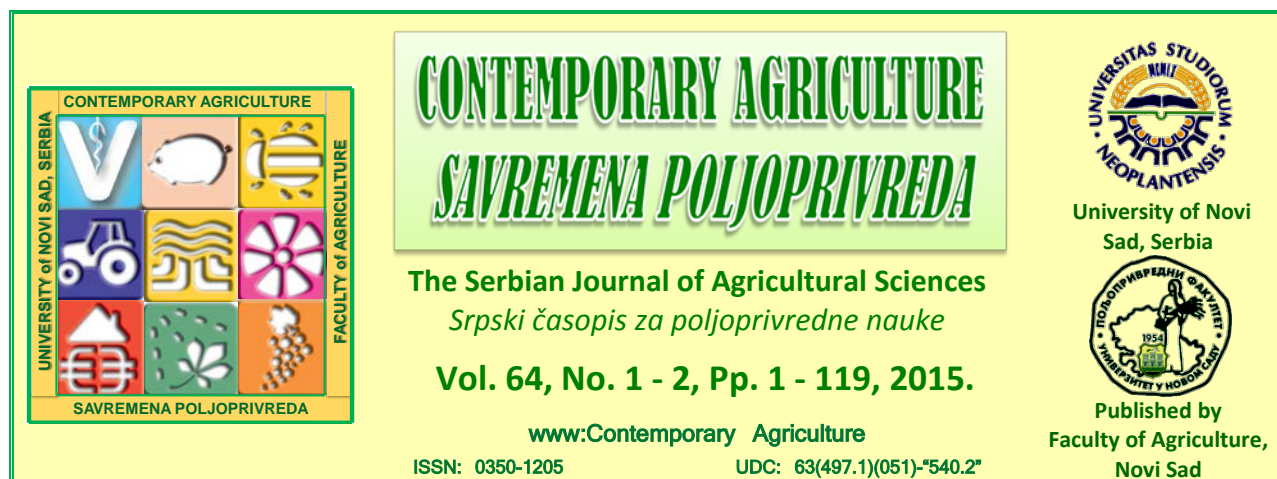
Dušica DORIĆ, Biljana KIPROVSKI, Đorđe MALENČIĆ, Vladislav OGNJANOV, Mirjana LJUBOJEVIĆ, Goran BARAĆ

Izvod: Uticaj egzogeno primenjenih auksina na oksidativni stres izazvan mehaničkom povredom izučavan je kod 6 selekcija vegetativnih podloga vrsta- *Prunus cerasus L.*, *P. mahaleb L.*, i *P. fruticosa Pall.* koje su uključene u oplemenjivački program na stvaranju slabobujnih podloga za višnju i trešnju. Kao kontrola korišćena je standardna vegetativna podloga za višnju i trešnju- PHL-A. Listovi ispitivanih selekcija sakupljani su 0, 1, 3 i 7 dana od termina tretiranja reznica u supstratu za ožiljavanje, u kontrolisanim uslovima vlaženja, sa 95-99 % relativne vlažnosti. Reznice su tretirane egzogeno primenjenom kombinacijom auksina- 0,8 % α - naftilsirćetna kiselina (NAA) i 0,5 % indolbuterna kiselina (IBA). Mehanička povreda nastala odvajanjem letorasta od matične biljke predstavlja jedan od faktora koji utiču na stvaranje oksidativnog stresa u biljkama. Intenzitet lipidne peroksidacije je korišćen kao pokazatelj reakcije biljaka na različite abiotičke faktore. U radu su praćene razlike intenziteta lipidne peroksidacije između reznica tretiranih auksinima i reznica bez primene egzogenih auksina. Skoro sve ispitivane selekcije su imale smanjen intenzitet LP nakon primene auksina (11,4- 47,2 % manje od kontrole) između prvog i trećeg dana. Najbolje smanjenje intenziteta LP je uočeno kod standardne podloge PHL-A (49,2 %). Smanjenje intenziteta LP je zabeleženo kod selekcije *P. fruticosa*, SV4 (56,9 %), sedmog dana, i kod selekcije *P. mahaleb*, M4, prvog i trećeg dana (5,9 i 5,8 %), u poređenju sa netretiranim reznicama. Kod selekcije *P. cerasus*- OV21 uočen je porast intenziteta LP, kako kod netretiranih, tako i kod tretiranih reznica, prvog, trećeg i sedmog dana. Vrednosti LP su bile manje kod tretiranih reznica, osim sedmog dana, dostižući 221,3 nmol MDA ekvivalenata g^{-1} svm. Pozitivan efekat primenjenih auksina na smanjenje intenziteta LP tokom postupka vegetativnog ožiljavanja može biti jedan od biohemijskih parametara u budućem selekcionom radu koji se odnosi na oplemenjivanje podloga za trešnju i višnju.

Ključne reči: zelene reznice, auksini, lipidna peroksidacija, oksidativni stress.

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ECOLOGICAL-PHYTOGEOGRAPHICAL CHARACTERISTICS OF WEED AND RUDERAL FLORA OF GOLUBINCI (SERBIA)

Marko Lj. NESTOROVIĆ[♦], Branko KONSTANTINOVIĆ¹

Summary: Presence of 244 species of vascular plants, from 180 genera and 44 families, was determined in various types of weed and ruderal habitats of village Golubinci near Stara Pazova. This paper presents the taxonomic, ecological and phytogeographical analyses of weed and ruderal flora. By an analysis of the present life forms of the plants belonging to the investigated weed flora, its hemicryptophytic – therophytic character has been established (47.13%:38.11%). Phytogeographic analysis of the weed flora revealed the presence of 18 different floristic elements grouped into 7 main area types. The most numerous were the group which belongs to Eurasian areal type (34.02%).

Key words: weed flora, ruderal flora, ecological indices, phytogeographical analysis.

INTRODUCTION

Weed and ruderal flora and vegetation is present in cities, villages, industrial zones and other areas under continuous or sporadic anthropogenic impact. Habitats of weed and ruderal flora and vegetation are situated on cultivated agricultural land, along the roads, alleys, railroads, in dilapidated buildings and at construction sites, on walls and roofs, various landfills, in hedges, along the moist and nitrified banks of streams and rivers in vicinity of settlements etc. As weed and ruderal flora and vegetation develop in closest vicinity of humans, it understandably besought considerable interest of researchers (Nestorović, 2002, 2003, 2005; Nestorović and Jovanović, 2002, 2003; Nestorović et al., 2005; Nestorović and Konstantinović, 2011a, 2011b; Jakovljević et al., 2008; Jovanović, 2004; Gavrilović et al., 2012; etc) for studies on taxonomic, phytogeographical and ecological analysis of weed and ruderal flora, monitoring of anthropogenous impact on changes in weed and ruderal flora and vegetation, and increased human activities influencing deliberate or accidental introduction of invasive plant species leading to disturbance of ecological equilibrium of weed and ruderal habitats (Jarić, 2009). The need for renewal and conservation of natural ecosystems in human environment also leads to need for research on urban flora and vegetation and impact of climatic and other conditions on vegetation cover of weed and ruderal habitats.

Research on weed and ruderal flora and vegetation was performed in area of village Golubinci, in Srem region of Vojvodina. Golubinci is a lowland area with poorly developed relief. Within this large complex there are various categories of weed and ruderal habitats with rich flora and vegetation.

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MATERIAL AND METHODS

The floristic research was performed in the territory within the cadaster limits of Golubinci (village near Stara Pazova). Collecting of plant material was performed in several weed and ruderal habitats from early April 2012 to late October 2013 (crops, orchards, vineyards, gardens, vegetable patches, road and path edges, hedges, trodden lawns, yards, landfills, levees, commons between fields etc.).

Following literature was used for determination of weed and ruderal flora of Golubinci: Takhtajan (2007); Tutin et al. (1999); etc.

Alignment of species to certain life forms was implemented according to the abridged Raunkier scheme presented by Ellenberg & Muller-Dambois, which was further developed and supplemented according to Stevanović (1992).

The phytogeographical placement of certain floristic elements was determined according to Gajić (1980).

The ecological (bioindication) indices for each species were determined for main ecological factors according to scheme devised by Kojić et al. (1994, 1997a).

RESULTS AND DISCUSSION

Presence of 244 species of vascular plants, from 180 genera and 44 families, was determined in various types of weed and ruderal habitats of village Golubinci near Stara Pazova (Table 1). The highest number of taxa within the weed and ruderal flora of Golubinci includes the same families that show the greatest richness in number of species and genera at the level of flora of whole Serbia. The best-represented families include *Asteraceae* (47), *Lamiaceae* (23), *Poaceae* (21) and *Fabaceae* (19). The high level of representation of families *Ranunculaceae* (12 species), *Scrophulariaceae* (11), *Boraginaceae* (10), *Brassicaceae* (10), *Polygonaceae* (9), *Rosaceae* (9) and *Apiaceae* (8) was expected due to the synanthropic character of many representatives of these families. This conclusion was supported by results of analysis of representation of certain genera in the weed and ruderal flora of village Golubinci. The dominance of typical weed and ruderal species from genera *Ranunculus*, *Chenopodium*, *Potentilla*, *Rumex*, *Trifolium* and *Veronica* shows a strong anthropogenous character of various weed and ruderal habitats, indicating that they are subjected to high level of nitrification, foot traffic, mowing and other anthropogenous impacts that define weed habitats as highly dynamic and unstable biotopes (Lososová et al., 2012).

Table 1. Overview of weed and ruderal flora of village Golubinci.

<i>Abutilon theophrasti</i> Medik.	<i>Ballota nigra</i> L.
<i>Achillea millefolium</i> L.	<i>Bassia scoparia</i> (L.) A.J.Scott
<i>Adonis aestivalis</i> L.	<i>Bellis perennis</i> L.
<i>Agrimonia eupatoria</i> L.	<i>Bidens tripartita</i> L.
<i>Agrostemma githago</i> L.	<i>Bilderdykia convolvulus</i> (L.) Dumort.
<i>Ailanthus altissima</i> (Mill.) Swingle	<i>Bromus commutatus</i> Schrad.
<i>Ajuga chamaepitys</i> (L.) Schreb.	<i>Bromus sterilis</i> L.
<i>Alcea rosea</i> L.	<i>Calamagrostis epigejos</i> (L.) Roth
<i>Alliaria petiolata</i> (M.Bieb.) Cavara & Grande	<i>Calystegia sepium</i> (L.) R.Br.
<i>Alopecurus pratensis</i> L.	<i>Campanula rapunculus</i> L.
<i>Althaea officinalis</i> L.	<i>Capsella bursa-pastoris</i> (L.) Medik.
<i>Althea cannabina</i> L.	<i>Cardaria draba</i> (L.) Desv.
<i>Alyssum alyssoides</i> (L.) L.	<i>Carduus acanthoides</i> L.
<i>Amaranthus retroflexus</i> L.	<i>Carthamus lanatus</i> L.
<i>Ambrosia artemisiifolia</i> L.	<i>Caucalis platycarpos</i> L.
<i>Amorpha fruticosa</i> L.	<i>Centaurea jacea</i> L.
<i>Anagallis arvensis</i> L.	<i>Centaurea pannonica</i> (Heuff.) Simonk.
<i>Anchusa arvensis</i> (L.) M.Bieb.	<i>Centaurea scabiosa</i> L.
<i>Anchusa officinalis</i> L.	<i>Cerinthe minor</i> L.
<i>Anthemis arvensis</i> L.	<i>Chamaecytisus supinus</i> (L.) Link
<i>Anthemis tinctoria</i> L.	<i>Chamomilla recutita</i> (L.) Rauschert
<i>Antirrhinum majus</i> L.	<i>Chelidonium majus</i> L.
<i>Arctium lappa</i> L.	<i>Chenopodium album</i> L.
<i>Aristolochia clematidis</i> L.	<i>Chenopodium hybridum</i> L.
<i>Armoracia rusticana</i> P.Gaertn., B.Mey. & Scherb.	<i>Chenopodium murale</i> L.
<i>Arrhenatherum elatius</i> (L.) P.Beauv. ex J.Presl & C.Presl	<i>Chenopodium rubrum</i> L.
<i>Artemisia vulgaris</i> L.	<i>Chondrilla juncea</i> L.
<i>Asclepias syriaca</i> L.	<i>Cichorium intybus</i> L.
<i>Aster lanceolatus</i> Willd.	<i>Cirsium arvense</i> (L.) Scop.
<i>Aster salignus</i> Willd.	<i>Cirsium vulgare</i> (Savi) Ten.
<i>Astragalus cicer</i> L.	<i>Clematis integrifolia</i> L.
<i>Avena fatua</i> L.	<i>Clematis vitalba</i> L.

