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OCCURENCE OF DEOXYNIVALENOL AND ZEARALENONE IN MAIZE*

IGOR JAJIĆ, VERICA JURIĆ, DRAGAN GLAMOČIĆ, SAŠA KRSTOVIĆ[1]

SUMMARY: A total of 65 samples of maize from the 2009 harvest were investigated for the presence of deoxynivalenol (DON) and zearalenone (ZON). Samples were collected from two different areas (Serbia and Republika Srpska) and were analyzed after the harvest. The samples were analyzed by ELISA test. The average incidence rate of DON in maize was 23.1% (concentration range 0.040−0.281 mg/kg, average value 0.142 mg/kg). The average incidence rate of zearalenone was 32.3% (concentration range 0.078−0.441 mg/kg, average value 0.224 mg/kg). Two of the tested samples was contaminated of zearalenone above the established maximum level adopted by the European Commission for unprocessed maize. Regardless of the established presence of DON (23.1%) and a significant presence of zearalenon (32.3%), tested corn which was the basis of complete mixture for pigs, can be considered appropriately safe for health care.

Key words: *deoxynivalenol, zearalenone, maize*

INTRODUCTION

Food safety is an important issue worldwide. Every year, a large number of crops are affected by fungal invasion, leading to considerable financial losses and impaired health in animals and humans. Toxicity is mainly caused by secondary metabolites of fungi, which are appropriately called mycotoxins (GIPSA, 2002). The most important agriculturally toxigenic fungi occurring in the moderate climatic zones of North America and Europe are *Fusarium* fungi (Kos et al., 2003). Three of the more prevalent mycotoxins that occur in grain are deoxynivalenol (DON), zearalenone and fumonisins (Schaafsma et al., 1998). The presence of these fungi is pronounced in cereals, wheat and corn being especially sensitive, causing their rotting. In literature, a disease of wheat caused by this fungus is known as *Fusarium head blight,* while in corn it is known as *Gibberella ear rot* (JECFA, 2001). Infection of these cereals has for a consequence a decrease in grain size and the amount of protein in the grain, as well as a harmful effect

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Original scientific paper / *Originalni naučni rad*

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on germination. The end result is a decrease in yield and feed quality.

Deoxynivalenol (DON or vomitoxin) is a polar organic compound, which belongs to trichotecenes, a group of mycotoxins, which, beside aflatoxin, zearalenone, ochratoxin and fumonisins, is most frequently found in foodstuffs and feed (Krska et al., 2001). The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (*Gibberella zeae*) and *Fusarium culmorum* (JECFA, 2001). Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world and its occurrence is considered to be an indicator of the possible presence of other, more toxic trichothecenes (Lombaert, 2002). Consumption of contaminated feed by livestock has been associated with a variety of adverse health effects including feed refusal, reduced weight gain, diarrhea, emesis and finally, the weight loss in livestock (Krska et al., 2001; Kuiper-Goodman, 2002). The most sensitive to the presence of deoxynivalenol are pigs, hence already at concentrations of 1 mg/kg in feed a certain percentage of these animals refuse food. EC was permitted level of deoxynivalenol in complementary and complete feedingstuffs (5 mg/kg), with the exception of complementary and complete feedingstuffs for pigs (0.9 mg/kg) and complementary and complete feedingstuffs for calves (< 4 months), lambs and kids (2 mg/kg) (EC, 576/2006). The maximum permitted level of deoxynivalenol in unprocessed maize is 1.75 mg/kg (EC, 1126/2007). The maximum permitted level of DON in feed has been set in our country (Službeni glasnik, 2010) and in complete feedingstuffs for pigs is 0.5 mg/kg.

Zearalenone (ZON) is a secondary fungal metabolite that is produced, like deoxynivalenol, by *Fusarium* species, mainly *Fusarium graminearum* and *Fusarium culmorum* (Betina, 1989). ZON and its derivatives can lead to hyperestrogenism and severe reproductive and infertility problems in animals, especially in swine (Mirocha, 1971). Additionally, bioassays have indicated a carcinogenic activity for ZON. Further studies have to be carried out to decide whether ZON has to be considered as a potential human carcinogen as well (Kuiper-Goodman et al., 1987). Depending on the quantity of zearalenone in the diet, and length of feeding such foods, clinical signs are stronger or weaker pronounced. Quantities of 0.5 to 1 mg/kg in the diet cause a rash (Erythema) around the tail and back, swelling of the vulva and mammary regions of piglets and adult females, and the amount of 1.5 to 5 mg/kg cause hyperestrogenism. In boars ZEA reduces testosterone concentrations, testicular weight and spermatogenesis, encourage feminization and reduce libido. Extremely high amounts (50-100 mg/kg) adversely affect ovulation, conception, implantation and embryo development and fetal pigs (Koselac and Pepeljnjak, 2004; Richard, 2007). Since the zearalenone and its metabolites are known for estrogen and anabolic properties, and growth effects, the synthetic compound zeranol (Ralgro) is included as an agent to improve growth and better utilization of animal feed (for cattle and sheep in the USA). The European Union banned in 1989. use of this preparation (Zinedine, 2009). In addition to feces and urine, zearalenone is excreted trough milk, and only 42-44 hours after contamination, and excretion is done in the next 5 days after cessation of intake of contaminated food. Organs where zearalenone and its metabolites are distributed are the uterus, intestines, testis, ovary and adipose tissue. Damage and pathological changes were observed in the form of cystic formations, and negatively affects the spermatogenesis (Sinovec, 2006). EC was permitted level of zearalenone in complementary and complete feedingstuffs for piglets and gilts (0.1 mg/kg) and complementary and complete feedingstuffs for sows and fattening pigs (0.25 mg/kg) (EC, 576/2006). The maximum permitted level of zearalenone

in unprocessed maize for foodstuffs is 0.350 mg/kg (EC, 1126/2007). The maximum permitted level of zearalenone in complete feedingstuffs for piglets in our country is 0.5 mg/kg and for fattening pigs is 1 mg/kg (Službeni glasnik, 2010).

Because of everything stated above, the aim of this work is to determine deoxynivalenol and zearalenone content in maize samples collected from different locations in the Republic of Serbia and Republic of Srpska, after the harvest, during 2009. Samples were analyzed by the ELISA test. In addition, this paper attempts to compare data with those found in relevant literature about incidence of this mycotoxin in countries of our region.

MATERIAL AND METHODS

Samples of maize from the 2009 harvest were collected from different locations in the Republic of Serbia and Republic of Srpska. Samples were taken immediately after the harvest. Immediately after sampling, 1000 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 0.8 mm. After that, the sample was homogenized by mixing. Samples thus prepared were packed in plastic bags and stored in a freezer at -20 ºC until analysis. Prior to each analysis, the samples were allowed to reach room temperature. 20 g of samples were weighed and mycotoxins were extracted on an Ultra Turax T18 homogenizer for 3 min at 11,000 rpm. Deoxynivalenol were extracted with distilled water (100 mL) and zearalenone with methanol:water (70:30, v/v) with add of 4 g NaCl. Crude extract was then filtered through 5B Advantec filter paper.

The immunochemical analysis was performed using the Veratox, DON HS (quantitative high sensitivity test, Neogen, USA) and zearalenone (Tecna, Italy) according to manufacturer's procedure.

RESULTS AND DISCUSSION

Samples of maize, collected during 2009. year from area of Serbia were analyzed and the results are presented in Table 1. Total of 35 samples were analyzed. As can be seen, the DON content was above the LOQ in 22.9% of samples of maize (concentration range 0.040−0.220 mg/kg, average value 0.121 mg/kg). Zearalenone was present in 30.0% of samples of maize (concentration range 0.078−0.348 mg/kg, average value 0.204 mg/kg).

Table 1. Content of deoxynivalenol and zearalenone in maize in Serbia from the 2009 harvest
Tabela 1. Sadržaj deoksinivalenola i zearalenona u kukuruzu iz Republike Srbije, žetva 2009

Mycotoxin	No. of samples	No. of positive samples (%)	Concentration in samples		
			average (mg/kg)	range (mg/kg)	median (mg/kg)
DON	35	8 (22.9)	0.121	0.040-0.220	0.135
Zearalenone	35	10 (30.0)	0.204	0.078-0.348	0.208

Samples of maize, collected during 2009. year from area of Republic of Srpska were analyzed and the results are presented in Table 2.

Table 2. Content of deoxynivalenol and zearalenone in maize in Republic of Srpska from the 2009 harvest
Tabela 2. Sadržaj deoksinivalenola i zearalenona u kukuruzu iz Republike Srpske, žetva 2009

Mycotoxin	No. of samples	No. of positive samples (%)	Concentration in samples		
			average (mg/kg)	range (mg/kg)	median (mg/kg)
DON	30	7 (23.3)	0.167	0.071-0.281	0.164
Zearalenone	30	11 (36.7)	0.242	0.108-0.441	0.212

Total of 30 samples were analyzed. As can be seen, the DON content was above the LOQ in 23.3% of samples of maize (concentration range 0.071−0.281 mg/kg, average value 0.167 mg/kg). Zearalenone was present in 36.7% of samples of maize (concentration range 0.108−0.441 mg/kg, average value 0.242 mg/kg).
Total content of both mycotoxins in samples from both areas is presented in Table 3.

Table 3. Total content of deoxynivalenol and zearalenone in maize from the 2009 harvest
Tabela 3.Ukupan sadržaj deoksinivalenola i zearalenona u kukuruzu, žetva 2009

Mycotoxin	No. of samples	No. of positive samples (%)	Concentration in samples		
			average (mg/kg)	range (mg/kg)	median (mg/kg)
DON	65	15 (23.1)	0.142	0.040-0.281	0.140
Zearalenone	65	21 (32.3)	0.224	0.078-0.441	0.212

Presented results show that the established presence of zearalenone (32.3%) was slightly higher compared to the presence of deoxynivalenol (23.1%) in all tested samples of maize. Also, the maximum detected amount of zearalenone (0.441 mg/kg) was higher than the maximum detected amount of deoxynivalenol (0.281 mg/kg).

None of the maize samples surpass the values, which are the maximum permitted level for DON and only two of the tested samples was contaminated of zearalenone above the established maximum limits for unprocessed maize in EU. Therefore the maximum permitted level of zearalenone in complete feedingstuffs for piglets (0.5 mg/kg) and for fattening pigs (1 mg/kg) in our country is higher than in the EU (0.1 and 0.25 mg/kg).

Rafai et al (2000) in Hungary, in the period 1991-1998 examined crops for the presence of DON and zearalenone. In 760 samples of maize the incidence rate was 10.8% for DON and 18.4% for zearalenone. By analyzing 30 samples of maize from 1997 harvest in Romania, Curtui et al. (1998) determined the presence of DON in 46% of the tested samples. In Croatia, Šegvić-Klaric et al. (2007) examined 30 samples of maize from 2004 harvest and established the presence of zearalenone in 26% of samples in a very high concentration range from 6.03 to 29.43 mg/kg. Manova and Mladenova (2009) in Bulgaria examined 19 samples of maize from 2007 harvest and established the presence of zearalenone in 21.1% of samples. In the study, which included samples from the whole world (JECFA, 2001) a large number of maize samples were examined (5.349) and the presence of DON was found in 40% of the tested samples.

According to the aforementioned data we can see that the presence of both mycotoxins in our investigations is in the level of those listed in the investigations in the region. While our neighboring countries belong to very similar climatic zone, as well as applied agricultural practice, we should bear in mind the fact that the samples in these surveys belonged to different harvest years, and that the impact of climatic conditions on the mycotoxin production is very high. Certain differences in the results of mycotoxin presence in this part of the world should be sought in a variety of techniques that are applied for sample analysis.

CONCLUSION

Based on the presented results we can conclude that the presence of the tested mycotoxins in maize is not alarming. The fact that only 2 samples of maize exceeded the maximum permitted level for zearalenone adopted in EU, does not seem particularly worrying. Although a significant presence of zearalenone is established (32.3%), it can be considered that investigated maize, which was the basis of complete mixture for pigs, is appropriately safe for health care, given the relatively low content of zearalenone in these samples

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PRISUSTVO DEOKSINIVALENOLA I ZEARALENONA U KUKURUZU

IGOR JAJIĆ, VERICA JURIĆ, SAŠA KRSTOVIĆ

Izvod

Na prisustvo deoksinivalenola (DON) i zearalenona (ZON) je ispitano ukupno 65 uzoraka kukuruza, žetve 2009. Uzorci su sakupljeni sa dva različita područja, Republike Srbije i Republike Srpske i analizirani neposredni nakon žetve. Uzorci su analizirani imunohemijskom metodom, tzv. ELISA testom. Prisutnost DON-a u kukuruzu je iznosila 23,1% (u koncentracionom opsegu od 0,040−0,281 mg/kg i prosečne vrednosti od 0,142 mg/kg), dok je prisutnost zearalenona iznosila 32,3% (u koncentracionom opsegu od 0,078−0,441 mg/kg i prosečne vrednosti od 0,224 mg/kg).

U samo dva od ispitivanih uzoraka je utvrđena prisutnost zearalenona koja je prevazilazila maksimalne količine koje su preporučene od strane Evropske komisije. Bez obzira na utvrđenu prisutnost DON-a od 23,1% i još značajniju prisutnost zea-

ralenone od 32,3%, ispitivani kukuruz, koji je bio osnov potpunih smeša za ishranu svinja, se može smatrati odgovarajuće zdravstveno bezbednim.

Ključne reči: deoksinivalenol, zearalenon, kukuruz

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ECONOMIC EFFECTS OF FRUIT PRODUCT PRODUCTION*

MIRJANA LUKAČ BULATOVIĆ[1]

SUMMARY: Possibilities of fruit production are pretty complex, having in mind both semi-finished and finished products. Within a wide assortment of fruit products, there are semi-finished products which can be directly marketed, but can also be used as raw material in higher phases of processing, which yields different economic effects. This paper presents indicators of economic effects (production value, production costs, particularly direct) in basic production and in all phases of processing for specific types of products. The analysis of basic economic indicators of production is made in 2008 in fruit processing plants located in Vojvodina.

Key words: *fruit processing, production value, production costs, financial result*

INTRODUCTION

Serbia currently disposes of significant processing and refrigerating capacities. However, in the last decade of development, this industry has been increasingly facing the problem of underutilization of the existing processing plants (Milić and Radojević, 2003). The actual utilization of capacities is far below those available and in 2002 it was approximately 30% in Serbia, and 55% in Vojvodina (Lukač-Bulatović, 2004). This is a consequence of a non-aligned development of primary production and processing industry. Another reason of underutilization of capacities is a low marketability of fruit products. Fruit products are still, to a large extent, home-made. That is a consequence of a low standard of living of consumers on the one, and high prices of fruit products of unsatisfactory quality and assortment, on the other hand (Rodić, 2002).

In addition to capacities and raw material base, product branding, that is, creation of a trademark is also an important factor for a fruit product to be successfull. By analyzing the assortment, it can be concluded that in fruit product production in Serbia there is no dominating specific product or products, either in terms of quantity or quality, as it is, e.g. peach compote in Greece, apricot products in Hungary, apple juice in Switzerland, tomato products in Italy etc. (Niketić- Aleksić, 1987).

The main aim of the research is to analyze all significant production and eco-

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Original scientific paper / *Originalni naučni rad*

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nomic aspects of fruit processing. The research focuses on quantification (measurement) of economic effects attainable in fruit product production, both individually and as a whole. The analysis of main economic indicators in fruit product production was made in 2008.

MATERIAL AND METHODS

The attained production and economic output in fruit product production was analyzed on the basis of data from the accounting records (fruit product production calculations, operating reports, fruit product recipes etc.) of companies involved in fruit processing, based in Vojvodina. The following products were analyzed: fruit spoon-meal, pressed fruit, industrial marmalade, jam, compote, fruit juices and fruit spreads.

RESULTS AND DISCUSSION

Production of fruit products

In 2008, 1,355,269 kg of fruit was processed in total. In the overall annual industrial production, marmalade was made of 354,700 kg of fruit, making 26.17%, taking a dominant position in the total quantity of processed fruit (Table 1). When other forms of processed fruit are concerned they range from, 49,800 kg of fruit used in the production of pressed fruit to 333,899 kg of fruit in the production of fruit spoon-meal (frozen).

Table 1. Structure of the processed fruit production in the studied fruit processing plant in the year 2008
Tabela 1. Struktura proizvodnje prerađevina od voća u ispitivanom objektu u 2008. godini

Type of processed fruit *Vrsta prerađevine*	Production (kg) *Proizvodnja*	Share (%) *Učešće*
Frut spoon –meal frozen *Voćna kaša zamrznuta*	333,899	24.64
Industrial marmalade *Industrijska marmelada*	354,700	26.17
Pressed fruit *Voćna rotativa*	49,800	3.67
Jam */ Džem*	82,029	6.05
Jam / *Marmelada*	113,360	8.36
Preserved fruit */ Kompot*	139,393	10.29
Fruit spread / *Voćni namaz*	70,431	5.20
Fruit juice / *Voćni sok*	211,657	15.62
Total / *Ukupno*	1,355,269	100.00

MAIN ECONOMIC INDICATORS FOR THE PRODUCTION OF PROCESSED FRUIT

It is possible to express operating results of companies by several indicators determining and measuring the achieved economic effectiveness of production in the course of a business year (Andrić, 1998). The most significant indicators of operating results are: value of production, production costs and economic (financial) result.

The value of production of the analyzed fruit is obtained by multiplying the output and the selling price per unit of product. The production value is proportionate to the production volume and selling price. With the increase in output and selling price, a greater production value can be expected and vice versa.

In the year under review (2008), the value of overall fruit product production amounted to 105.8 million dinars, whereby pressed fruit production value amounted to only 3.3 million dinars, and industrial marmalade production amounted to 43.8 million dinars, being the highest amount.

In the structure of overall costs, direct production costs of the stated fruit products make 72.31%, whereas indirect costs make 27.69% (Tab. 2 and 3).