- Clinopodium vulgare* L.
Conium maculatum L.
Consolida orientalis (J.Gay) Schrödinger
Consolida regalis Gray.
Convolvulus arvensis L.
Conyza canadensis (L.) Cronquist
Coronilla varia L.
Crepis biennis L.
Crepis foetida L.
Cynodon dactylon (L.) Pers.
Dactylis glomerata L.
Datura stramonium L.
Daucus carota L.
Digitalis lanata Ehrh.
Digitaria ciliaris (Retz.) Koeler
Digitaria sanguinalis (L.) Scop.
Diplotaxis muralis (L.) DC.
Dipsacus fullonum L.
Dipsacus laciniatus L.
Echinochloa crus-galli (L.) P. Beauv.
Echium vulgare L.
Elymus repens (L.) Gould
Equisetum arvense L.
Erigeron annuus (L.) Pers.
Erodium cicutarium (L.) L'Hér.
Eryngium campestre L.
Eupatorium cannabinum L.
Euphorbia cyparissias L.
Euphorbia esula L. subsp. *tommasiniana* (Bertol.) Nyman
Euphorbia helioscopia L.
Falcaria vulgaris Bernh.
Festuca arundinacea Schreb.
Fumaria officinalis L.
Galeopsis tetrahit L.
Galinsoga parviflora Cav.
Galium aparine L.
Galium mollugo L.
Galium verum L.
Geranium molle L.
Geum urbanum L.
Glechoma hederacea L.
Hedera helix L.
Helianthus annuus L.
Heliotropium europaeum L.
Helleborus odoratus Waldst. & Kit.
Hibiscus trionum L.
Hieracium bauhini
Hordeum murinum L.
Humulus lupulus L.
Hyoscyamus niger L.
Hypericum perforatum L.
Inula britannica L.
Iva xanthifolia Nutt.
Knautia arvensis (L.) Coult.
Lactuca serriola L.
Lamium amplexicaule L.
Lamium purpureum L.
Lapsana communis L.
Lathyrus aphaca L.
Lathyrus tuberosus L.
Leonurus cardiaca L.
Ligustrum vulgare L.
Linaria genistifolia (L.) Mill.
Linaria vulgaris Mill.
Lithospermum arvense L.
Lolium perenne L.
Lotus corniculatus L.
Lycopus europaeus L.
Lysimachia vulgaris L.
Lythrum salicaria L.
Lythrum virgatum L.
Malva sylvestris L.
Matricaria perforata Mérat
Medicago lupulina L.
Medicago sativa L. subsp. *falcata* (L.) Arcang.
Melampyrum arvense L.
Melilotus officinalis (L.) Pall.
Melissa officinalis L.
Mentha arvensis L.
Mentha longifolia (L.) Huds.
Mentha piperita L.
Myosotis arvensis (L.) Hill
Myosotis sylvatica Hoffm.
Nigella arvensis L.
Nonnea pulla (L.) Lam. et D.C.
Oenothera biennis L.
Ononis spinosa L.
Orlaya grandiflora (L.) Hoffm.
Ornithogalum umbellatum L.
Oxalis stricta L.
Papaver rhoeas L.
Parietaria officinalis L.
Petasites hybridus (L.) P.Gaertn., B.Mey. & Scherb.
Phragmites australis (Cav.) Trin. ex Steud.
Picris echioides L.
Picris hieracioides L.
Pimpinella saxifraga L.
Plantago lanceolata L.
Plantago major L.
Plantago media L.
Poa annua L.
Poa pratensis L.
Polygonum aviculare L.
Polygonum lapathifolium L.
Polygonum persicaria L.
Portulaca oleracea L.
Potentilla anserina L.
Potentilla argentea L.
Potentilla cinerea Chaix ex Vill.
Potentilla reptans L.
Prunella vulgaris L.
Prunus spinosa L.
Ranunculus acris L.
Ranunculus arvensis L.
Ranunculus ficaria L.
Ranunculus repens L.
Ranunculus sardous Crantz
Raphanus raphanistrum L.
Reseda lutea L.
Robinia pseudoacacia L.
Rorippa sylvestris (L.) Besser
Rosa canina L.
Rubus caesius L.
Rumex acetosa L.
Rumex acetosella L.
Rumex crispus L.
Rumex sanguineus L.
Salvia nemorosa L.
Salvia pratensis L.
Salvia verticillata L.
Sambucus ebulus L.
Saponaria officinalis L.
Scutellaria hastifolia L.
Senecio erucifolius L.

Senecio vernalis Waldst. & Kit.
Senecio vulgaris L.
Setaria pumila (Poir.) Schult.
Setaria viridis (L.) P. Beauv.
Sideritis montana L.
Silene latifolia Poir. subsp. *alba* (Mill.) Greuter & Burdet
Silene vulgaris (Moench) Garcke
Sinapis arvensis L.
Solanum dulcamara L.
Solanum nigrum L.
Solidago virgaurea L.
Sonchus arvensis L.
Sonchus oleraceus L.
Sorghum halepense (L.) Pers.
Stachys annua (L.) L.
Stachys germanica L.
Stellaria media (L.) Vill.
Symphytum officinale L.
Tanacetum vulgare L.
Taraxacum officinale Weber
Teucrium chamaedrys L.
Thlaspi arvense L.
Thymus serpyllum L.
Torilis arvensis (Huds.) Link
Tragopogon dubius Scop.
Trifolium campestre Schreb.
Trifolium hybridum L.
Trifolium pretense L.
Trifolium repens L.
Tussilago farfara L.
Urtica dioica L.
Verbascum blattaria L.
Verbascum phlomoides L.
Verbena officinalis L.
Veronica austriaca L. subsp. *austriaca* Baumg.
Veronica chamaedrys L.
Veronica hederifolia L.
Veronica persica L.
Vicia cracca L.
Vicia sativa L.
Vicia villosa Roth.
Vinca minor L.
Viola arvensis Murray
Viola kitaibeliana Schult.
Viola odorata L.
Xanthium strumarium L.

The analysis of representation of plant life forms in the weed and ruderal flora of village Golubinci indicates pronounced dominance of hemicryptophytes (47.13%), matching the representation of this life form in the overall flora of Serbia (Table 2). The perennial scapose hemicryptophytes, along with biennial forms, are best represented with a total of 95 species. Regarding the phenological dynamics of this life form, there is a dominance of aestival-flowering species (135), while regarding the height categories the tall plants are particularly pronounced. All this characteristics match the perennial character of hemicryptophyte life form and the general ecological-climatic characteristics of the study area.

The annual herbaceous plants (therophytes) are represented with 93 species (38.11%), placing them at the second place within the biological spectrum of weed and ruderal flora of village Golubinci (Table 2). Besides the fact that structure of this life form is also dominated by stem (scapose) forms, in contrast to hemicryptophytes there is a significant increase in number of vernal species as well as shorter plants, which is explained by their annual character and need to complete their life cycle (from seed to seed) within a single vegetation season.

The life form of geophytes is represented in the weed and ruderal flora of village Golubinci by 14 species (5.74%). At the same time, most present geophytes belong to the rhizome-bearing type, which is one of most adaptable life forms among the polycarpous perennials in such unstable anthropogenized habitats. This is contrasted with insignificant presence of bulb and root geophytes, which are more characteristic of stable or less degraded forest, steppe or meadow habitats. The life forms of chamaephytes and phanerophytes show the same tendencies, as they are primarily characteristic of stable habitats with primary vegetation and insignificant zooanthropogenous impact.

Table 2. Overview and representation of life forms in weed and ruderal flora of Golubinci.

Life forms	n	%	Life forms	n	%
Hemicryptophytes (H)			Geophytes (G)		
H scap	76	31.15	G rhiz	2	0.82
H scap-bienn	19	7.79	G rhiz scap	3	1.23
H caesp	7	2.87	G rhiz caesp	2	0.82
H rept-ros	4	1.64	G rhiz rept-caesp	1	0.41
H rept-scap	1	0.41	G tub rept	1	0.41
H ros	4	1.64	G rad scap	4	1.64
H ros bienn	1	0.41	G bulb scap	1	0.41
H ros-rept	1	0.41	Total	14	5.74
H semiros	2	0.82			
Total	115	47.13	Hydrophytes (Hyd)		
			Hyd G rhiz	2	0.82
Therophytes (T)			Total		
T scap	76	31.15		2	0.82
T scap-semiros	3	1.23	Chamaephytes(Ch)		
T caesp	6	2.46	Ch suff caesp	4	1.64
T caesp-rept	2	0.82	Ch herb rept	2	0.82
T rept	3	1.23	Total	6	2.46
T semiros	1	0.41			
T semiros-scap	1	0.41	Phanerophytes (P)		
T ros	1	0.41	NP caesp	3	1.23
Total	93	38.11	NP rept	1	0.41
			Mi caesp	1	0.41
Scandentophytes (S)			P scap	2	0.82
S herb	4	1.64	Total	7	2.87
S lig	3	1.23			
Total	7	2.87			

The analysis of ecological indices for five main ecological factors (humidity, acidity, amount of nitrogen, light and temperature) has shown dominance of plants preferring submesophytic and subxerophytic habitats, with mostly

neutral to weekly alkaline reaction, mostly of semiopen to open character, medium rich or rich in mineral materials, and mesothermic to thermophilous regarding the temperature regime (Table 3).

Table 3. Representation of species with certain values of ecological indices.

Ecological indices	Values of ecological indices										Mean of ecological indices
	1		2		3		4		5		
	No. of species	%	No. of species	%	No. of species	%	No. of species	%	No. of species	%	
V	6	2.46	107	43.85	112	45.9	17	6.97	2	0.82	2.59
K	1	0.41	7	2.87	157	64.34	79	32.38	0	0	3.29
N	2	0.82	30	12.3	119	48.77	86	35.25	7	2.87	3.27
S	0	0	4	1.64	95	38.93	141	57.79	4	1.64	3.59
T	0	0	1	0.41	117	47.95	106	43.44	20	8.2	3.59

The phytogeographical analysis of weed and ruderal flora of village Golubinci shows high diversity of floristic elements, with 18 recorded floristic elements (Table 4). The spectrum of floristic elements is dominated by elements with extensive distribution, matching the findings of most authors studying this issue in various weed habitats (Gavrilović et al. 2012; Nestorović, 2005; Nestorović and Konstantinović, 2011).

The spectrum of range types (Table 4) indicates that the most numerous group is Eurasian with 83 species (34.02%), followed by Pontic-Central Asian with 43 species (17.62%), Central European with 40 species (16.39%) and circumpolar-cosmopolitan with 30 species (12.3%). Far lower percentage was recorded for plant species from Atlantic group (2.46%), while no endemic or relict species were recorded. The total number of 244 recorded weed and ruderal species and dominant participation of species with extensive ranges is matching the ecological characteristics of weeds growing in immediate vicinity of human settlements and under strong anthropogenous and zoogenous influences.

The most prominent characteristic of weed and ruderal flora overall is significant participation of adventive (9.02%) and cosmopolitan species (8.2%). The increased abundance of adventive and cosmopolitan species indicates instability of weed habitats.

Table 4. Spectrum of range types.

Group	No. of species	%	Floristic element	No. of species	%
Euroasian	83	34.02	Euroasian	41	16.8
			Subeuroasian	35	14.34
			Subsoutsiberian	7	2.87
Middle-European	40	16.39	Submiddle European	40	16.39
Circumpolar-cosmopolitan	30	12.3	Circumpolare	5	2.05
			Subcircumpolar	5	2.05
			Cosmopolitan	20	8.2
Pontic-Central-Asian	43	17.62	Pontic-Panonian	1	0.41
			Subpontic	10	4.1
			Subpontic-Centralasian	8	3.28
			Subpontic-Submediterranean	12	4.92
			Pontic-Centralasian	3	1.23
			Pontic-Centralasian-Submediterranean	7	2.87
Submediterranean	20	8.2	Submediterranean	13	5.33
			Pontic- Submediterranean	7	2.87
Adventive	22	9.02	Adventive	22	9.02
Atlantic	6	2.46	Subatlantic- Submediterranean	6	2.46
Total				244	100

The analysis of adventive flora of Golubinci has shown presence of 22 plant species (9.02% of total flora). This percentage is lower than in studies of some other urban floras (Pyšek et al., 2009a, 2009b; Yavorska and Mosyakin, 2001). Comparison of percentage of adventive plants in total weed and ruderal flora of Golubinci with data in 4 other urban areas in Serbia: Smederevska Palanka (13.76%) (Jakovljević and Jovanović, 2005), Kragujevac (12.4%) (Marinković, 2002), Mladenovac (12.3%) (Anđelković, 2002), Loznica (10.49%) (Jovanović and Mitrović, 1998) indicates that representation of this plant group is the greatest at Smederevska Palanka, which was expected due to the larger size of this urban area.