Table 2. Amount and structure of costs in the production of semi-finished fruit products in the year 2008

Tabela 2. Iznos i struktura troškova u proizvodnji poluproizvoda od voća u 2008. godini (RSD/kg)

Costs elements *Elementi troškova*		Semi-finished fruit products *Poluproizvodi od voća*		
		Industrial marmalade *Industrijska marmelada*	Pressed fruit *Voćne rotative*	Frut spoon - meal frozen *Voćna kaša - zamrz.*
I	Direct costs / *Direktni troškovi*	95.72	52.14	48.10
	Share (%) / *Učešće*	72.31	72.31	72.31
1.	Material costs / *Materijalni troškovi*	81.90	41.26	41.68
	Share (%) / *Učešće*	61.87	57.22	62.67
1.1.	Ingredients / *Sirovine*	35.72	39.27	38.22
	Share (%) / *Učešće*	26.98	54.46	57.47
1.2.	Additives / *Dodaci*	36.63	/	/
	Share (%) / *Učešće*	27.67	/	/
1.3.	Packaging / *Ambalaža*	0.68	0.47	0.47
	Share (%) / *Učešće*	0.51	0.65	0.71
1.4.	Energy (all types) / *Energija (svi oblici)*	8.87	1.52	2.99
	Share (%) / *Učešće*	6.70	2.11	4.50
2.	Gross earnigs / *Bruto lični dohoci*	5.12	6.14	2.05
	Share (%) / *Učešće*	3.87	8.51	3.08
3.	Amortization / *Amortizacija*	8.70	4.74	4.37
	Share (%) / *Učešće*	6.57	6.57	6.57
II	Indirect costs / *Opšti troškovi*	36.65	19.97	18.41
	Share (%) / *Učešće*	27.69	27.69	27.69
III	Total costs / *Ukupni troškovi*	132.37	72.11	66.51
	%	100.00	100.00	100.00

The overall material costs in the average amount of 64.67din./kg make 60.28% of the overall costs. Among these costs, costs of basic raw materials prevail, making 32.65% on average.

The average absolute amount of overall costs of (basic) raw materials per 1 kg of products amounted to 32.65 dinars, ranging between 24.57 dinars in compote production, to 39.27 dinars in pressed fruit production. A relative participation of basic raw material costs in the structure of overall costs ranged between 21.27% in marmalade

production to 57.47% in the production of frozen-fruit spoon-meal.

Table 3. Amount and structure of costs in the production of finished fruit products in the year 2008

Tabela 3. Iznos i struktura troškova u proizvodnji gotovih proizvoda od voća u 2008. godini (RSD/kg)

Costs elements *Elementi troškova*		Finished fruit products *Gotovi proizvodi od voća*				
		Preserved fruit *Kompot*	Jam *Marmela-da*	Jam *Džem*	Fruit spread *Voćni namaz*	Fruit juice *Voćni sok*
I	Direct costs / *Direktni troškovi*	71.25	97.04	104.09	90.26	61.67
	Share (%) / *Učešće*	72.31	72.31	72.31	72.31	72.31
1.	Material costs / *Materijalni troškovi*	56.25	79.52	85.93	77.96	52.88
	Share (%) / *Učešće*	57.08	59.25	59.69	62.45	62.00
1.1.	Ingredients / *Sirovine*	24.57	28.54	33.04	31.07	30.80
	Share (%) / *Učešće*	24.93	21.27	22.95	24.89	36.11
1.2.	Additives / *Dodaci*	9.56	32.73	34.33	30.52	8.58
	Share (%) / *Učešće*	9.70	24.39	23.85	24.45	10.06
1.3.	Packaging / *Ambalaža*	16.68	12.30	12.61	12.66	11.47
	Share (%) / *Učešće*	16.93	9.17	8.76	10.14	13.45
1.4.	Energy (all types) / *Energija (svi oblici)*	5.44	5.95	5.95	3.71	2.03
	Share (%) / *Učešće*	5.52	4.43	4.13	2.97	2.38
2.	Gross earnigs / *Bruto lični dohoci*	8.53	8.70	8.70	4.10	3.19
	Share (%) / *Učešće*	8.66	6.48	6.04	3.28	3.74
3.	Amortization / *Amortizacija*	6.47	8.82	9.46	8.20	5.60
	Share (%) / *Učešće*	6.57	6.57	6.57	6.57	6.57
II	Indirect costs / *Opšti troškovi*	27.29	37.16	39.86	34.57	23.62
	Share (%) / *Učešće*	27.69	27.69	27.69	27.69	27.69
III	Total costs / *Ukupni troškovi*	98.54	134.20	143.95	124.83	85.29
	%	100.00	100.00	100.00	100.00	100.00

The absolute amount of other ingredients per 1 kg of product amounted to 19.04 dinars on average. Costs of packaging make on the avereage 7.54% of the total production costs in the year under review. depreciation costs make 6.57% on average. gross salaries make 5.46% on average, whereas electric power and heavy fuel oil make 4.09% on average.

The economic (financial) result in the plant under review was determined as a differential between the production value and overall costs. A positive financial result (profit) was made in fruit product production (Tab. 4 and 5).

Table 4. Basic economic indicators in the production of semi-finished fruit products in the year 2008

Tabela 4. Osnovni ekonomski indikatori u proizvodnji poluproizvoda od voća u 2008. godini (000 RSD)

Pokazatelj *Indicator*		Semi-finished fruit products *Poluproizvodi od voća*		
		Industrial marmalade *Industrijska marmelada*	Pressed fruit *Voćne rotative*	Frut spoon –meal frozen *Voćna kaša - zamrz.*
A.	**Value of production** Vrednost proizvodnje	43,804	3,337	4,873
B.	**Total costs** Ukupni troškovi	39,988	2,891	4,387
C.	**Profit** Dobit	3,816	446	486
	Production costs savings *Ekonomičnost proizvodnje*	1.10	1.15	1.11
	Processed fruit production rentability rate (%) *Stopa rentabilnosti proizvodnje*	9.54	15.43	11.07
	Productivity of production *Produktivnost proizvodnje*	2.21	2.65	0.88
	Labour consumption in man-hour per 100 kg *Utrošak ljudskog rada (č/100kg)* Machine utilization in hours per 100 kg *Utrošak mašinskog rada (č/100kg)*	0.30	0.08	0.10

In the year under review, the overall financial result in fruit product production amounted to 12.2 million dinars. The highest financial result (profit) was in the production of industrial marmalade (3.8 million dinars).

Production is cost-effective if the cost-effectiveness ratio is greater than 1.0 (E>1), it is break-even if the cost-effectiveness ratio equals one (E=1), and it is not cost-effective if the ratio is below 1.0 (E<1). The cost-effectiveness ratio expressed this way indicates the value of production attained per unit of costs incurred (Gogić, 2005).

The obtained coefficients indicate that the fruit product production in the year under review was cost-effective. The average ratio of cost-effectiveness was 1.13, ranging between 1.10 in the production of industrial marmalade to 1.20 in the production of fruit spread.

Profitability is a significant indicator of success of operations, or in other words, a decisive factor in the evaluation of financial success and particularly for further development of any company. Actually, profitability is an indicator of justifiableness and utility of an industry. Cost-effectiveness of production and profitability of resources used can be the subject of a review. As the calculations made do not present data on average funds employed, the rate of cost-effectiveness is used in this paper. The cost-effectiveness rate can be obtained from the ratio of profit and overall production costs (Elenov, 2002). Production can be said to be cost-effective if a positive financial result is obtained. Due to that, the cost-effectiveness rate is often called the rate of profit and is expressed as a percentage.

Tabela 5. Basic economic indicators in the production of of finished fruit products in the year 2008

Table 5. Osnovni ekonomski indikatori u proizvodnji gotovih proizvoda od voća u 2008. godini (000 RSD)

Indicator *Pokazatelj*		Finished fruit products *Gotovi proizvodi od voća*				
		Preser-ved fruit *Kompot*	Jam *Marmela-da*	Jam *Džem*	Fruit spread *Voćni namaz*	Fruit juice *Voćni sok*
A.	**Value of production** Vrednost proizvodnje	10,416	13,098	14,553	7,764	7,968
B.	**Total costs** Ukupni troškovi	,8,965	11,319	12,721	6,469	6,859
C.	**Profit** Dobit	1,451	1,779	1,832	1,295	1,109
	Production costs savings *Ekonomičnost proizvodnje*	1.16	1.16	1.14	1.20	1.16
	Processed fruit production rentability rate (%) *Stopa rentabilnosti proizvodnje*	16.19	15.72	14.40	20.02	16.17
	Productivity of production *Produktivnost proizvodnje* Labour consumption in man-hour per 100 kg *Utrošak ljudskog rada (č/100kg)*	3.25	3.75	3.75	1.76	1.37
	Machine utilization in hours per 100 kg *Utrošak mašinskog rada (č/100kg)*	0.06	0.28	0.28	0.23	0.04

In fruit product production in the period under review, a positive profitability rate was achieved. Viewed by types of products, the percentage of a positive profitability rate was 13.10% on avarage. The highest profitability rate was in the production of fruit spreads (profitability rate = 20.02%). In the production of other fruit products, the profitability rate ranged from 9.54% (production of industrial marmalade) to 16.19% (compote production).

Fruit product production may yield better production and economic results than selling fresh fruit (Rott, 1996). Higher processing phases require higher individual costs, however, the increased costs enable higher revenue and "residual" revenue. The economic effect of apple processing into clear juice and brandy compared to the effect of selling apples as consumer goods on the local market is a higher revenue by 26.9% and a higher residual revenue by 1.7% (Lukač-Bulatović, 2006).

CONCLUSION

In 2008, the overall fruit product production in the plant under review was 1,355 t. At the average selling price of 78.10 din./kg, the production value amounted to 105.8 million dinars.

In the overall production cost structure, direct production costs make 72.31% on average, whereas indirect costs make 27.69%. Material costs are the highest in the direct cost structure (60.28% on average). In the structure of material costs, costs of basic raw materials are the highest (50.50% on the average).

The economic (financial) result in the plant under review was determined as a differential between the production value and overall costs. A positive financial result (profit) was made in fruit product production. In the year under review, the overall financial result in fruit product production amounted to 12.2 million dinars.

In the fruit product production an average profitability rate of 13.10% was achieved. The highest profitability rate was in fruit spread production (profitability rate = 20.02%). In the production of other fruit products, the profitability rate ranges between 9.54% (industrial marmalade production) to 16.19% (compote production).

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EKONOMSKI EFEKTI U PRERADI VOĆA

MIRJANA LUKAČ BULATOVIĆ

Izvod

Mogućnosti prerade voća su veoma složene, kako po asortimanu poluproizvoda, tako i gotovih proizvoda. U okviru širokog asortimana prerađevnina od voća postoje i poluproizvodi koji se mogu plasirati direktno na tržište, ali isto tako mogu poslužiti i kao sirovina za više faze prerade, pri čemu, se ostvaruju i različiti ekonomski efekti. U radu su prikazani pokazatelji ekonomskih efekata (vrednost proizvodnje, troškovi proizvodnje, posebno direktni) u osnovnoj proizvodnji i u svim fazama prerade za određenu vrstu prerađevina. Analiza osnovnih ekonomskih indikatora proizvodnje je izvršena u objektima za preradu voća lociranim na području Vojvodine, u 2008. godini.

Ključne reči: prerada voća, vrednost proizvodnje, troškovi proizvodnje, finansijski rezultat

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ECONOMIC ASPECTS OF ORGANIC PRODUCTION DEVELOPMENT*

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SUMMARY: Organic foodstuff trade has been rising continually. International organisations, countries and corporations can have a significant impact on trade flows of organic products, which poses a significant challenge. More than ever before, consumers' demands for high-quality and healthy food of plant and animal origin produced in unimpaired nature are becoming more and more pronounced. Agricultural enterprises today are faced with many problems in organic production organization. The main research aim of this paper is to provide an analysis and comparison of organic productions in the world. The paper presents the growth of world market of organic foodstuff products, also providing explanations related to functioning of organic retail sale sector in the most developed countries of the world. Profit, as the motive for organic products producers, has been shaken by the current economic crisis which has additionally increased the financial risk of investing financial assets in organic production.

Key words: *organic agriculture, sustainable development, organic production, organic retail sector.*

INTRODUCTION

Man has, by his activities, caused the changes in natural ecosystems and biosphere, thus hazarding not only their existence but his own existence, too. The results of such activities are numerous negative effects of destruction and pollution of the environment. Instead of natural ecosystems which have been gradually disappearing, there are more and more man-made ecosystems, planted woods, industrial and urban systems, etc.

The economic reasons are the main culprits of big environmental disasters, as the desire of both individuals and companies to achieve greater economic profit, as well as the lack of motive for preservation of the environment, are increasingly taking a toll. Striving for greater profit leads to even larger-scale negative environmental effects.

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Economic thinking means searching for the best solutions and possibilities in given circumstances, i.e. looking for the best ways of exploitation of the limited production resources on one hand and for the smallest negative effects of their exploitation on the other hand (Sredojević, 2002).

In some parts of Serbia, certain forms of sustainable and organic agriculture have been applied, based on various principles, as is the case in hilly and mountainous regions. Grassland resources in these regions are of great importance for the development of the overall economy, as the basis for organic agriculture in hilly and mountainous regions can be breeding of small ruminants Difficulties and problems related to economic justification of agricultural production can be solved by focusing animal production on producing highly valuable food like meat, milk and dairy products. The production of this food is one of the directions in agricultural production which enables preservation of environment as well as improvement of the quality of life.

MATERIAL AND METHODS

Today agricultural enterprises, farms as well as individual producers face many problems in the organization of organic production. Profit as a motive for selling organic products has been shaken by the current economic crisis. The crisis has even more increased the financial risk of investing financial assets in organic production. According to the previous researches, it can be concluded that the crucial condition for increasing the area under organic production is awareness of the importance of organic products primarily for the people from the developed European countries and the whole world. For this analysis we have used published works covering these issues both from our country and from abroad.

RESULTS AND DISCUSSION

Modern and contemporary agriculture has affected the biodiversity resulting in a very small number of species of cultivated plants and animals. In agricultural ecosystems worldwide in all climatic zones only 12 kinds of grains, 23 kinds of vegetables and 35 kinds of fruit are grown, which makes 70 kinds on approximately 1,440 million of hectares of arable land in the whole world (Willer, Helga et all., 2008, 2009). According to certain data, only 150 plant species are grown for human consumption while only 5 types of grains (wheat, corn, rice, barley and millet) are needed to satisfy 50% of nutrition demands. Certain American researches showed that organic food has approximately 63% potassium, 73% iron, 60% zinc, and 125% calcium more than products produced in conventional agriculture; the content of dry matter in organic products is up to 30% higher, which means that there is a higher concentration of nutrients per mass unit and higher quality for processing industry; also, the levels of magnesium, phosphor and vitamin C are higher. The sale of organic products has been tripled in the EU countries and, according to surveys, 46% of consumers buy this kind of food for health reasons and 40% because of their better taste. Less developed countries which still have their ecosystems preserved as they do not use expensive chemicals have a chance to increase their export through organic products. The WHO data state that about 3 million people are poisoned with pesticides every year.

The main purpose of legislative regulations is protection of people's health and biodiversity while the legislation of environment protection is related to the standards of agricultural products, pollution of stationary and mobile resources, pollution of water, air and to the nature protection. In our country ISO 9000 standards for product quality management and ISO 14000 standards for the environment quality management were accepted. Hereby, our country entirely supported the conclusions of the Rio de Janeiro Convention. However, when the International Community imposed sanctions on Yugoslavia (Serbia) in 1992, our society was not capable of sufficiently investing in improvement and protection of environment. Seven years later (1999) the members of the NATO alliance attacked our country which affected catastrophically all living beings both directly and indirectly by harmful and hazardous substances through contaminated natural resources.

According to the research conducted primarily in economically developed countries, the prevailing opinion today is that contemporary organized agricultural production significantly contributes to pollution and destruction of our environment; only the production performed in natural environment and depending on the effects of natural factors can contribute to the preservation and stability of environment and ecosystem.

Organic agricultural production means that the system of production prohibits or excludes application of synthetic mineral fertilizers, pesticides, growth regulators and feed additives. This kind of production depends substantially on crop rotation, plant residues, on manure, green manure, biological measures against weed, on diseases and pests. At the level of an agricultural enterprise or farm the following regulation must be met: the overall economic results and profit can be maximized only under the condition of achieving ecological optimum. Agricultural enterprises and farms with organic production are supposed to produce seed, fertilizers and feed by themselves (Milić and Sredojević, 2004).

The products produced in accordance with the principles of organic technology must have the label: Organic Farming-EEC Control System only if they are actually produced by the rules of such production and if they passed the required inspection.

The consumers' demand for high quality and healthy food of plant and animal origin produced in undisturbed natural environment are becoming more and more prominent.

The opponents of organic agriculture state that organic products are more expensive than those produced in conventional production. On one hand, it is true because the higher prices of organic products result from larger engagement of labour force which is very expensive in developed countries, and on the other hand there is a great market deficiency of these products, which affects the price.

WORLD ORGANIC PRODUCTION TODAY

Organic agriculture has been developing very fast. The portion of land under organic production has been continuously growing in many countries (Willer, Helga et all., 2008, 2009). According to the latest measuring, almost 30.4 million ha have been used for organic agricultural production (Table 1).

Africa has over 400,000 ha of land under organic production. The countries with largest areas of land with organic production are Tunisia (155,000 ha), Uganda (88,500 ha) and South Africa (50,000 ha). The organic products grown on this continent are

mostly exported to EU countries because the African countries have generally accepted the standards regulated by EU. Asia has 3.1 million ha under such a production. Countries with the largest land areas are China (2.3 million ha), India (530,000 ha) and Indonesia (40,000 ha). The greatest demand for organic products is in Japan, South Korea, Singapore, Taiwan and Hong Kong. At the beginning of 2007 Europe had 7.4 million ha of land under organic production. The countries with the greatest land areas with organic production are Italy, Spain and Germany (Graph 1). The largest markets of organic products are in Germany and the UK, while the greatest consumption of organic food per capita is in Switzerland. South America has 4.9 million ha under such organic production. The three countries with the largest areas of such a production are Argentina (2,220,000 ha), Uruguay (930,000 ha) and Brazil (880,000 ha). Almost all organic products on this continent are exported. North America has 2.2 million ha under organic production. The largest share of 1.6 million ha is in America while the rest of 600,000 ha is in Canada. Australia, including New Zealand and island countries of Fuji and Papua New Guinea have around 12.4 million ha of land with organic production. The countries with largest areas under this production are Australia (12,295,000 ha) and New Zealand (64,000 ha).