Number of species shared by all study areas was high, but according to available data some of the species recorded in other studied urban areas are absent in subsponaneous form at Golubinci. Species recorded at Smederevska Palanka (Jakovljević and Jovanović, 2005) include: *Amaranthus crispus* (Lesp. & Thévenau) N.Terracc., *Callistephus chinensis* (L.) Nees, *Ipomoea purpurea* Roth. Species recorded at Mladenovac (Anđelković, 2002) include: *Callistephus chinensis* (L.) Nees, *Catalpa bignonioides* Walt., *Tecoma radicans* (L.) Juss., *Symphoricarpos racemosus* Mill., *Convolvulus tricolor* L., *Ipomoea purpurea* Roth, *Parthenocissus inserta* (A.Kern.) Fritsch. Species recorded at Kragujevac (Marinković, 2002) include: *Cosmos bipinnatus* Cav., *Cosmos sulphureus* Cav., *Rudbeckia laciniata* L., *Euphorbia lathyris* L., *Pyraecantha coccinea* M. Roem., *Sorghum technicum* (Körn.) Batt. & Trab., *Sorghum bicolor* (L.) Moench. Species recorded at Loznica (Jovanović and Mitrović, 1998) include: *Catalpa bignonioides* Walt., *Ipomoea purpurea* Roth, *Philadelphus coronarius* L. These species belong to the group of decorative plants and they obviously managed to escape from cultivation and appear subsponaneously, but only as ephemerophytes. It may be assumed that all this species are also present at the territory of Golubinci but as cultivated plants, and it is only a matter of time before they are recorded in escaped state in this area. It is however still insufficiently explained if records of all the listed adventive species in other towns in Serbia satisfy the criteria of adventive character presented by Rejmánek et al. (2002).

CONCLUSION

Presence of 244 species of vascular plants, from 180 genera and 44 families, was determined in various types of weed and ruderal habitats of village Golubinci near Stara Pazova.

The best-represented families were *Asteraceae*, *Lamiaceae*, *Poaceae* and *Fabaceae*, which are also characterized by the greatest richness in number of species and genera in flora of the whole territory of Serbia. However, the pronouncedly high participation of species from families *Ranunculaceae*, *Scrophulariaceae*, *Boraginaceae*, *Brassicaceae*, *Polygonaceae*, *Rosaceae* and *Apiaceae*, as well as species from genera *Ranunculus*, *Chenopodium*, *Potentilla*, *Rumex*, *Trifolium* and *Veronica*, indicate pronounced anthropogenous character, dynamic and unstable conditions in weed habitats.

The analysis of representation of life forms in weed and ruderal flora of village Golubinci has shown its hemicryptophyte-therophyte character, where aestival, mostly tall and scapose hemicryptophytes are represented much more (47.13%) than therophytes (38.11%). On the other hand, among the therophytes there is a more significant increase in number of vernal species and shorter plants, which is explained by their annual character and need to complete their life cycle (from seed to seed) within a single vegetation season. The greater percentage representation of hemicryptophytes and lower participation of annual weed plants in area of Golubinci was explained by greater stability and lower openness of weed and ruderal habitats in a typical non-urban area with much less pronounced anthropogenous impact.

The analysis of ecological indices for five main ecological factors has shown dominance of plants preferring submesophytic and subxerophytic habitats, of mostly neutral to weakly alkaline reaction, medium rich to rich in mineral materials, mostly of semi-open to open character, and mesothermic to thermophilous regarding the temperature regime.

The phytogeographical analysis of weed and ruderal flora has shown presence of 18 recorded floristic elements, grouped in seven main range types. The analysis of floristic elements and their representation indicate dominant participation of species with extensive ranges. The most numerous group is Eurasian with 83 species (34.02%), followed by Pontic-Central Asian with 43 species (17.62%), Central European with 40 species (16.39%) and circumpolar-cosmopolitan with 30 species (12.3%). Far lower percentage was recorded for plant species from the adventive group, submediterranean and Atlantic species. This type of phytogeographical structure matches the ecological characteristics of weeds growing in immediate vicinity of human settlements and under strong anthropogenous and zoogenous influences.

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**EKOLOŠKO-FITOGEOGRAFSKE KARAKTERISTIKE KOROVSKJE I
RUDERALNE FLORE GOLUBINACA (SRBIJA)**

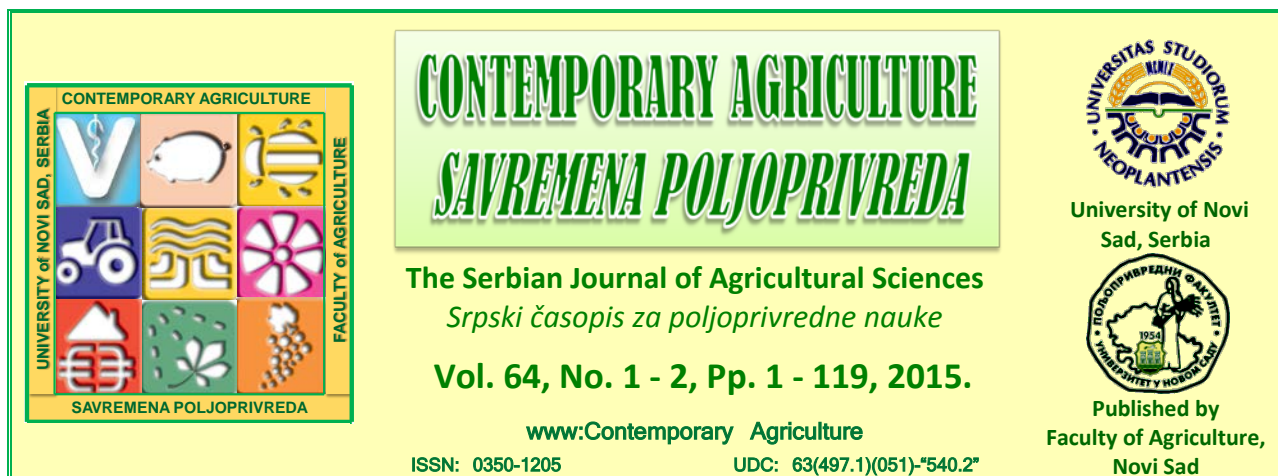
Marko Lj. NESTOROVIĆ, Branko KONSTANTINOVIĆ

Izvod: Na različitim tipovima korovskih i ruderalnih staništa sela Golubinci kod Stare Pazove utvrđeno je prisustvo 244 vrste vaskularnih biljaka, iz 180 rodova i 44 familije. U radu je predstavljena taksonomska, ekološka i fitogeografska analiza korovske i ruderalne flore. Analizom zastupljenosti životnih formi biljaka u ispitivanoj korovskoj flori ustanovljen je hemikriptofitsko-terofitski karakter (47,13%:38,11%). Fitogeografskom analizom utvrđeno je prisustvo 18 različitih flornih elemenata, grupisanih u 7 osnovnih areal tipova. Najbrojnija je grupa koja pripada evroazijskom areal tipu (34,02%).

Ključne reči: korovska flora, ruderalna flora, ekološki indeksi, fitogeografska analiza, Golubinci, Stara Pazova.

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AFLATOXIN PRESENCE IN SERBIAN POULTRY FEED DURING 2013-2014 PERIOD*

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Summary: The aim of this study was to compare the presence and the content of aflatoxin in poultry feed samples from two years (2013-2014). A total of 349 samples of complete feeding mixtures for turkeys, broiler chicks and laying hens were investigated. In 203 samples from 2013 year (10 samples of feed for turkeys, 145 samples of feed for broilers and 48 samples of feed for laying hens), overall aflatoxin content was ranged from 2 to 87 µg/kg, with the average value of 19 µg/kg. On the other hand, in 146 samples from 2014 year (14 samples of feed for turkeys, 78 samples of feed for broilers and 54 samples of feed for laying hens), overall aflatoxin content was notably lower (12 µg/kg) and ranged from 5 to 35 µg/kg. The obtained aflatoxin occurrence may lead to a conclusion that the high aflatoxin content during 2013 was an exception in comparison with 2014 and the studies from previous years. However, these results were not surprising taking into account a high degree of corn infestation by *A. flavus* molds during 2012, which came as a result of favorable agricultural conditions for the development of these molds.

Key words: aflatoxin, poultry feed, ELISA, Serbia.

INTRODUCTION

Secondary fungal metabolites, as a natural food and feed contaminants, became an important factor in terms of food safety and public health. Mycotoxin production can occur during plant growth, maturity, harvesting, storage, processing of grains and is influenced by various factors (temperature, relative humidity, oxygen availability, damaged or broken grain kernels) (Bernardo, 2004; Lanyasunya et al., 2005). The Food and Agriculture Organization (FAO) estimated that 25% of the world's crops are affected by mycotoxins, of which the most notorious are aflatoxins (WHO, 1999). Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2) are secondary metabolic products of some *Aspergillus* spp.: *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, and belong to

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a group of potent carcinogenic and teratogenic compounds (Kurtzman et al., 1987; Martins, 1989). The most potent known hepatocarcinogenic substance is aflatoxin B1 (AFB1); recently, after a thorough risk evaluation, it has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011).

Many authors investigated aflatoxin toxicity and presence of its residues in poultry (Ghosh et al., 1990; Quezada et al., 2000; Oguz et al., 2002; Bintvihok and Kositcharoenkul, 2006; Iqbal et al., 2014). The highest concentrations of aflatoxins are usually found in liver and kidneys. This is in accordance with generally accepted fact that these organs play key role in metabolism of xenobiotics (Iqbal et al., 2014). Chickens that consume aflatoxin-contaminated feed have increased levels of hepatic enzymes in their blood, likely due to necrosis of liver tissue (Oguz et al., 2002). Gosh et al. (1990) found that AFB1 decreased the values of T-lymphocyte counts in broilers. These conditions lower immune response and make chickens highly susceptible to various bacteria and fungal infections. Chronic exposure of chickens to aflatoxins is often related to the loss of weight and has impact on reproductive capabilities (Quezada et al., 2000; Bintvihok and Kositcharoenkul, 2006). These problems may cause great economic losses in poultry industry. Aflatoxins (AFs) also show carcinogenic, teratogenic and mutagenic properties. The concentrations of aflatoxin residues in muscles depend on the concentrations of aflatoxins in feed as well as on the age of the chicken. The metabolism of aflatoxins and their elimination from tissues is more rapid in older chickens. After 15 days of feeding chickens with meals without of aflatoxin, the concentration of aflatoxins in meat decreases below the limit of detection (Hussain et al., 2010). Aflatoxin residues in poultry meat represent a great concern for human health, since poultry meat is very common food commodity (Herzallah, 2009).

To reduce the risk of exposure, many countries have regulated the levels of aflatoxin in feed. Currently, the legal limits of aflatoxin in feedstuffs are highly variable from the European Union (EU) Countries to other countries. The EU has a limit of 20 µg/kg for complete feedstuffs for pigs and poultry (except young animals) and 10 µg/kg for other complete feedstuffs (Commission Directive, 100/2003). In Serbia, proposed maximum permissible levels of aflatoxins in complete and complementary feedstuffs for pigs and poultry (except young animals) is 20 µg/kg and in complete and complementary feedstuffs for calves, lambs, kids, piglets, chicks, turkey poult, ducklings is 5 µg/kg (Službeni glasnik RS, 27/2014).

Aflatoxin was monitored by the United States Food and Drug Administration (FDA), and a level of 20 µg/kg has been set as the limit of aflatoxin content for corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals (FDA, 2000). The mentioned regulation also establishes maximum level of aflatoxin in cottonseed meal intended for beef, cattle, swine, or poultry (300 µg/kg) and corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry (100 µg/kg) (FDA, 2000).

The purpose of this study is to give the information on the occurrence of aflatoxin in complete feeding mixtures for poultry during two years period (2013-2014).

MATERIAL AND METHODS

Samples of poultry feed were collected randomly from different farms in Serbia during 2013 and 2014 year. These samples (349) included complete feeding mixtures for turkeys, broiler chicks and laying hens. Immediately after sampling, 200 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. Then, sample was homogenized by mixing and packed in plastic bags. Samples were stored in a freezer at -20 °C until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

Extraction. Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper.

Analysis. The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 µg/kg). Analytical procedure was carried out according to manufacturer's procedure. Optical densities on the basis of which aflatoxin content was calculated, were read using the reader of microtitration plates with a 630 nm filter (BioTec Instruments, USA).

Quality control. In order to ensure quality of obtained results, method for determination of aflatoxin was validated. Validation parameters of the method were estimated according to European Commission (2006). Limit of detection (LOD) for aflatoxin in feed samples was 0.45 µg/kg while limit of quantification (LOQ) was 1.37 µg/kg. Average recovery value, based on analysis of certified reference material TR-A100 (Trilogy, USA) was 106.2% which is within acceptable limits according to Commission Regulation 401/2006. Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable" according to Commission Regulation 401/2006. Therefore, the method was suitable for the determination of aflatoxin in animal feed.

RESULTS AND DISCUSSION

The occurrence of aflatoxin was investigated in 349 samples of complete feeding mixtures for turkeys, broilers and laying hens, and the results are presented in table 1. By analyzing 203 samples from 2013 year (10 samples of turkey feed , 145 samples of broiler chicken feed and 48 samples of laying hen feed), overall aflatoxin content was ranged from 2 to 87 µg/kg, with the average value of 19 µg/kg. In complete feed for turkeys and broilers, average aflatoxin content (18 µg/kg) was somewhat lower than in complete feed for laying hens (21 µg/kg). On the other hand, in 146 samples from 2014 year (14 samples of turkey feed , 78 samples of broiler feed and 54 samples of laying hen feed), overall aflatoxin content was notably lower than in 2013 (average value 12 µg/kg) and ranged from 5 to 35 µg/kg.