Table 1. Area of land under organic production in 2007.
Tabela 1. Površine zemljišta pod organskom proizvodnjom u 2007.

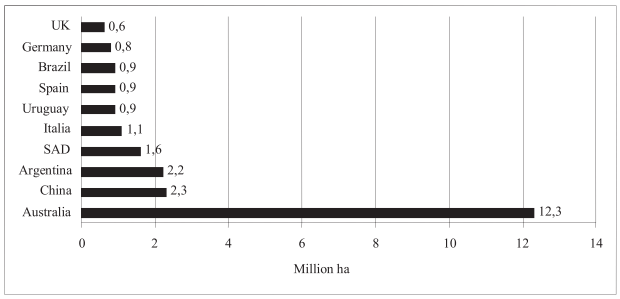
Continents *Kontinent*	Organic land (ha) *Organsko zemljište*
Africa	417,059
Asia	3,090,924
Europe	7,389,085
South America	4,915,643
North America	2,224,755
Australia and Oceania	12,380,796
TOTAL / *UKUPNO*	30,418,262

(Izvor: Willer, Helga et all., 2008)

The greatest importers of organic products are the EU, the USA and Japan, thus their regulations have significant impact on world trade and standard improvement in other areas and regions. In 2007 the EU completely changed the regulations for organic production, which came into force on January 1st, 2009.

The continent with the largest areas under organic agricultural production is Australia and Oceania (12.4 million ha), the second largest is Europe (7.4 million ha) and the third large is South America (4.9 million ha), followed by Asia (3.1 million ha), North America (2,2 million ha) and Africa (0.4 million ha).

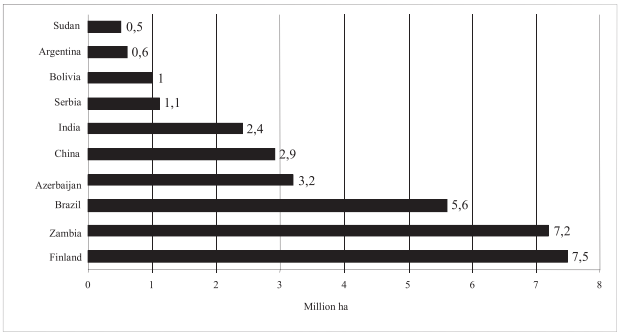
As in the previous years, Australia has been the country with largest areas under organic production, China takes the second place while Argentina takes the third place.



Graph 1. Countries with the largest areas of land under organic production in 2007.
Grafikon 1. Zemlje sa najvećim površinama zemljišta pod organskom proizvodnjom u 2007

(Izvor: Willer, Helga et all., 2008)

Today we know all the forms and results of using organic land. Of course, it should be noted that not all information from all the countries is available. Arable land is mostly used for grains, flowers, green fodder, industrial crops, aromatic and spicy herbs, root-rhizome crops and vegetables. There are the areas with permanent crops like citruses, cocoa, coffee, fruit, grapes, industrial crops, aromatic and spice herbs, olives, sugar cane and tea.



Graph 2. Countries with the greatest land areas under wild-growing plants in 2007.
Grafikon 2. Zemlje sa najvećim površinama pod samoniklim biljem u 2007.

(Izvor: Willer, Helga et all., 2008)

The land for organic production under wild-growing plants is equal on all four continents: Africa, Asia, Europe and South America. Australia and Oceania are almost without such kind of land while there are very small areas in North America. Out of the total world land areas under wild-growing plants, Europe accounts for 28%, Africa for 25%, Asia for 24% and South America for 22%.

EUROPEAN MARKET OF ORGANIC FOOD

Europe has the largest and most complex market of organic products in the whole world, worth about 20 billion US dollars (2006). Europe has taken the leadership partly due to the depreciation of American dollar, so we can expect Europe to take a very

important role related to the issue of organic agriculture in the years to come. Western Europe, or precisely, its four countries: Germany, France, Italy and UK, account for over 75% of the overall regional income. The other countries like Denmark, Sweden or Holland show a constantly high growth but they have smaller markets because of their small retail stores. German market has been growing most rapidly in Europe. Such high market growth is the result of the fact that organic food is widely available in supermarkets, drugstores but also in retail shops. The populations of Switzerland and Scandinavian countries are among the largest consumers of organic food, followed by the Danish, the Swiss and the Austrians. The populations of south, central and south-east Europe take the last place regarding organic food consumption.

Germany has the largest market of organic products in Europe. During 2006 retail sale increased for 18% reaching 4.6 billion euro. The demand for organic products increased faster than the supply: the demand for organic milk in retail sale increased for 35%, for cheese for 70%, frozen vegetables for 60% and yogurt for 50%. There was also higher demand for butter, pastry, fruit juice etc. The sale of cookies, sweets, cakes and other confectionaries increased for more than 100% and thus it represents the highest rate of increase. The retail sale market, which is traditionally insignificant, had the growth rate above the average. Organic supermarkets also had the above average growth of 25% while traditional organic stores lost their market share. Organic retail sale sector in Germany is aimed at increasing interaction with conventional marketing structures. For example, after having researched organic retail market, the national retailer REWE with 3,000 conventional supermarkets all over the country opened two very successful organic supermarkets, with plans for further expansion and capacity enlargement.The market growth of the UK was estimated to 22% in 2006 which represents the highest growth in comparison with the previous years. The retail sale of organic products was estimated to almost 2.8 billion euro (or about 2 billion pounds) in 2006. Sale of organic products by mail or by customer order increased by 53%. Children organic food (baby food) increased for 7%, the organic poultry market has been increasing and the production of organic eggs was larger than "cage" egg production for the first time. 79% meat, 96% milk and eggs and 73% vegetables were produced by domestic producers, but with the demand larger than the supply, the import of organic products increased. The Italian market reached the estimated value of about 2.65 billion euro in 2006. The results of organic food retail sale reached the value of 1.9 billion euro while the export was estimated to 750 million euro. The sale in specialized stores increased by 10%, but it also increased in conventional stores by 7.5%. The sale in processing companies and whole sale stores increased by 55%, while in supermarkets it increased only by 2%. The export of organic products increased by 25.8% in 2006. Retail sale of organic cooking oil, sugar, coffee, tea, bread and confectioneries increased, while the sale of soft drinks, milk products, vegetables, fruit, pastry, baby food and eggs decreased. While the turnover of food in conventional retail sale sector stagnated, organic retail sale sector increased its sale by 15% in Italy in 2006. The largest whole sale trader ECOR opened its own chain of organic supermarkets on 60 locations all over the country. Organic whole sale traders in Italy realize great profit (income) from continual increase of number of children's meals in school cafeterias. In France the value of retail sale market was estimated to 1.7 billion euro and it is still increasing. The number of consumers buying organic products keeps on growing (Willer, Helga et all., 2008, 2009).

The market value of Switzerland was evaluated to 764 million euro in 2006. The

consumers of that country spend averagely 102 euro per capita on organic products more than the consumers from any other European country or any other country in the world (Tab. 2).

Organic products account for 4.5% of the overall food market. In Switzerland there are currently two chains of specialized stores of healthy food. These are MUELLER and EGLI. Other companies, like the supermarket of organic products YARDO and the chain of national cosmetic stores, are still at the beginning of their development. In 2007 three leading trade chains of whole sale merged and established a new company which currently has the market share of about 80% of retail organic trade.

Table 2. European market of organic products – consumption per capita in 2006.
Tabela 2. Evropsko tržište organskih proizvoda – potrošnja po stanovniku u 2006.

Country *Zemlja*	Consumption per capita (€) *Potrošnja po stanovniku (€)*
Switzerland	102
Lichtenstein	86
Denmark	80
Austria	64
Germany	56
UK	47

(Izvor: Willer, Helga et all., 2008)

CONCLUSION

Organic agriculture is developing rapidly and the share of land under organic production is growing in many countries. According to the latest data (from 2009) there are about 32.2 million ha of land under this type of production. The demand for organic products is the largest in North America and in Europe, while Asia, South America and Australia are significant producers and exporters of organic food. According to the data from 2009, land with organic production increased from 7.4 million ha to 7.8 million ha in Europe and the countries with the largest land areas under such a production are still Italy, Spain and Germany.

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EKONOMSKI ASPEKTI RAZVOJA ORGANSKE PROIZVODNJE

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Izvod

Trgovina proizvodima organskog porekla beleži stalni rast. Međunarodne institucije, države i preduzeća mogu značajno uticati na trgovinske tokove organskim proizvodima što predstavlja poseban izazov. Zahtevi potrošača za kvalitetnom i zdrastveno bezbednom hranom biljnog i životinjskog porekla koja je proizvedena u nenarušenoj prirodnoj sredini sve su više izraženi. Poljoprivredna preduzeća se danas susreću sa velikim brojem problema prilikom organizovanja organske proizvodnje.

Predmet izučavanja ovog rada je analiza i poređenje organske proizvodnje u svetu. Prikazan je razvoj svetskog tržišta organski proizvedene hrane, a objašnjen je i kako funkcioniše organski maloprodajni sektor u najrazvijenijim zemljama sveta. Dobit kao motiv koju proizvođači mogu da ostvare prodajom organskih proizvoda je uzdrman zbog postojeće svetske ekonomske krize, koja dodatno povećava finansijski rizik prilikom ulaganja novčanih srestava u organsku proizvodnju.

Ključne reči: organska poljoprivreda, održivi razvoj, organska proizvodnja, organski maloprodajni sektor

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ALLELOPATHIC RELATIONSHIPS BETWEEN SOME WEED SPECIES AND SMALL GRAINS BY GRAIN HARVEST INDEX ASPECT*

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SUMMARY: The aim of this investigation was to study allelopathic relationships between weeds and small grains by grain harvest index (GHI) of small grain in order to provide more information about these complex relationships. The study included small grains (wheat, barley, oat, triticale and rye) and some weed species: Agropyron repens, Cirsium arvense, Sonchus arvensis and Sorghum halepense. Applied extracts of weed species mainly expressed suppressed effect on GHI, but stimulating actions were noticed, too. Positive influence on GHI was registered with Agropyron repens and Sonchus arvensis variant in wheat and with Agropyron repens segments variant in oat.

Key words: *allelopathy, extract, weed, small grains, harvest index.*

INTRODUCTION

Grain harvest index (GHI) presents the ratio between economic (grain yield) and total biological yield (Donald and Hamblin, 1976). It is an important indicator of small grains productivity. The higher value of GHI means better distribution of dry matter between vegetative and generative small grains organs. It means, in fact, better physiological efficiency of genotype in assimilates translocation from stems and leaves into spikes and grains (Đokić, 1988). After positive correlation between GHI and grain yield was found out, this parameter was recommended as a criterion in small grains breeding for genotypes productivity improvement (Mayo, 1987; Sharma et al., 1987; Richards, 2002). Some authors (Richards, 2002) stated that increasing grain yield was obtained owing to grain harvest index increasing, without increasing total biomass. Also, Moradi et al., (2008) set down further that oat and other small grains yield improvement might be achieved by combining high biological yield with high grain harvest index.

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Stressing factors cause decreasing of small grain yield, that can be observed on the basis of its parameters. Studying weeds and small grains relationships only by physical weed presence aspect is partial and incomplete. Many authors observed action of phytogenic substances of one species towards the same or other species by physiological aspect. That chemical reaction between plants, based on metabolites, secreted out from root and other organs or products of plant residuals decomposition, is allelopathy (Gračanin and Ilijanić, 1977; Singh et al., 2003).

Weed allelopathic influences on small grains can be stimulating or inhibitory (Muminović, 1987; Sary et al., 2009). It has been still since the earliest plant growth phases by germination energy and total germination (Ognjanović et al., 1995). The most important allelopathic effect of weed is on small grains yield. Knowing the yield is a complex trait, studying weed and small grains allelopathy by aspect of some indicators and components of productivity therefore could contribute to clearer explanation of that phenomenon. It can be especially useful if investigation is conducted in different conditions of cultivation, fertilization, sowing, crop protection etc. (Johnson et al., 2000).

The allelopathy studying offers new possibilities for sustainable weed management. Qasem and Foy (2001) evaluated and discussed the importance, characteristics, positive and negative impacts and future role of weeds as an integral part of the natural and agroecosystems. Interference between plants in nature and the importance of differentiating between competition and allelopathy are interpreted. The use of allelopathy for controlling weeds could be either through directly utilizing natural allelopathic interactions, particularly of crop plants, or by using allelochemicals as natural herbicides (Singh et al., 2003).

The aim of this investigation was to study allelopathic weed effect on grain harvest index, as an important yield indicator in small grains in order to contribute to more complete investigation allelopathy between weed and small grains.

MATERIAL AND METHOD

The investigation was carried out in vegetative pots in glasshouse of Small Grains Research Center, Kragujevac during 1999/00-2001/02. The following genotypes were included in the trial: wheat cv. Takovčanka, barley cv. Jagodinac, oat cv. Vranac, triticale cv. KG-20 and rye line L-307/4. The sowing was conducted during December, in density 25 seeds per pot. Each sowed pot was watered by each one glass of made extracts in accordance with trial variants.

The influences of the following weed species extracts were explored: *Agropyron repens, Sonchus arvensis, Cirsium arvense* and *Sorghum halepense*. The extracts were made by grinding dried whole plant (with root, in green phase). Thereupon, each 100 g of grinded mass was diluted by 4l water. Such made extracts were left for two days. Besides these, the influence of the segments of *Agropyron repens* was studied by putting 10 buds of that weed in each pot. The trial included control variant without weed, too, watered by clear water and weeding by each 7-10 days. The trial was set up in three replications. During winter period the pots were located in the glasshouse and in vegetative house in spring and summer periods. In the middle of February the number of small grains plants was reduced down to 12 plants per pot and top dressed by 1.5 g NPK fertilizer per pot.

The wheat plants were harvested in phase of wax maturity and dried. Afterwards,

biomass (underground part) and grain yield per pot were measured. The obtained results were elaborated by analysis of variance and showed in tables.

RESULTS AND DISCUSSION

Three years average GHI values for small grains species treated with weed extracts are presented in Table 1.

By analyzing average GHI values (Tab. 1) it can be observed its varying from 28.2% in rye (*Sorghum halepense* var.) to 45.8% in barley (control var.). The highest average values of GHI in all small grains species are recorded in weedout variant.

By analyzing GHI values in investigation years it was observed its varying from 26% in rye (1999/00, *Cirsium arvense* var.) to 47% in barley (2000/01, weedout control). There were extra low values of this parameter even 18% in rye in some trial replications. Almost all extracts of studied weed species caused GHI reduction. There were however the examples of stimulate influences of some weed extracts on grain harvest index in wheat, 2001/02 in *Agropyron repens* and *Sonchus arvensis* var. and in oat in 1999/00 in variant with *Agropyron repens* segments. The *Agropyron repens* extract influence on oat GHI then ranged from neutral to negative. Kojić (1961) reported *Agropyron repens* growing together with oat has not expressed negative influence on oat grain yield, even some improvement in oat grain yield was registered. This weed did not cause unfavorable changing of many other traits, either. In the field conditions,

Table 1. Grain harvest index for various small grains species, average 1999/00-2001/02
Tabela 1. Žetveni indeks zrna kod različitih vrsta strnih žita, prosek 1999/00-2001/02

Weed species/Vrste korova (B)	*Small grains species*/Vrste strnih žita (A)					$\overline{X}$ (B)
	Wheat Pšenica	*Barley* Ječam	*Oat* Ovas	*Triticale* Tritikale	*Rye* Raž	
Control Kontrola	44.2	45.8	43.0	43.6	31.4	41.6
Segments of Agropyron repens Delovi *Agropyron repens*	39.3	41.6	41.7	40.9	29.2	38.5
Extract Agropyron repens Ekstrakt *Agropyron repens*	41.0	38.5	40.5	39.8	31.8	38.3
Extract Cirsium arvense Ekstrak*t Cirsium arvense*	41.6	36.6	37.6	39.1	30.4	37.1
Extract Sorghum halepense Ekstrakt *Sorghum halepense*	41.1	38.9	38.3	39.3	28.2	37.1
Extract Sonchus arvensis Ekstrakt *Sonchus arvensis*	41.8	38.5	38.8	39.0	30.8	37.8
$\overline{X}$ (A)	41.5	40.0	40.0	40.3	30.3	38.4

LSD	A	B	A x B	A – *small grains species*/vrsta strnih žita
0,05	1,9	2,1	4,7	B – *weed species*/vrsta korova
0,01	2,5	2,8	6,2	A x B – *interaction*/interakcija

Chad et al. (2000) concluded that adequate soil moisture can accelerate weed residue decomposition and, so, decrease allelopathy effects. Tepe et al. (2004) noticed more decreasing GHI values in conditions of physical weed presence, varying of 6.0% to 13.6%.

On average for all variants, rye had highly significantly lower GHI (30.3) related

to other small grains. Differences between wheat, barley, oat and triticale were not statistically significant. On average for all small grains species, control variant had statistically highly significantly higher GHI (41.6%) related to all weed extracts variants. There were not statistically significant differences between all weed variants.

Analyzing interaction between small grains species and weed extract variant, only suppressed weed extracts effects on GHI wheat, barley, oat, triticale and rye can be noticed. Except in rye and triticale, GHI decreases in the rest of small grains in almost all variants were significant. Chad et al. (2000), exploring influence of *Sorghum halepense* residuals in soil expressed different (positive or negative) effect on GHI of various wheat cultivars. They emphasized the influence of quickness of weed residuals decomposition on value of this parameter. So, regular soil cultivating, rapid residual ploughing in and due-time wheat sowing instigate residual decomposition and hence make conditions for eliminating of allelopathy between *Sorghum halepense* and wheat as well as other small grains. Ben Hammouda et al. (1995) reported that many factors affect intensity and direction of sorghum allelopathy. In fact, allelopathy is associated with concentration and effect of allelochemicals. The level of phenolics in residue, furthermore, depends on environmental conditions, plant part and development stage. They pointed out that prompt tillage, after harvest, could alleviate allelopathy in field conditions.