Overall aflatoxin incidence was higher in samples from 2013 year (54.2%) than in samples from 2014 (24.0%). This is not a surprise because the cereal grains used for preparation of feeding mixtures in 2013 originated from 2012 harvest when the aflatoxin presence and content were found to be surprisingly high (Kos et al. 2013; Jajić et al. 2013b). Namely, Jajić et al. (2013b) reported about aflatoxin contamination in 63.6% of the analyzed maize samples, with concentration range of 5-367 µg/kg. Somewhat higher contamination (68.5%) was reported by Kos et al. (2013), with a concentration range of 1.01-86.1 µg aflatoxin/kg. All this has contributed to the aflatoxin contamination of compound feed in 2013 (Jajić et al. 2013a).

As can be seen in table 1, very high rate of positive samples (80%) was established in samples of feeding mixtures for turkeys originating from 2013, but this probably does not represent the actual situation because rather small number of samples (10) was analyzed. On the other hand, on the basis of more representable number of samples (48) of feeding mixtures for laying hens originating from 2013, the incidence of aflatoxin was also very high 81.3%. In the most representable group of 145 samples (feeding mixtures for broiler chicks) originating from the same year, the presence of aflatoxin was found in 43.4% of analyzed samples.

In 2014, aflatoxin presence was found to be quite lower than in 2013. In feeding mixtures for broilers, the presence of aflatoxin was 15.4% but in feeding mixtures for laying hens and turkeys it was quite higher (31.5% and 42.9%, respectively).

Table 1. Aflatoxin occurrence in poultry feed samples in the year 2013. and 2014.

	2013.				2014.			
	Turkeys	Broilers	Laying hens	Total	Turkeys	Broilers	Laying hens	Total
No of samples	10	145	48	203	14	78	54	146
Average (µg/kg)	18	18	21	19	10	13	11	12
Coefficient of variation (%)	15	18	19	18	6	8	7	7
MIN (µg/kg)	7	2	5	2	5	6	5	5
MAX (µg/kg)	48	78	87	87	22	35	32	35
No of positive samples (%)	8 (80.0)	63 (43.4)	39 (81.3)	110 (54.2)	6 (42.9)	12 (15.4)	17 (31.5)	35 (24.0)
No of samples above regulation (%)	2 (20.0)	53 (36.6)	8 (16.7)	63 (31.0)	1 (7.1)	12 (15.4)	2 (3.7)	15 (10.3)

Regarding legislative in our country (Službeni glasnik RS, 27/2014) and the European Union (Commission Directive, 100/2003), contamination levels in 2013 were above the maximum limit in 2 (20.0%) samples of feeding mixtures for turkeys, 53 (36.6%) samples of feeding mixtures for broilers and in 8 (16.7%) samples of feeding mixtures for laying hens. As was expected, much lower number of samples from 2014 contained aflatoxin levels above the maximum permissible limit. Namely, this was in 1 (7.1%) sample of feeding mixtures for turkeys, 12 (15.4%) samples of feeding mixtures for broilers and in 2 (3.7%) samples of feeding mixtures for laying hens.

As for our neighboring countries, aflatoxin contamination was monitored in Romania (Braicu et al., 2008; Tabuc et al., 2010; Macri et al., 2014) and Croatia (Šegvić Klarić et al., 2009; Pleadin et al., 2014). In 22 feedstuff samples, collected between January and August 2013 in Romanian province Transylvania, Macri et al. (2014) found that 100% of the samples were positive for aflatoxin B1, with the concentration range of 1.13–92.92 µg/kg. Samples were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA). Tabuc et al. (2010) analyzed 56 cereal

samples (corn, wheat, barley and oats). They determined the level of fungal contamination and aflatoxin content; *Aspergillus* species were present in over 80% of the samples, and aflatoxin B1 has been identified in almost 30% of the samples, mainly in corn with the low concentrations <10 µg/kg. Braicu et al. (2008) investigated forty-three samples of different cereals (wheat, maize, rye and Triticale). It was found that 58.1% of the samples were contaminated with various concentrations of different mycotoxins: aflatoxin B1 (1.6–5.7 µg/kg), aflatoxin B2 (0.89–4 µg/kg), aflatoxin G1 (1.2–5.76 µg/kg), aflatoxin G2 (0.96–3.4 µg/kg) and 4.3–30 µg/kg ochratoxin A. The concentration of total aflatoxin ranged from 11.2 to 10.8 µg/kg. Among different cereals, the highest number of contaminated samples was found to be in the wheat samples (62.5%). Pleadin et al. (2014) analyzed 633 maize samples from 2013 using ELISA technique as the screening method and High Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) as the confirmatory method. They reported the mean value for AFB1 of 81 µg/kg, and the maximal concentration of 2072 µg/kg. The authors gave information about validation method for both techniques that were used. Šegvić Klarić et al. (2009) analyzed 37 samples of cereals and feed randomly collected in 2007 from households of an endemic nephropathy area in Croatia. The incidence of aflatoxins was 24.3 % with the average value of 4.6 µg/kg.

Based on everything stated above, it can be said that the high aflatoxin content during 2013 was an exception in comparison with 2014 and the studies from previous years. These results can be explained by the uncommonly high frequency and incidence of *A. flavus* infestation of maize grain in 2012 (Lević et al., 2013). Lević et al. (2013) described that this infestation was caused by extremely stressful agrometeorological conditions, high temperatures and drought over the period from flowering to waxy maturity of maize in the same year.

CONCLUSION

Based on the results presented in this paper it can be concluded that the presence of aflatoxin and aflatoxin content during 2013 were rather high as the consequence of the outbreak of *A. flavus* in 2012. It was an exception in comparison with the results from 2014 and the studies from previous years when environmental conditions were unfavorable for *A. flavus* growth and toxin production. Namely, the average aflatoxin content in 2013 was 19 µg/kg with the obtained maximum value of 87 µg/kg. The overall presence was 54.2% with 31.0% of samples above the maximum limit permitted by Serbian and EU regulations. In the opposite, samples from 2014 showed significantly lower average values of the same mycotoxin (12 µg/kg), maximum value (35 µg/kg), overall presence (24.0%) and most importantly, percentage of samples with aflatoxin concentration above the maximum limit permitted by Serbian and EU regulations (10.3%).

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PRISUSTVO AFLATOKSINA U SMEŠAMA ZA ISHRANU ŽIVINE U SRBIJI U PERIODU 2013-2014 GODINE

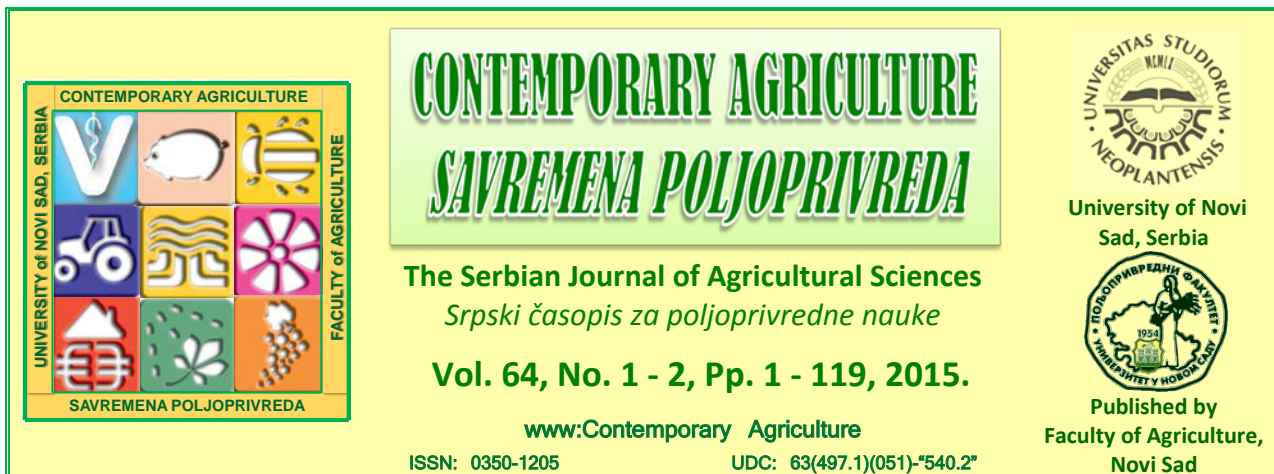
Igor JAJIĆ, Vojislava BURSIC, Sandra JAKŠIĆ, Gorica VUKOVIĆ, Saša KRSTOVIĆ

Izvod: Cilj istraživanja je bio da se uporedi prisustvo i sadržaj aflatoksina u uzorcima smeša za ishranu živine koje potiču iz dve različite godine (2013-2014). Ispitano je ukupno 349 uzoraka potpunih smeša za ishranu za čurki, brojlera i koka nosilja. Ispitivanjem 203 uzoraka iz 2013. godine (10 uzoraka hrane za čurke, 145 uzoraka hrane za brojlere i 48 uzoraka hrane za koke nosilje) utvrđen je ukupni sadržaj aflatoksina u opsegu od 2 do 87 μg/kg, sa prosečnom vrednošću od 19 μg/kg. S druge strane, analiziranjem 146 uzoraka iz 2014. godine (14 uzoraka hrane za čurke, 78 uzoraka hrane za brojlere i 54 uzoraka hrane za koke nosilje), utvrđen je znatno niži sadržaj aflatoksina (12 μg/kg) koji se kretao u koncentracionom opsegu od 5 do 35 μg/kg. Dobijeni rezultati prisutnosti aflatoksina mogu navesti na zaključak da je visok sadržaj aflatoksina tokom 2013. predstavljao izuzetak u odnosu na 2014. ali i na rezultate istraživanja iz prethodnih godina. Ovi rezultati ipak ne predstavljaju naročito iznenađenje uzimajući u obzir pojavu visokog stepena infestacije kukuruza plesnima *A. flavus* tokom 2012. godine, nastalu kao posledica povoljnih agroekoloških uslova za razvoj ovih plesni.

Ključne reči: aflatoksin, hrana za živinu, ELISA, Srbija.

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RESULTS OF HUNTING THE RINGED PHEASANTS IN HUNTING GROUNDS OF AP VOJVODINA (SERBIA)*

Zoran RISTIĆ[♦], Gordana UŠČEBRKA, Danica BOŠNJAK,
Slobodan STOJANOVIĆ, Dragan ŽIKIĆ¹

SUMMARY: Pheasant is the main game species in most of the Europe, therefore a large number of pheasant chicks is being artificially bred and released to hunting grounds every year in order to increase "natural population" and enable higher catch. This paper gives results of one-year investigation in hunting grounds of Autonomous Province of Vojvodina, showing that 19.67% (236) from total number of ringed and previously released pheasants (1200) was caught, so one of five released pheasant chicks were bagged. In hunting grounds Sonta, both ringed (170) and non-ringed (185) pheasants were released immediately before hunting. In total catch, there were 131 ringed ones (77.06%). Results of this investigation point that, regarding survival of artificially bred and released pheasants, they should be kept in shelters made according to regulated standards, with good conditions for pheasant game, and only after that they should be released into natural habitat; also the approach and the way of hunting this game should be modified.

Key words: pheasant, artificial breeding, catch percent

INTRODUCTION

The common pheasant (*Phasianus colchicus*) is naturally distributed from Caucasus Mountains, along the Black Sea, and to the east in Asia – in Korea, Manchuria, China, Japan and Formosa (Fuller and Garson, 2000). This natural range was widened due to its introduction in a much larger area. Mustin et al. (2011) notes introduction of this pheasant in almost fifty countries, and presently it is naturalized in most of the Europe and North America.

Pheasant is presently the main game species in most of the Europe, therefore a need has arisen to artificially breed large number of pheasant chicks which are released to hunting grounds every year in order to increase "natural population" and enable higher catch. During 1960's, when in Central, Eastern and South Europe number of

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partridges began to dwindle rapidly, a mass production of pheasants began with its organized release into hunting grounds. Since then, in larger European countries pheasant is the main game bird and this is the case in our country too. Such practice was continued until present days, although in our country number of pheasant released had been manifold decreased in last 15 years.

A number of studies that had been done did not establish a precise percent of catch for pheasants released in such a way, from artificial breeding centers with help of hunters-breeders (Klinger and Riegner, 2008). There are a relatively small number of studies that give details regarding weather conditions or causes of losses of released pheasants from artificial production and some studies point to the large losses due to intensive agricultural production using modern equipment and pesticides (Stoate, 2002).

Pheasant is the important factor in maintaining diversity in agricultural systems, mostly through planting, management and protection of forests (Nelli et al., 2012) and one of few game species which protection may be of financial importance.

The aim of these investigations is to, from data obtained and from literature data, find the percent of usage for seasonal populations, i.e. the populations where a significant number of pheasant chicks was released, and to establish effect of seasonal factors and ways of hunting on sustainability of this game species.

MATERIAL AND METHODS

These investigations were conducted during 2013 in five hunting grounds (Kovačica, Ada, Maradik, Rusko Selo, Sonta), with different habitat conditions and at different age of pheasant chicks. After the chicks were hatched and raised in pheasantries according to routine technology, at the age of 7-8 weeks they were ringed using plastic rings in different colors (depending on a hunters society) and transferred into enclosures. At the age of 12-13 weeks they were released into open hunting grounds (Kovačica, Ada, Maradik, Rusko Selo) and the first hunt was two months later (mid-October), with 9-12 hunting days per hunting grounds during 2013/14 hunting season, or were released immediately before every hunt (Sonta) with total of seven hunting days during pheasant hunting season.