The highest GHI reduction was noticed in variant with *Agropyron repens* segment, in wheat and in *Cirsium arvense* variant in barley, oat and triticale. There were not differences in allelopathy between wheat and *Cirsium arvense, Sonchus arvensis* and *Sorghum halepense* almost. Kojić (1961) found out that *Cirsium arvense* extract strongly provokes decreasing of germination and keeping back of wheat root, leading to less values of grain yield and many of its components and indicators.

CONCLUSION

Allelopathy between weeds and small grains species can be investigated by aspect of grain harvest index as an important genotype productivity indicator.

Applied extracts of weed species mainly expressed suppressed effect on GHI and the highest were on extract of *Cirsium arvense* and *Sorghum halepense.* But in separate year stimulating actions were noticed, too. Positive influence, by GHI aspect was registered in *Agropyron repens* and *Sonchus arvensis* variant in wheat and in *Agropyron repens* segments variant in oat.

Barley, among small grains, showed the highest sensitiveness towards weed extracts, while the most unfavorable allelopathy was observed between small grains and *Cirsium arvense* and *Sorghum halepense,* by grain harvest index aspect.

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ALELOPATSKI ODNOSI IZMEĐU NEKIH KOROVSKIH VRSTA I STRNIH ŽITA SA ASPEKTA ŽETVENOG INDEKSA ZRNA

OLIVERA NIKOLIĆ, SNEŽANA ŽIVANOVIĆ – KATIĆ

Izvod

Istraživanje je izvedeno u sudovima u staklari Centra za strna žita u Kragujevcu, tokom 3 godine. Obuhvatalo je po jednu sortu pšenice, ječma, ovsa, tritikalea i raži, a od korovskih vrsta *Agropyron repens, Cirsium arvense, Sonchus arvensis* i *Sorghum halepense*. Cilj istraživanja je bio da se ispita alelopatski uticaji između korova i strnih žita sa aspekta žetveni indeks zrna, kako bi se imalo više rezultata o njihovim složenim

odnosima. Primenjeni ekstrakti korova su uglavnom ispoljili negativan uticaj na žetveni indeks zrna, mada je bilo i stimulativnih efekata. Najveće smanjenje žetvenog indeksa zrna zabeleženo je u varijantama sa *Cirsium arvense* i *Sorghum halepense*. Ječam je među ispitivanim vrstama strnih žita ispoljio najveću osetljivost, sa aspekta ŽIZ, dok je najnepovoljnija alelopatija postojala između strnih žita i korovskih vrsta *Cirsium arvense* i *Sorghum halepense*.

Ključne reči: alelopatija, ekstrakt, korovi, strna žita, žetveni indeks.

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THE APPLICATION OF CONSTRUCTED WETLAND FOR WASTE WATER TREATMENT OF THE NOVO MILOSEVO*

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SUMMARY: The results of the constructed wetland application for treatment of waste waters of the Novo Milosevo settlement show a high degree of efficiency based on the analyzed physico-chemical parameters (suspended matter, ammonium ion, total phosphorus, chloride, and BOD_5). The results presented in this work are concerned with the first year of the wetland operation, and they justify the application of the wetland systems under the natural conditions in our country.

Key words: *waste water, purification, constructed wetlands*

INTRODUCTION

In many places, the water quality has dramatically deteriorated in the last several decades. Increasing amounts of waste waters are being discharged without any pretreatment to the recipients, endangering thus their quality and further usability. Hence, many water bodies reached the state of unsatisfactory quality, which is reflected on the health of humans and whole living world in the environment. The first step in preserving satisfactory quality of water resources is certainly the purification of waste waters. This has to be done as close as possible to the place where the waste waters are generated. Such an approach assumes the building of decentralized, local systems of small capacities, with the accompanying infrastructure.

The European Commission for Water proposed in 1991 the document: Implementation of Council Directive 91/271/EEC of May 1991 Concerning Urban Waste Water Treatment, which serves as the guidelines for treatment of urban waste waters, where the method of WETLANDS is mentioned on equal footing as the approach to be used for small and medium settlements from 500 to 5000 inhabitants. Despite of the fact that none of the national legislative systems imposes the use of any particular method, the practice in many developed countries (England, France, Austria, Germany, Italy, Slov-

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enia, etc.) has shown certain advantages of the application of wetlands (Kadlec, 2004). In our country, the application of this method began in 2004, by putting into operation the wetland system at Glozan (Ratkovic and Belic, 2004). After that, several projects have been completed and several wetland systems were constructed, among them the wetland at Novo Milosevo (Ivković, 2005).

The wetland is located east of the settlement, on the right side of the railway Belgrade-Kikinda. The terrain is horizontal, with the altitude of about 78 m above sea level. Treatment of waste water is carried out in several stages that are by passing it through the following sections: *primary treatment lagoon*, *pre-wetland, primary wetland, secondary wetland,* and *tertiary wetland.* The maximum daily inflow of waste water is 2408 m^3/day, and maximal flow rate 55.74 l/s.

Primary treatment lagoon is the first stage in the waste water treatment, where larger suspended particles are separated, to prevent their potential settling in the filling and pipes. Through an overflow construction and distributing piping system, the waste water from the lagoon comes to the pre-wetland. The lagoon dimensions are 44x44 m, and the maximal depth 6 m. The lagoon volume is about 5000 m^3, and at a maximal load, the water residence time is two days. The water from the lagoon comes to the pre-wetland, with the field dimensions of 30x20 m, filled with fraction No. 4 and equipped with the pipe distributing system. The purpose of this unit, which is covered with common reed, is to separate the suspended particles that have not been separated in the settling lagoon. From here, the collected water is led to the primary wetland with horizontal flow, of an area of 7600 m^2. Here, the water from the distribution pipes of Ø 300 is applied onto the field by the overflow, via the distributive pipes of Ø 90 and 33 m long, which are arranged in the corresponding trenches. The purified water, via a system of half-perforated (drainage) pipes is led to a collecting shaft, located at the end of this wetland section. The overflow from the shaft regulates the water level in the wetland. The next section is the secondary treatment wetland, of an area of about 4000 m^2, filled in with fraction No. 4, and involving vertical flow. From the distribution pipeline, the water is spread over through a system of half-perforated pipes, arranged over the upper zone of the wetland. With the aid of drainage pipes placed at the bottom, the purified water is collected and led to the collection canal with a drainage collector at the end of the cell, and then to the overflow shaft 2. The water level is maintained by adjusting the overflow. The last section of the system is the tertiary wetland. The task of this unit, of an area of about 4000 m^2, is to improve the purification effects. The water quality is brought to a level corresponding to the waste water which can be discharged to a water body, in this case to the Canal M-4-4, that is the Kikinda Canal, as the end recipient.

MATERIAL AND METHOD

Studies encompassed three series of samples taken in September, October and November of 2008. Samples of the waste water were taken at the particular treatment objects, at four measurement points: Measurement point 1 – the lagoon for primary treatment; Measurement point 2 – the shaft at the inflow to the primary wetland; Measurement point 3 – the shaft at the inflow to the secondary wetland; Measurement point 4 – the shaft at the outflow from the secondary wetland.

In view of the fact that the wetland system was still in the founding phase, its last section (tertiary wetland) had not attained yet its full functioning at the time of sam-

pling, so that it was not possible to take water sample at the planned measuring point 5 – the shaft at the outflow from the tertiary wetland.

The purification degree of the waste water was assessed on the basis of the analysis of selected parameters measured in the autumn period of 2008, and this was the first evaluation of the operation efficiency of the constructed wetland at Novo Milosevo. The analysis encompassed the following parameters: suspended matter, electric conductivity, ammonium ion, total phosphorus, chloride, and BOD_5. Table 1 shows the overall effect of treatment of waste waters from the Novo Milosevo settlement, based on the above parameters.

RESULTS AND DISCUSSION

Effects of the treatment – September 2008

Suspended matter content at the inflow to the wetland system, measurement point 1 (lagoon for primary treatment) was 80 mg/l. After passing through the pre-wetland, the concentration was 100 mg/l; after the primary wetland it was 20 mg/l, and after the secondary wetland 77 mg/l. The reduction achieved in the primary wetland was 80.0 %. **Electric conductivity** at the inflow to the system (lagoon for primary treatment) was 1724 μS/cm; after passing through the pre-wetland it decreased to 1710 μS/cm; at the outflow from the primary wetland it was 1800 μS/cm, and the lowest value of 1652 μS/cm was measured after passing the secondary wetland. The **ammonium ion** concentration at the inflow was 56.2 mg/l, and at the measurement point 4, 37.3 mg/l. Therefore, the treatment lowered this parameter by 33.6 %. The **total phosphorus** at the inflow was 13.58 mg/l, and at the measurement point 4, 10.35 mg/l, so that the decrease was 23.8 %. The **chloride** concentration at the inflow to the system was 95.6 mg/l, and at the outflow (measurement point 4) 91.5 mg/l, the reduction being 4.3 %. The **BOD_5** measured at the inflow to the system was 375.0 mg/l, and at the measurement point 4, 49.5 mg/l, the corresponding decrease being 86.8 %. The flow through all the system units (pre-wetland, primary and secondary wetlands) ensured essential reduction of the organic load (Figure 1).