After every hunt all pheasants caught were counted, males and females separately, and number of ringed individuals was noted. Thus obtained data were processed and shown in tables.

RESULTS AND DISCUSSION

During the hunting season, all ringed pheasants were noted and results of investigations regarding percent of bagged pheasants that were released into hunting grounds two months before the start of the hunting season are shown in Tables 1 and 2.

In four hunters' societies, with different biotopes, 1200 pheasant chicks aged 7-8 weeks were ringed in shelters. The ring color was coded for every hunting grounds in order to avoid errors in calculating hunting percent per hunting grounds. Number of hunt days in hunting grounds ranged between 9 and 12, and number of hunters and hunt method was differing significantly among hunting grounds. In Maradik and Rusko Selo hunting grounds hunt was organized every seven days as group hunt with battue. During the hunting season there was total of 54 hunt days, with 724 hunters participating.

Table 1. Results of hunting of pheasants that were released into hunting grounds two months before the hunting season

Hunting grounds	Number of hunt days	Number of hunters	Pheasants released (ringed)	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed released
Kovačica	12	63	200	28	19	67.86	9.50
Ada	9	80	200	77	37	48.05	18.50
Maradik	12	180	200	119	54	45.38	27.00
Rusko Selo	11	401	600	209	126	60.29	21.00
Total	54	724	1200	433	236	54.50	19.67

Table 2. Results of hunting pheasants that were released into hunting grounds two months before the hunting season, according to sex

Hunting grounds	Pheasants bagged			Number of ringed pheasants caught		
	Total	Males	Females	Total	Males	Females
Kovačica	28	19	9	19	14	5
Ada	77	54	23	37	28	9
Maradik	119	89	30	54	41	13
Rusko Selo	209	169	40	126	74	52
Total	433	331	102	236	157	79

From total of 433 pheasants bagged, there were 236 ringed ones, so share of ringed pheasants in total number of birds caught was 54.5%, and from total number of ringed pheasants released into hunting grounds 19.67% were caught. Real parameter of catch is 19.67%, pointing to conclusion that in such approach and technology for releasing pheasants into hunting grounds one of five released chicks was bagged.

Results of investigations by Gruychev (2014) point to a high percent of pheasant chicks losses in conditions of natural habitat (80%). The main causes for pheasant chick losses were carnivorous animals and human activities.

The similar issue was explored by Bagliacca et al. (2008) who examine the dispersion and habitat use after release of pheasants. They concluded that if pheasants are reared according to the disciplinary rules stated for the production of pheasants for wildlife reproduction programs, good survival rates and breeding succes of the released pheasants can be expected.

Present results from authors for hunting grounds in Vojvodina, with highly different percent of pheasants caught, may be explained by different habitat conditions and by omissions in sheltering. For instance, in hunters society in Mali Radinci 52% of released pheasant chicks were bagged and in hunting grounds of hunters' society Novi Sad it was under 10% (Ristić, 2005).

Based on investigations carried out in hunting grounds of Vojvodina regarding survival rate of artificially released pheasants (Ristić et al., 1995), it was established that percent of pheasants caught was ranging between 7.40% and 39.30% or 22.53% in average. From total number of branded pheasants (1720), 367 or 21.34% were caught, while in comparison to total caught (1575) percent of branded pheasants caught was 23.30%.

In our investigation, extremely low percent of caught pheasants (9.50%) was found in Kovačica hunting grounds, which may be explained by the fact that there was small number of hunters and hunt was organized near the settlements which limited free shooting. Better percent of pheasants bagged (60.29%) was in hunting grounds Rusko Selo, but their total number of hunters during pheasant hunting season (401) was considerably higher than in other hunters' societies.

Regarding results from the Sonta hunting grounds, data show that percent of ringed pheasants caught in comparison to those released immediately before hunt is much higher (Table 3).

Table 3. Results of hunting of ringed pheasants that were released immediately before hunt (hunting grounds Sonta)

	Released	Bagged	%
Non-ringed	185.00	125.00	67.57
Ringed	170.00	131.00	77.06
Total	355.00	256.00	72.11

Data from Table 3 show pheasant chicks released into hunting grounds immediately before each hunt, during the pheasant hunting season. There were seven hunts organized, with total of 170 ringed and 185 non-ringed pheasants released. Percent of caught ringed pheasants during the hunting season was 77.06%, which is rather high, but also a high percent of both ringed and non-ringed released pheasants was bagged (72.11%), and total number of bagged birds included also a number of previously existing pheasants from the same area, so this result is not quite exact.

Considering this issue, other authors also reached similar conclusions. FAWC (2008) emphasize importance of respecting all principles of breeding and releasing technology. In optimal environmental conditions and with good

care on pheasants, catch of 50% for released males may be attained. This is not the maximal result, since there are hunting grounds with catch higher than 60%, but the average value is about 35% from total number of chicks released. Losses in released pheasant chicks are difficult to estimate. As a rule, dead individuals are being noted only in immediate surroundings of the shelter, and even that is not thorough. According to the same authors, losses between release and hunt period may be about 30% from number of released pheasants, from which about 20% is caused by the habitat change (stress, diet disturbances etc) and 10% by predators.

Ristić (2005) noticed that it had been regarded as normal that 20 do 30% females are being caught. Where considerable decrease in number of pheasant game occurred, it is necessary to minimize number of females bagged in order to develop structure of basic flock and to locate it mostly in a center of the hunting grounds (at the circle of supposed dispersion of released birds), and at the same time to spare females at the edges since these are mostly populated by pheasants from existing local population.

By investigations of Ristić (2005) for 1973-2000 period in hunting grounds of Vojvodina about 5.4 million of pheasant chicks were released, mostly aged 5-6 or 7-8 weeks, with only about 10% of older categories (10, 12, 14 and 20 weeks, mature individuals and basic flock pheasants, so-called "old hens"). Most intensive period for introduction was from mid-seventies to beginning of 1990's. In 1973-2000 period, catch percent ranged from 10.54% minimum in 2000 to maximal 28.77% in 1973, with average catch of 22.02% for the entire period.

Based on investigations by Ristić (2006) percent of pheasant catch was 38.67% in hunting grounds "Rit" Beograd where immediate release method was used (during seven year period, 20,004 pheasants were released in mornings and 7,736 were bagged in the same day hunt). Using the same principle, in "Ristovača" hunting grounds result was 61.62% (for six year period, 25,175 pheasants were released in the mornings and 15,512 were bagged in the same day hunt).

CONCLUSION

From results obtained and their analysis, the following conclusions may be made:

Regarding survival of artificially bred, released and bagged pheasant chicks – they should be kept in shelters constructed according to regulation norms, with favorable conditions for pheasant game, since average catch percent in comparison to pheasant chicks released two months prior to hunt was 19.67%.

Pheasant should be hunted from the beginning of the hunting season, using group hunt with battue, and to let the grounds in peace at least seven days after every hunt.

Regarding ways of release, more appropriate are the hunting grounds with stable shelters: results are better with pheasant chicks between 5 and 6 weeks of age, while in hunting grounds with temporary shelters it is better to use 7-8 weeks old chicks.

In organizations where habitats have no perennial plants and the crops are harvested quickly, it is recommended to build enclosures in order to keep pheasants until the hunting season and to release birds into hunting grounds immediately before hunt. In such way losses are minimized, and catch percent is higher, in our case 77.06%.

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REZULTATI ODSSTRELA PRSTENOVANIH FAZANA U LOVIŠTIMA AP VOJVODINE (SRBIJA)

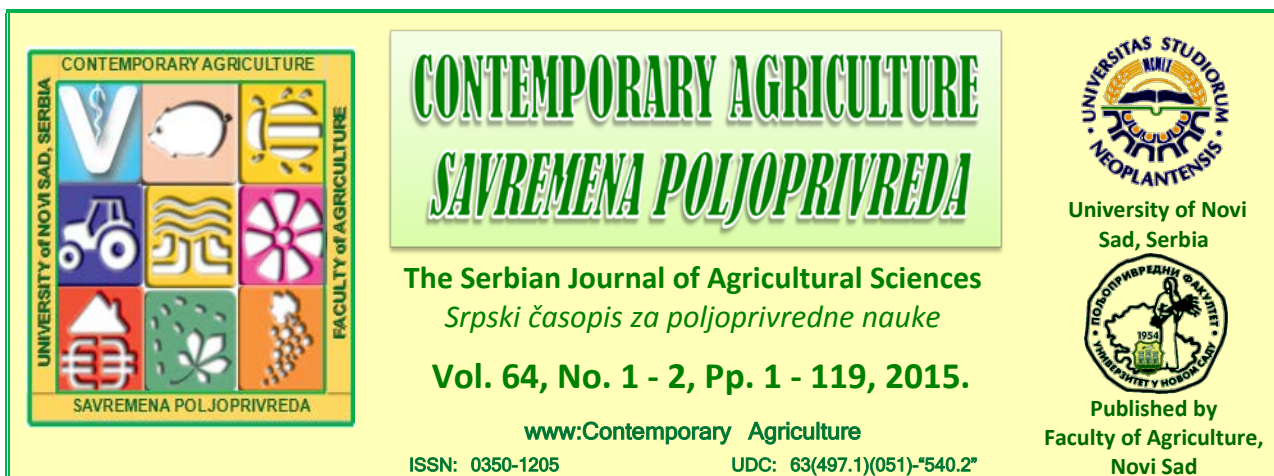
Zoran RISTIĆ, Gordana UŠĆEBRKA, Danica BOŠNJAK, Slobodan STOJANOVIĆ, Dragan ŽIKIĆ

Izvod: Fazan je glavna vrsta lovne divljači u većem delu Evrope, što je dovelo do činjenice da se veliki broj fazančića veštački uzgaja i svake godine ispusti u lovišta, u pokušaju da se poveća "prirodna populacija" i mogućnost većeg izlova. U ovom radu su izneti rezultati jednogodišnjeg istraživanja u lovištima AP Vojvodine, koji ukazuju da je izlovljeno 19,66% (236) u odnosu na ukupan broj prstenovanih i prethodno puštenih fazana (1.200), što znači da je odstreljeno svako peto fazansko pile koje je ispušteno u lovište. U lovištu Sonta puštani su "pred pušku" i prstenovani (170) i neprstenovani fazani (185). Ukupno je odstreljeno 131 prstenovanih fazana što iznosi 76,47%. Rezultati ovog istraživanja ukazuju da kada je u pitanju preživljavanje veštački proizvedenih i unetih fazančića – iste treba čuvati u prihvatilištima koja su napravljena po propisanim normama, i u kojima su stvoreni dobri uslovi za fazansku divljač, tek onda ispuštati u prirodni ambijent, ali isto tako i promeniti pristup u načinu lova ove divljači.

Ključne reči: fazan, veštačka proizvodnja, procenat odstrela.

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Review paper

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EMERGENCE OF PSEUDORABIES VIRUS (*MORBUS AUJESZKY*) INFECTION AT LARGE SWINE FARMS IN AP VOJVODINA (SERBIA)*

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Jelena APIĆ, Igor STOJANOV, Vladimir POLAČEK¹

Summary: The aim of this paper was to evaluate current epidemiological situation concerning occurrence of Aujeszky's disease (AD) outbreak in large swine farms in Vojvodina Province. The material for this research included the samples from five swine farms, where certain disorders and health problems in suckling piglets i.e. clinical signs suggesting AD were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and anamnestic evaluation, clinical investigation, gross pathological examination, standard bacteriological examination for detection the presence of aerobic and anaerobic bacteria in the organs and tissue samples (lungs, tonsils, lympho nodes, spleen, cerebral tissue) derived from diseased, died pigs. Viral isolation on the susceptible cell culture and molecular diagnostic method (RT-PCR) were applied. Also, the material for the investigation included 300 blood samples collected from breeding animals, in order to evaluate the presence of specific antibodies against AD virus. The achieved results suggest that swine population in AP Vojvodina province is enzootically infected with AD virus. Besides vaccination, which represents one of the measure in AD eradication, it is necessary to apply strict trade control and checking the health status of herds from where breeding animals are purchased.

Key words: Aujeszky's disease, Pseudorabies, swine farms, Vojvodina.

INTRODUCTION

Aujeszky's disease (Pseudorabies, *Morbus Aujeszky*) is an acute, contagious viral disease, of wide range of domestic and wild-life species (Allepuz et al., 2009). The causative agent is designated as suis alpha herpes virus 1, a member of the *Herpes viridae* family and *Alpha herpes virinae* subfamily (Mettenleiter et al., 2012). The Aujeszky's disease virus (ADV) is an important pathogen of pigs and infects almost all mammalian species except man. However,

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pigs are the only animal species that can survive the infection with ADV, which accounts for its ability to be subclinically (latently) infected (Martinez-López et al., 2009) while infected individuals of all other animal species succumb to the disease without shedding the virus (Komáromi and Szabó, 2005).

During the last forty years vaccination against Aujeszky's disease (AD) on large swine farms in Vojvodina Province was performed with modified-live vaccine (MLV) (Došen et al., 2002; Pušić et al., 2009). However, in Serbia, especially in Vojvodina region, an endemic infection with ADV is a major problem in spite of long lasting vaccination (Pušić et al., 2009; Prodanov-Radulović et al., 2011). Vaccination with MLV alone is unlikely to eradicate the disease due to the problem of latent carriers (Muller et al., 2003). The epidemiological investigation revealed that during the period 2000-2010 the outbreaks of AD were recognized on the territory of 13 municipalities in Vojvodina (Pušić et al., 2007; Pušić et al., 2009). The main reasons for spreading the infection were displacement of latently infected breeding animals from large farms to individual producers and quitting of vaccination on some of the large farms (Pušić et al., 2011). Several years ago the production of MLV type vaccine in Serbia was completely stopped and most of swine farms ceased with vaccination against AD because of lack of vaccine on the market. This resulted in existence of highly susceptible population of pigs on large swine farms in Vojvodina region. As a result, in the beginning of 2011 to 2014, a severe AD outbreaks occurred on several large swine farms.

The aim of this paper was to evaluate current epidemiological situation concerning occurrence of AD outbreak in large swine farms in AP Vojvodina region.

LITERATURE REVIEW

The Aujeszky's disease has a great economic impact to the swine industry, causing losses due to high mortality in suckling and weaner piglets (Casal et al., 2004; Mettenleiter et al., 2012). Adult pigs do not show obvious clinical signs of infection, except for mild respiratory disorders, fever and weight loss; however, among pregnant females, the disease can cause reproductive disorders (abortion, embryonic death, mummified fetuses) or stillbirths and the birth of avital, weak piglets with neurologic symptoms that lead to death (Stančić et al., 2010; Stančić et al., 2011). The primary means of transmission of the AD among swine herds is thought to be by aerosol suspensions of the virus and direct contact between infected and susceptible pigs. Indirect contacts by vehicles, equipment, personnel or artificial insemination can also spread the virus among herds (Allepuz et al., 2009; Prodanov-Radulović et al., 2011). Inapparent infections rarely occur when ADV is introduced into a herd for the first time where neonatal pigs are present, because pigs of this age are highly susceptible (Casal et al., 2004). Highly virulent strains produce a non suppurative meningoencephalitis that causes fatal disease in piglets, with nervous clinical signs (Martinez-López et al., 2009).

Many European countries have established eradication programs, with the objective of initially controlling and finally eradicating the disease (Martinez-Lopez et al., 2009). The disease has been eradicated from domestic pig populations in most of the European Union (EU) countries, as well as from the United Kingdom, Canada, New Zealand, and the United States (Komáromi and Szabó, 2005; Ketusing et al., 2014). Different Member States of the EU have used different approaches to ADV eradication (veterinary administrative measures, serological tests, culling infected animals, using inactivated vaccines, prohibition of use of live vaccines) (Komáromi and Szabó, 2005). Restrictive regulations on the trade of pigs to regions or countries, which are free of ADV have been imposed in the EU. However, the risk of moving infected animals into free or low prevalence areas remains an important problem for the control and eradication of ADV (Martinez-Lopez et al., 2009).

The control of the disease in endemic countries is based on compulsory vaccination of the entire swine population (Casal et al., 2004; Müller et al., 2003). In the last 40 years, in Serbia pigs were immunized with MLV to minimize clinical disease and death loss (Došen et al., 2002; Pušić et al., 2011). The virus strain used in this non glycoprotein-E (gE) deleted vaccines was Ercegovac field strain isolated in 1956, and attenuated through 200 passages on chicken fibroblast tissue culture (Pušić et al., 2009). The main goal of this vaccination programs was to reduce economic losses caused by clinical episodes of AD. However, only vaccination for clinical purposes has not been successful in preventing the spread of the virus under field conditions (Ketusing et al., 2014; Müller et al., 2003; Pušić et al., 2011).

By serological examination of blood samples, obtained from unvaccinated pigs in Vojvodina, Pušić et al. (2009) discovered that specific antibodies against ADV were present in 32.7% animals. Relatively high percentage of seropositive breeding pigs owned by individual producers, where no vaccination against AD is performed, suggests that the infection in pig population is present on a significant level in Vojvodina region. The systematic vaccination of breeding pigs, although it reduces the probability of infection and the spread of ADV, generally does not prevent infection and may produce subclinically (latently) infected pigs that will likely not be detected serologically (Martinez-Lopez et al., 2009; Prodanov-Radulović et al., 2011). There is a concern that MLV, after vaccination may

revert back towards virulence during replication in the host. In such cases, it is possible that the vaccinal virus circulates on farms immunising pigs, although the presence of a field strain of low virulence should not be excluded (Pušić et al., 2009). The vaccinated animals do not suffer from the symptoms of the disease, but they may contract a field infection, excrete live virus and become carriers (Casal et al., 2004; Müller et al., 2003).

Aujeszky's disease is mandatory notifiable disease in Serbia (Pušić et al., 2007). However, there is no special decree issued by the Veterinary Authorities that regulate AD control neither does national or regional eradication program exist. (Pušić et al., 2009). Voluntary vaccination with MLV vaccines precludes the serological diagnosis of infected pigs, thus making eradication efforts impossible (Komáromi and Szabó, 2005). In many EU countries, efforts were made to eradicate MA (Müller et al., 2003). To reach this goal, the development and approval of a gene deleted (gE) vaccine was the decisive break-through, as this vaccine made it possible for the first time to distinguish infected from vaccinated pigs (Ketusing et al., 2014). The gE-negative strains are used the most widely, as the absence of the gene fragment encoding the gE protein and the consequent absence of the gE protein itself does not affect either growth of the virus or the protection induced by it. The advantage of such gene-deleted vaccines is that the detection of antibodies to the lost protein by enzyme-linked immunosorbent assay (ELISA test) indicates serological positivity induced by a field virus and not by the vaccine virus (Pasik, 2004). Recently, in Serbia the new type of attenuated vaccine for active immunisation of pigs against ADV was registered. The vaccine contains a modified live viral strain NIA-3, which is gE antigen and thymidine kinase deleted (a marker vaccine). However, it is not so far, possible to differentiate between vaccinal and infectious titer because the vaccination campaign using marker vaccines in Serbia is not yet operational.

THE RESULTS OF OUR INVESTIGATIONS

Material and methods

The material for this research included the samples from five swine farms, located in Južnobački and Sremski district in Vojvodina, where certain disorders and health problems in suckling piglets i.e. clinical signs suggesting AD were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and anamnestic evaluation, clinical investigation, gross pathological examination, standard bacteriological examination for detection the presence of aerobic and anaerobic bacteria in the organs and tissue samples (lungs, tonsils, mediastinal and mandibular lymph nodes, spleen, cerebral tissue - cerebellum, medulla oblongata) derived from diseased, died pigs. Viral isolation (VI) on the susceptible cell culture of tissues originating from the dead animals and molecular diagnostic method, reverse transcriptase - polymerase chain reaction (RT-PCR) were applied (Ma et al., 2008). Also, the material for the investigation included blood sera collected from breeding animals, in order to evaluate the presence of specific antibodies against ADV.

History of the examined swine farms: The following details were ascertained by the epidemiological examination and from farm records: number and category of pigs on the unit, production details (nucleus or commercial), disease status, current veterinary health plan (vaccination, medication) and biosecurity protocols used. The control of indoor pig environment was inspected with regards to basic zootechnical conditions for swine (temperature, lighting, ventilation, stocking density, bedding, hygiene). The animals were inspected for clinical signs of disease and abnormal behavior. The clinical inspection was followed by the necropsy of dead pigs.

Bacteriological testing: Isolation of bacteria from clinical samples was performed by aerobic and microaerophilic cultivation. Microscopic examination determined whether the isolated bacteria were Gram positive or not and whether it is a coccoid or rod-like organisms. The determination was carried out by determining the biochemical characteristics of the isolated bacteria (Quinn et al., 2011).

Virology testing - Aujeszky's disease virus isolation : Isolation of ADV was done by cultivation of tissue samples on cell culture line PK-15 (porcine kidney - ATCC CCL-33). Samples of brain, tonsil, and lung of tested animals were homogenized by mortar and pestle and diluted in PBS 1:10 (1g of tissue and 9 ml of PBS) supplemented with antibiotics (200 IU/ml penicillin; 100 µg/ml gentamicin and 5 µg/ml amphotericin B) to prevent bacterial growth. The tissue homogenate is centrifuged on 2000 g for 10 min and 1 mL of supernatant was used for inoculation of 24 hours old PK-15 cell culture with 75% confluent cell layer in 25 cm² tissue culture flask. Before inoculation, the cell culture growing medium is decanted from the flask and 1 mL of tissue homogenate was added to the cell monolayer, gently shaking to distribute the inoculated material over the whole cell monolayer, and incubated for 1 hour on 37°C. After the incubation 10 ml cell growing medium (Eagle MEM, Sigma) with 10% fetal calf sera (EU grade, PAA, Austria) was added to the cells and cell monolayer was microscopically observed daily for the development of the characteristic herpes virus cytopathic effects (CPE - with rounded birefringent cells, followed by

complete detachment of the cell sheet) in the next 7 days. In the absence of any obvious CPE, after the 7 days incubation period, one blind passage into the new 24 old cell monolayer was performed with 1 ml suspension of the first cell passage after 3 cycles of freezing-thawing steps. If the visible CPE is observed, the virus presence was confirmed by neutralization with specific antiserum.

Serology testing: The serum neutralizing test (SNT) was applied in order to estimate the specific antibody titer against ADV, following standard procedure as described before (Office International des epizooties, Manual of Epizootic, 2004). A total number of 300 blood samples were examined.

Results

The first examined farm represent the modern commercial swine farm. In the time of examination, on the farm the following swine categories were included: 680 sows, 515 pregnant gilts, 50 breeding gilts, 15 boars, 1060 suckling piglets, 5051 weaned piglets and 6050 fatteners. The farm represent the one-site production system (farrowing-to-finish). Applying control of all production stages, the correct stocking densities and housing requirements were detected. The farm have organised own veterinary services and swine health control programme is conducted according to Law (Stojanac et al., 2014). Anamnestically, the health disorders in sows and higher percent of mortality in their litters were observed. By gross pathological examination of 25 dead suckling piglets the pathology lesions that are considered to be characteristic for ADV infection were detected: diphtheroid and necrotic tonsillitis, swollen and occasionally hemorrhagic lymph nodes, and focal necrosis on the liver and spleen in the form of yellow-white foci size 2-3 mm scattered. By clinical examination in suckling piglets in one chamber, the signs of severe disturbance of the central nervous system (CNS) were observed (hypersalivation, incoordination, trembling, paddling, convulsions, opisthotonus). In some cases the whole litter of piglets died within 48 hours. In lactating sows the signs of inappetence, mild apathy, agalactiae and constipation was noticed. Anamnestically, within the farm perimeter, the carcass of dead cat was found recently. By bacteriological examination of organs and tissues from succumbed suckling piglets *Streptococcus sp.* was isolated. Applying viral isolation (VI), from tissue samples derived from dead piglets, ADV was isolated and by RT-PCR technique the genome of ADV was detected. Serological testing (SNT) of 50 sera samples (sows, gilts, boars) has shown a large variety of specific antibody values against ADV (antibody titer of 1:4 to 1:128). Interestingly, in one boar, beside the serological positive result (titer 1:8), clinically the bilateral testes oedema was noticed. By additional epidemiological examination, it was discovered that all breeding animals on the examined farm are imported from the EU countries 6-7 years ago and vaccination against AD was never been performed. Also, the serious failures in implementation of biosecurity measures on the swine farm were discovered: the farm-fence is not entirely enclosed, the vehicles for transporting fatteners to the slaughterhouse are entering directly in the farm perimeter, the workers employed to repair some parts of the farm objects are entering without changing the clothes and boots.

On the other two evaluated commercial swine farms, the capacity 450-500 sows, by pathomorphological examination of 38 carcasses of the dead suckling piglets, the existence of gross pathology changes indicative for infection with ADV were detected (*Tonsillitis purulenta et diphtheroides necroticans*, *Dilatatio vesicae urinariae porcelli*, *Necroses submiliares et miliares hepatis*). Clinically, the health disturbance was found sporadically in suckling piglets (yellowish diarrhoea, paddling, trembling, convulsions). In weaned piglets and fatteners the clinical signs of respiratory disease were noticed (dyspnoea, sneezing, coughing). Applying VI, from tissues of dead suckling piglets ADV was isolated. Applying SNT of 100 sera samples, the positive serological finding (antibody titer of 1:4 to 1:128) was detected. By epidemiological evaluation, it was discovered that on the examined swine farms the vaccination against AD was continuously performed in the last 8-10 years. However, because of vaccine shortage on the market, the vaccination was stopped in the last 3 years.

The fourth examined case refers to the modern commercial swine farm, the capacity 2500 sows. The farm represent the one-site production system (farrowing-to-finish). Applying control of all production stages, the correct stocking densities and housing requirements were detected. The farm have organised own veterinary services and swine health control programme is conducted according to Law (Stojanac et al., 2014). The health issue was established at weaning of piglets with clinical signs of respiratory illnesses and CNS diseases (dyspnoea, coughing, paddling, trembling, egzophthalmia). However, gross pathology finding considering the suspicion of AD, was negative. Epidemiological analysis showed that the farm performs vaccination against AD, but the first vaccination of piglets is 10 days before weaning. Serological testing of sera samples derived from weaners (50 samples) and sows (50 samples) has shown a large variety of specific antibody values against ADV (antibody titer of 1:4 to 1:128). From tissue samples of dead weaned piglets in cell culture ADV was isolated and the presence of viral genome was confirmed by RT-PCR.

In the last examined commercial swine farm, the capacity 650 sows, anamnesticly, the health disorders in sows and in their litters were observed. By epidemiological investigation it was discovered that on the swine farm in total 50 new sows had been introduced 2 months ago. Serologically, in 25 sows the presence of specific antibodies against ADV was detected (antibody titer from 1:16 to 1:64). However, despite the fact that these animals were serologically positive, the origin of that immunological status from the aspect of AD remained unknown: vaccination or infection. From other hand, the evaluated swine farm 5 years before stopped with the vaccination against AD. By clinical examination in lactating sows the signs of inapetence, mild apathy and agalactiae were observed. In suckling piglets the signs of severe disturbance of the CNS were clinically detected (wide open eyes, paddling, convulsions, ataxia, opisthotonus, epileptiform-like seizures). The onset of clinical signs in 2- to 3-day-old piglets was sudden, sometimes spanning 8-10 hours from onset to death. Clinically the fatteners also become anorectic, listless and apathic. The pathomorphological changes that were detected in dead sucklings indicated the lesions characteristic for ADV infection: diptheroid and necrotic tonsillitis, swollen and occasionally hemorrhagic lymph nodes, focal necrosis on the liver and spleen in the form of yellow-white foci size 2-3 mm, oedema and haemorrhages on the meninges and brain tissue. By bacteriological testing on tissue samples from dead piglets the following bacteria were isolated: *Escherichia coli*, *Escherichia coli haemolytica* and *Streptococcus sp.* Applying VI, from tissues from dead piglets ADV was isolated.

DISCUSSION

Regarding the occurrence of AD outbreak in large swine farms in Vojvodina Province, the achieved results can be grouped in several categories: the existing problem of farm biosecurity, cessation of vaccination against AD, inappropriate immunoprophylactic program and introducing of new breeding animals on the farm without previous quarantine.

In the first three examined cases of AD, individually non-vaccinated swine herd were surrounded by vaccinated or poorly vaccinated herds and backyard stocks. A primary outbreak of AD in a naive immunologically unprotected herd can be a devastating event, with spread through the entire herd within 1 week and ending with more than 90% of suckling pigs dead, nursery pigs stunted in their growth, and with respiratory disease in adults (Došen et al., 2007; Mittenleiter et al., 2012). In evaluated farms, the established gross pathology lesions i. e. herpetic and splenic yellow-white foci of necrosis are most frequently seen in young pigs because that lack passive immunity (Mittenleiter et al., 2012). In the examined sera samples, ADV antibodies were detected by SNT as early as 7 days after occurrence clinical disease. The high antibody titre detected in sows gives a rise to suspicion that a mildly virulent strain of wild virus was latently present on the farm, because infection with a mildly virulent strain may pass clinically unnoticed (Müller et al., 2003). However, protection against ADV is not closely related to the level of antibody because the virus is nonviraemic and spreads predominantly by mucosal infection and neuronal innervations (An et al., 2013; Mittenleiter et al., 2012). Quitting of vaccination on some of the large farms in the Vojvodina with the high density pig population without implementation of strict biosecurity measures can be hazardous. The biosecurity and management practices in backyard herds are generally lacking, which makes them a possible threat for large farms (Došen et al., 2012; Prodanov-Radulović et al., 2011).

In the fourth examined case, an inadequate immunoprophylaxis program against AD was applied and disease occurred primarily in weaning piglets. Generally, vaccines must be used in sows, in order to give protection to the young piglets via the colostrum (Casal et al., 2004; Dosen et al., 2002). The aim of the vaccination programme is to create a good level of immunization in sows and uniform level of protection in the piglets during the first weeks of their life (Casal et al., 2004). One of the main limitations of the immunization of neonates from vaccinated or previously infected mothers is the interference with maternally derived antibodies (MDA). The half-life of MDA that pigs receive in the colostrum of immune sows is approximately 18 days (Mittenleiter et al., 2012). It is considered that due to inadequate immunization program, clinical form of the AD occurs primarily in piglets 14 days after weaning. However, in early phase of infection, by gross pathological examination in dead sucklings, often the negative pathological finding can be detected (Dosen et al., 2002).

In the last examined case, it was demonstrated that ADV can be latently present in apparently healthy breeding animals without showing any clinical signs. The obtained results indicate that purchasing of breeding animals, with different or unknown health status regarding AD, represents the important route of transmission and spreading of the infection. The outbreak of AD on the swine farm, where immunoprophylaxis was not carried out and purchasing the breeding animals where vaccination against AD was applied, may lead to devastating consequences. The reason for this is the phenomenon of latent infection with reactivation and shedding of the virulent virus (Allepuz et al., 2009; Prodanov-Radulović et al., 2014). Because of subclinical infection, before purchasing breeding animals, the quarantine measures need to be taken (Došen et al., 2002; Došen et al., 2012). However, during quarantine, latently

infected pigs may not be detected. Even if infected pig shows the signs of the disease during quarantine, these signs may be unspecific (respiratory problems, fever, anorexia) and not associated with AD (Prodanov-Radulović et al., 2011). Serological monitoring was shown to be successful in detecting infectious animals (Martinez-López et al., 2009).

Although successful in preventing herds from severe clinical outbreaks, voluntary vaccination in the past did not result in a marked reduction of the spread of ADV in pig-dense parts of Vojvodina region (Pušić et al., 2007; Pušić et al., 2009; Prodanov-Radulović et al., 2011). In addition to the above problems, eradication of ADV from large pig herds in Vojvodina is extremely difficult due to the characteristics of pig production: the dominance of farrow-to-finish type farms, resulting in the simultaneous presence of a large number of susceptible animals; frequent absence of all in-all out principle; the often deficient technological discipline (Prodanov-Radulović et al., 2014). The diversity of production technologies does not make it possible to develop a single procedure that could equally be used on all pig farms (Došen et al., 2007). The herd-specific risk factors such as biosecurity measures, vaccination schemes or the health status of the purchased pigs might be also related to the success of the AD eradication.

CONCLUSION

It may be concluded that the swine population in Vojvodina province is enzootically infected with ADV. Individually vaccinated herds are surrounded by non-vaccinated or poorly vaccinated herds and backyard stocks. Such surrounding herds create pockets of susceptibility which can serve as reservoirs of ADV. Also, the obtained results indicate that purchasing of breeding animals, with different or unknown health status regarding MA, represents the important route of transmission and spreading of the infection.

The cessation of vaccination in the regions with the high pig density, if is not supported with the appropriate eradication strategy, represent a great risk from re-introducing of the virus in the herd with all the consequences. Great economic losses, caused by this diseases, as well as restrictions in trade with ADV free countries point on a need of making regulations about prophylaxis and control, as well as to launch a National eradication programme which would allow regional approach. In the future, the MA eradication programme in Serbia should be based on vaccination of swine population with gE deleted vaccines. Generally, the MA eradication should include three different stages. During the first stage, all pig herds are vaccinated with a gE-deleted marker vaccine and the transmission of ADV must be reduced sharply. Therefore, additional biosecurity measures need to be taken, including strict quarantine measures, serological monitoring, consequent management and movement restrictions. The estimated duration of the large-scale vaccination is 2-3 years, depending on the epidemiological situation. In the second stage of eradication programme, the remaining sources of virus need to be traced and eliminated by compulsory serological testing of pig herds for gE and total AD-specific antibodies. During the final stage of eradication, vaccination should be forbidden.

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POJAVA INFEKCIJE VIRUSOM AUJESKIJEVE BOLESTI (*MORBUSAUJESZKY*) NA VELIKIM FARMAMA SVINJA U AP VOJVODINI (SERBIJA)


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Jelena APIĆ, Igor STOJANOV, Vladimir POLAČEK

Izvod: Aujeskijeva bolest (MA) je infektivno virusno oboljenje, čiji je uzročnik svinjski alfa herpes virus 1, koji inficira široki opseg domaćina izuzev ljudi i primata. Svinje predstavljaju jedinu životinjsku vrstu koja može preživeti infekciju sa virusom Aujeskijeve bolesti, nakon čega se uspostavlja latentna infekcija. Cilj rada je bio sagledavanje trenutne epizootiološke situacije, vezano za nedavne slučajeve izbijanja MA na velikim farmama svinja u Vojvodini. Materijal za ispitivanje je obuhvatio uzorke poreklom sa pet farmi svinja, na kojima su registrovani određeni zdravstveni problemi u kategoriji prasadi na sisi, a koji su ukazivali na MA. U zavisnosti od specifičnosti ispitivanog slučaja i dostupnog materijala, primenjene su sledeće metode ispitivanja: epizootiološka ispitivanja, klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bakterija, virusološko ispitivanje (izolacija virusa na kulturi ćelija) i molekularna metoda dijagnostike, reverzna transkripcija-lančana reakcija polimeraze (RT-PCR), iz uzoraka organa i tkiva obolele, uginule prasadi (tonzile, deo pluća, medijastinalni i mandibularni limfni čvorovi, deo slezine, moždano tkivo). Takođe, material za ispitivanje je obuhvatio 300 uzoraka krvi priplodnih jedinki, u cilju utvrđivanja prisustva specifičnih antitela protiv MA. Postignuti rezultati ukazuju da je populacija svinja na velikim farmama u Vojvodini enzooski inficirana virusom MA. Pored vakcinacije, koja predstavlja jednu od osnovnih mera u okviru eradikacije MA, neophodna je primena stroge kontrole prometa i zdravstvenog statusa zapata iz kojih se kupuju priplodne jedinke.

Ključne reči: Aujeskijeva bolest, lažno besnilo, farme svinja, AP Vojvodina.

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METABOLIC AND HAEMATOLOGICAL PARAMETERS IN PARETIC COWS THAT RECEIVED OR NOT RECEIVED KETOPROFEN (Case report)*

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Summary: In the period after calving in cows there are many endocrin and metabolic changes because of the increase in energy demand which can cause numerous diseases including puerperal paresis, which is caused by hypocalcemia. The aim of this study was to compare the metabolic and hematological parameters in the first and second week after calving in paretic cows which is administered ketoprofen during three days after calving. Blood samples were taken from cows with postpartum paresis 7 and 14 days after calving. We examined the blood count (number of leukocytes, neutrophils, lymphocytes, red blood cells and hemoglobin concentration) and metabolic profile of cows (total protein, albumin, bilirubin, Ca, P, glucose, cholesterol, AST, GGT, NEFA, BHB). Application ketoprofen does not lead to significant differences in the absolute values of the parameters. However, there was a significant difference in the dynamic changes in the blood picture and metabolism in the second week after birth compared to the first, so that cows that received ketoprofen have less lipid mobilization and ketogenesis, less load hepatocytes and favorable ratio of neutrophils and lymphocytes. We conclude that the use of ketoprofen may be useful as a form of supportive therapy, as it reduces the intensity of lipid mobilization and ketogenesis, and relieves the symptoms of inflammatory response and liver dysfunction in paretic cows.

Key words: ketoprofen, paresis, cows, metabolism, blood count.

INTRODUCTION

During the transition period, the cows go through many physiological adaptations, or endocrine changes due to calving and metabolic changes due to the sharp increase in energy requirements that are necessary for the synthesis of milk (Djokovic et al., 2014). During this period, in order to compensate the shortage of energy in the cow's body fatty acids are mobilized from adipose tissue and are released in the plasma as a non esterified fatty acids (NEFA) (Drackley, 1999; Ingvarsten, 2006). Partial oxidation NEFA then leads to the formation of ketone bodies, which are released from the liver and peripheral tissues are used as the energy source. Reducing the effectiveness of insulin

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facilitates the transition of available glucose in the mammary gland to be used as energy for the synthesis of milk (Giesy et al., 2012).

There are many ways in which different disorders during the transition period in cows can trigger an inflammatory response. The acute inflammatory response is associated with changes in lipid metabolism and glucose, which involves increasing lipolysis and NEFA levels (Hardardottir et al., 1994). From the aspect of glucose metabolism, during an acute inflammatory response there is an increase in plasma glucose (Kushibiki et al., 2001). In the period after calving may occur many diseases, most commonly mentioned: ketosis, metritis, mastitis, lameness, abomasum, puerperal paresis and degeneration of the liver (Divers and Peek, 2008). In addition, it comes to significant changes in the metabolism of certain mineral matter where and the most common disorder is puerperal paresis which is developed as a result of hypocalcemia. Factors contributing to the occurrence of hypocalcemia are inability of cows to quickly mobilize enough calcium from the bones and to establish absorption from the small intestine. Beside the metabolic disorders of Ca, causes syndrome recumbent cows may be a metabolic disorder and P, Mg and K, fatty liver, muscle damage, and nerve damage, inflammatory and toxic processes (Djokic et al., 2014).

In veterinary practice, ketoprofen is used to reduce body temperature of animals with fever, to reduce respiratory signs in cows and pigs with pneumonia, to alleviate pain in a variety of conditions such as colic in horses, injuries of joints in horses and dogs, as well as control traumatic and postoperative pain in all animal species (Lees et al., 2004). In a study performed by Donalisio et al. (2013) was found that ketoprofen in comparison with the flunixin-meglumine has similar antiinflammatory effects in cows, or in vitro was noted that the drugs tested reversibly inhibit both isoforms of COX enzymes, but much more COX-1.

The aim of this study is to compare the metabolic and hematological parameters in the first and second week after calving in paretic cows who received ketoprofen.

MATERIAL AND METHODS

In the group of cows that received ketoprofen three cows have developed signs of paresis in the period 24 to 72 hours after calving. That values were compared with a control group of cows that developed paresis and who have not received ketoprofen. Blood samples of the control and experimental groups of cows were taken at 7th and 14th day after calving. We examined the blood count (number of leukocytes, neutrophils, lymphocytes, red blood cells and hemoglobin concentration) and metabolic profile of cows (total protein, albumin, bilirubin, Ca, P, glucose, cholesterol, AST, GGT, NEFA, BHB; Randox reagents). We used a device Siemens ADVIA hematology and biochemical results were obtained on the device type Rayto.

Statistics: the values of haematological and biochemical parameters were presented in the first and second week after calving, the percentage change in the values of the parameters in the second week compared to the first was determined and it was examined whether there is a difference in the dynamics of change in cows received and who are not received ketoprofen.

RESULTS AND DISCUSSION

The results show that there are no significant variations in the values of haematological and biochemical parameters in blood of paretic cows who were receiving and who were not receiving ketoprofen. However, the application of ketoprofen leads to differences in the dynamic changes in the blood picture and metabolism in the second week after birth compared to the first as presented in Table 1 and Figure 1. Paretic cows that did not received ketoprofen show an increase in the number of neutrophils and decrease in the number of lymphocytes, with a decrease of albumin concentration, an increase in the concentration of bilirubin, GGT, NEFA and BHB in relation to the paretic cows that received ketoprofen, where they take place opposite processes as presented in Table 1. The more intense inflammatory response characterized by an increase in the number of neutrophils, lymphocytes decline in value and decline in value albumin with increased lipid mobilization and ketogenesis, and there is a greater load hepatocytes probably due to the intense at the accumulation of fat in the liver (which is discussed in greater rise in GGT and more pronounced drop in cholesterol levels in cows that did not receive ketoprofen).

The above results indicate that in cows that did not receive ketoprofen there is intense inflammatory response that in their study confirmed Donalisio et al. (2013). Inflammatory reactions and differential white grapes in cows must be viewed differently, because there are specific blood counts, namely: a high percentage of lymphocytes (60-80%) and little or no reserve of young granulocytes in the bone marrow. During the inflammatory response in cattle occurs in the number of neutrophils with a decline in the total number of leukocytes, but neutropenia and leukopenia were the first signs of acute inflammation. Only after several days accelerates the leukopenia and young neutrophils out of the circulation, but is often clinically undetected due to rapid wear. In acute purulent inflammation guy there

leukopenia, lymphopenia and neutropenia have to turn left. If the animal survives the acute phase compensation occur in the white line, so that the number of neutrophils and lymphocytes decreases, and their relationship becomes inverse (Kovacevic Filipovic, 2007). Here are described changes in the classical inflammatory reaction. Peripartum period demonstrates specific characteristics when the ratio of neutrophils and lymphocytes is in question. The ratio of neutrophils and lymphocytes (N: L ratio) is an important indicator of the health burden and stress of animals (Davis et al., 2005). This relationship in adult cattle is about 0.5:1. In the period around calving there is no is no typical stress leukogram in cows, which is characterized by neutrophilia, lymphopenia, eosinopenia and variable monoitosis. Acute stress and hypercortisolemia lead to an increase in the concentration of neutrophils dragging these cells into the central pool of blood flow, whereas lymphocytes migrate to the periphery, which leads to the increase in the neutrophil and lymphocyte (N: L ratio is often above 1) (Tornquist and Rigas, 2010). After adaptation to stress N: L ratio returns to the physiological level in the period 2-7 days (Lynch and al., 2010). The value of the relationship N: L is over 1 and it indicates that cows are loaded inflammation or other stressors (Latimer and Prasse, 2003).

Metabolic adaptation of the organism, which is reflected in the intensive mobilization of fats and their use for energy purposes a significant burden on the liver, which can quickly enter the dekompenzacione processes. In fact, higher concentrations of NEFA in the peripartum period ends in the liver where they are exploited for energy purposes. It can be completely metabolized to carbon-4-oxide and water, which provides energy for the liver and physiological functions of the organism. However, it is possible to turn the metabolic processes which burdens the health of the liver and it is: a) partial oxidation of NEFA and production of ketone bodies (especially BHB) going into the circulation and b) NEFA reesterification and the formation of triglycerides that remain in hepatocytes. These two processes often occur together and lead to ketosis cows and fatty infiltration of the liver, which are a major problem in the production and health of cows (Đjokovic, 2010; Šamanac, 2009).

Table 1. Hematology and metabolic findings in paretic cows

	KETOPROFEN						NEGATIVE CONTROL					
	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6	
	Ned1	Ned2	Ned1	Ned2	Ned1	Ned2	Ned1	Ned2	Ned1	Ned2	Ned1	Ned2
WBC x10 ⁹	10,41	10,21	9,05	9,15	6,02	7,39	7,8	9,1	8,2	8,8	8,1	8,9
NEUTRO	6,99	5,48	5,95	5,41	2,53	4,13	4,5	6,1	4	4,7	4,1	5,2
LY	2,53	3,14	2,42	3,3	2,68	2,52	2,6	2,6	3,5	2,9	3,5	2,3
RBC x10 ¹²	5,96	5,48	5,62	5,8	7,03	7,19	6,9	6,5	7,1	6,7	7,2	7,2
Haemoglobine	10,80	10,10	9,2	9,9	10,40	10,60	10,40	9,90	9,87	9,55	9,23	9,23
Platelet	406	213	443	392	394	316	399	401	389	404	412	415
Total protein g/dl	7,0	7,5	6,5	7,0	7,7	7,7	7,1	7,05	7,5	6,9	7,2	6,9
Albumini g/dl	3,3	3,4	2,6	2,6	3,1	3,2	3,1	2,8	3,2	3,05	3	2,75
Total bilirubin	21,1	24,3	12,4	5,8	7,3	12,0	14,5	22,1	9,1	16,7	8,4	15,9
Ca mmol/l	2,4	2,1	2,0	1,9	2,2	2,3	2,1	2,4	2,0	2,3	2,3	2,3
P mmol/l	1,6	1,0	1,5	1,3	1,9	1,9	1,5	1,7	1,6	1,7	1,8	1,8
Glukoza mmol/l	2,7	3,1	3,8	2,6	2,7	3,1	2,5	2,9	2,9	3,3	3,1	2,6
Holesterol mg/dl	104,9	109,9	98,4	78,2	146,6	128,4	106	87,5	99,5	71,5	89,5	81,1
AST IU/L	176,3	389,1	163,7	113,3	114,7	134,3	150	321	98	240	171	299
GGT IU/L	34	22	36	39	31	25	35	39	29	33	28	37
NEFA mmol/l	0,65	0,35	0,5	0,2	0,34	0,31	0,56	0,62	0,51	0,49	0,63	0,58
BHB mmol/l	0,92	0,51	0,83	0,55	0,71	1,1	0,9	1,2	0,84	1,1	0,83	0,99

The change in transcription and protein postranscription contained in VLDL affect the onset of infiltration of liver fat (Bernabucci et al., 2009). Increased mobilization of lipids and their metabolism in the liver with a reduced ability of the liver to produce transport lipoproteins leads to accumulation of lipids in the liver, fatty infiltration and fatty degeneration, which has a negative impact on health and productivity of cows. The most severe form of the disorder called hepatic coma, also known as gravidity toxemia, which usually has a lethal outcome (Đjokovic, 2010).

Elevated concentrations of bilirubin in paretic cows that did not receive ketoprofen with intense ketogenesis indicates the existence of load hepatocytes.

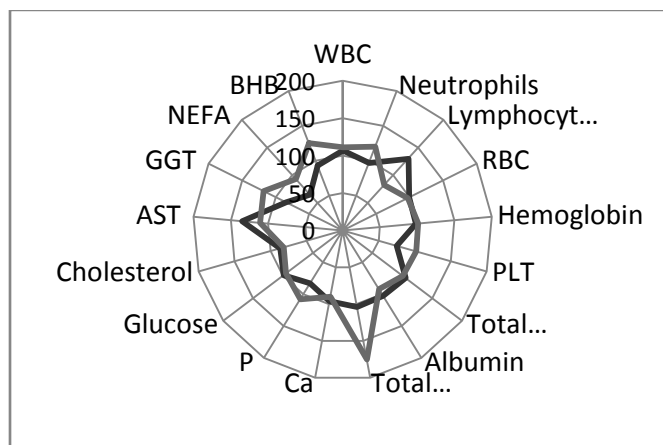


Figure 1. The average relative change of selected haematological and biochemical parameters in the second week compared to the first week postpartum in cows receiving (dark line) and who have not received ketoprofen (lighter line).

Although the mechanism of action who helps lipids to induce inflammatory response is not known, there are several ways in which lipids trigger this response. Studies in humans and different animal models confirm the importance of fatty acids as modulators of inflammatory reaction (Calder, 2008; Serhan et al., 2008). Also, many studies have confirmed that excessive amounts of fat and elevated NEFA concentrations represent a positive risk factors for the development of many proinflammatory peripartum diseases in dairy cows, including mastitis and metritis (Bernabucci et al., 2005; Goff, 2006; Douglas et al., 2007).

In their study Trevisi and al. (2009) have confirmed that inflammatory mediators directly induce metabolic changes giving oral interferon- α (cytokine) in the last 2 weeks of gestation, leading to liver inflammation and release of acute phase proteins. Compared to the control group of cows treated cows had significantly higher concentrations in plasma ketones in the first 2 weeks after calving. In other studies, Travis et al. (2001) also estimated the inflammatory response after calving based on the level of albumin, vitamin A (index RBP) and total cholesterol (index lipoprotein) in the blood and developed a composite index of activity of the liver (LAI), with the explanation that the lower the index is, the inflammatory condition greater is the. The same authors (Travis et al., 2010) have shown that cows with higher inflammatory response after calving are characterized by differences in inflammatory markers was expressed in the preceding dry period.

CONCLUSION

According to the statistical analysis, it was noticed that the application of ketoprofen after calving in paretic cows may be useful as a form of supportive therapy. Ketoprofen, as non-steroidal anti-inflammatory drug reduces the intensity of lipid mobilization and ketogenesis, and also relieves the symptoms of inflammatory response and liver dysfunction.

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METABOLIČKI I HEMATOLOŠKI NALAZ KOD KRAVA SA POSLEPORODAJNOM PAREZOM KOJE SU PRIMALE ILI NISU PRIMALE KETOPROFEN (Prikaz slučaja)

Zorana KOVAČEVIĆ, Marko R. CINCOVIĆ, Branislava BELIĆ,
Dragica STOJANOVIĆ, Ivana DAVIDOV

Izvod: U periodu posle teljenja kod krava dolazi do brojnih endokrinih i metaboličkih promena zbog povećanja energetske potrebe usled čega mogu nastati brojna oboljenja uključujući i puerperalnu parezu, nastalu kao posledica hipokalcemije. Cilj ove studije je bio da se uporede metabolički i hematološki parametri u prvoj i drugoj nedelji posle teljenja kod paritčnih krava kojima je aplikovan ketoprofen tokom tri dana nakon teljenja. Uzorci krvi su uzeti od krava sa posleporodajnom parezom 7. i 14. dana posle teljenja. Ispitana je krvna slika (broj leukocita, neutrofila, limfocita, eritrocita i koncentracija hemoglobina) i metabolički profil krava (ukupni protein, albumin, bilirubin, Ca,

P, glukoza, holesterol, AST, GGT, NEFA, BHB). Aplikacija ketoprofena ne dovodi do značajnih razlika u apsolutnoj vrednosti parametara. Međutim, nađena je značajna razlika u dinamičnim promenama u krvnoj slici i metabolizmu u drugoj nedelji posle porođaja u odnosu na prvu, tako da kod krava koje su primale ketoprofen postoji manja lipidna mobilizacija i ketogeneza, manje opterećenje hepatocita i povoljan odnos neutrofila i limfocita. Zaključujemo da upotreba ketoprofena može biti korisna kao vid potporne terapije, jer smanjuje intenzitet lipidne mobilizacije i ketogeneze, a ublažava i znake inflamatornog odgovora i disfunkcije jetre kod paretičnih krava.

Ključne reči: ketoprofen, pareza, krave, metabolizam, krvna slika.

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