Effects of the treatment – October 2008

Suspended matter content at the inflow to the wetland system (measurement point 1) was 180 mg/l; after passing through the pre-wetland it was 150 mg/l; after the primary wetland it was 340 mg/l, and after the secondary wetland 48 mg/l. Therefore the reduction in the content was achieved in the pre-wetland and secondary wetland. The **electrical conductivity** showed an increase in the course of passing through the wetland system. The value measured at the inflow to the system was 1730 μS/cm; after passing through the pre-wetland it was 1738 μS/cm; at the outflow from the primary wetland it was 1804 μS/cm, and after the secondary wetland 1810 μS/cm. The **ammonium ion** concentration at the inflow was 56.5 mg/l, and at the outflow (measurement point 4) 49.8 mg/l, the decrease being 11.9 %. At the outflow from the pre-wetland the ammonium content was lowered to 43.9 mg/l; after the primary wetland it increased to 54.0 mg/l, to fall to 49.8 mg/l after passing the secondary wetland. **Total phosphorus** concentration at the system inflow was 13.5 mg/l, while at the measurement point 4, 11.0 mg/l, and the reduction being 11.9 %. The decrease in the total phosphorus content was observed as the result of the treatment in the pre-wetland, while in the other sections its increase was observed. No reduction in **chloride** content was observed since the value

measured at the inflow to the system was 86.1 mg/l, and at the measurement point 4, 100 mg/l. A lower chloride concentration (61.2 mg/l) was measured for the measurement point 2. The **BOD_5** value measured at the inflow to the system was 394.0 mg/l and at the measurement point 4, 59.5 mg/l, so that the reduction was 84.9 %. The decrease in the organic load was observed for each unit of the wetland system (Figure 1).

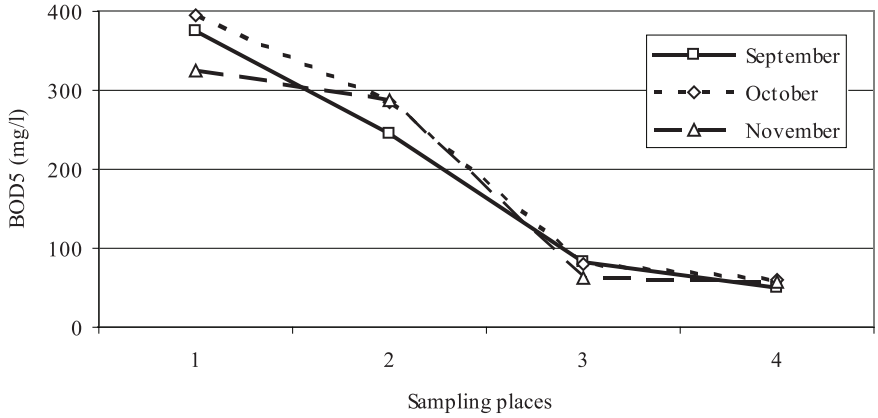

Figure 1. Values of BOD_5 measured in the autumn of 2008
Slika 1. Vrednosti BPK_5 izmerene tokom jeseni 2008. godine

Effects of the treatment – November 2008

Suspended matter content at the inflow to the wetland system (measurement point 1) was 110 mg/l; after passing through the pre-wetland it was 190 mg/l; after the primary wetland it was 10 mg/l, and after the secondary wetland it was 130 mg/l. The reduction achieved in the primary wetland was 94.0 %. The **electric conductivity** measured at the inflow to the system was 1785 μS/cm; after passing the pre-wetland it fell to 1757 μS/cm; at the outflow from the primary wetland it was 1872 μS/cm, and after the secondary wetland it was 1817 μS/cm. The concentration of **ammonium ion** at the inflow was 59.8 mg/l and at the measurement point 4, 32.6 mg/l, the reduction being 45.5 %. At the outflow from the pre-wetland the ammonium ion concentration was increased to 62.2 mg/l, the reduction being observed in the primary and secondary wetlands. **Total phosphorus** concentration at the inflow was 16.00 mg/l, while at the measurement point 4 it was 14.95 mg/l, the reduction being 6.6 %. The decrease in phosphorus content was observed for the pre-wetland and primary wetland, whereas at the outflow from the secondary wetland its concentration was increased. There was no reduction in the chloride content since the value measured at the inflow to the system was 89.5 and at the other measurement points 93.5 mg/l. The **BOD_5** value measured at the inflow was 324.0 mg/l, while at the measurement point 4 it was 57.5 mg/l, the observed decrease being 82.2 %. The decrease in the organic load was observed for each section of the wetland system (Figure 1).

The first study of the constructed wetland at the Novo Milosevo settlement showed a relatively high efficiency of removal of organic pollutants (Table 1). The reduction was also observed in another two parameters, ammonium ion and total phosphorus. However, the reduction effects were not achieved in regard of the contents of chloride and suspended matter.

Table 1. Summarized efficiency of waste water treatment on Novo Milosevo constructed wetland (%) according to analyzed parameters during autumn 2008.
Tabela 1. Ukupni efekti prečišćavanja otpadnih voda naselja Novo Miloševo na mokrom polju (%) na osnovu analiziranih parametara u jesenjem periodu 2008.

Parameter / *Parametar*	September / *Septembar*	October / *Oktobar*	November / *Novembar*
Suspended matter / *Suspendovane materije*	3.7	73.3	+
Electric conductivity / *Elektroprovodljivost*	4.2	+	+
Ammonium ion / *Amonijum jon*	33.6	11.9	45.5
Total phosphorus / *Ukupan fosfor*	23.8	18.5	6.6
Chloride / *Hloridi*	4.3	+	+
BOD5 / *BPK5*	86.8	84.9	82.2

+ the parameter had a higher value at the wetland outflow

CONCLUSION

Under the conditions of the lack of plants for waste water treatment, surface waters in our country are most frequent recipients of pollutants originated from households, industry, and agriculture. In the recent years, the application of simple and economically feasible wetland systems for waste water treatment appear to be promising solutions in the protection of water courses and accumulations. Wetlands make a link between aquatic systems that are exposed to the pollution risk and terrestrial systems that are sources of polluting agents. They also make part of the hydrological cycle because the waste waters after treatment may be reused. By their nature, these systems assume the decentralization and engagement of local authorities, aiming at the, so actual nowadays, sustainable development (Belić, 2007). Aqueous macrophits are the basic part of wetland systems because of their multiple functions that contribute to the efficient treatment of waste waters. In addition to the physical and chemical effects that they have in water treatment, these plants contribute to the esthetic aspects of the overall system, giving to it a natural and pleasant outlook (Belić and Josimov-Dunđerski, 2007).

This article presented the first results obtained for the wetland constructed with the purpose to treat waste waters from the Novo Milosevo settlement. By analyzing the change in the particular parameters at the inflow and outflow of the system's sections it was possible to get an insight into the effect of waste water treatment, as well as in the state of the effluent before its discharge to the recipient. Generally, it may be concluded that the results demonstrate the reliability of the wetland. The Novo Milosevo wetland does not work in its full capacity yet, because not all households are connected to the sewerage system, and the reed bed is still poor and non-uniform. Despite of this, the data show high percentage of reduction of organic load, ammonium ion, total phosphorus, which is in agreement with the findings reported for wetlands in developed countries. The non-uniform values of some parameters at the outflow suggest the need

for a further study of this issue. Only continuous monitoring may provide answers to the pertaining problems and serve as the basis for the improvement of the wetland functioning.

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PRIMENA MOKROG POLJA ZA PREČIŠĆAVANJE OTPADNIH VODA NOVOG MILOŠEVA

JASMINA JOSIMOV–DUNĐERSKI, ANĐELKA BELIĆ, MILICA RAJIĆ

Izvod

Rezultati primene mokrog polja za prečišćavanje otpadnih voda naselja Novo Miloševo ukazuju na visok stepen efikasnosti kada su u pitanju pojedini fizičko-hemijski parametri. Na osnovu vrednosti analiziranih parametara (suspendovane materije, elektroprovodljivost, amonijum jon, ukupni fosfor, hloridi i BPK_5) može se zaključiti da sistem mokrih polja uspešno prečišćava otpadne vode naselja. Rezultati prikazani u ovom radu odnose se na prvu godinu primene mokrog polja u Novom Miloševu i oni opravdavaju primenu sistema mokrih polja u prirodnim uslovima koji vladaju u našoj zemlji.

Ključne reči: otpadne vode, prečišćavanje, mokra polja

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PHEASANT REPRODUCTION IN OPEN HUNTING GROUNDS

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SUMMARY: Success percent in natural reproduction of pheasant is 64%. In 50 nests observed, 533 eggs were noted. Average egg number per nest was 11.52; minimum found was 6, and maximum was 21 eggs per nest. Total of 356 one-day chicks hatched (or 64.38%), which is 7.12 chicks per nest, or 11.13 one-day pheasant chicks per successful nest. From 50 nests observed, 44 were the first, 4 the second and 2 the third one, or 88%, 8% and 4%, respectively. Egg-laying period in first nest was between March 18 and May 12. Hatching period for first nests was between May 8 and 15. In 2009, from 25 nests, 14 have been destroyed (56%). From those, 2 were destroyed by unknown doers (14.29%), 5 by magpies (35,71%), 2 by badgers (14.29%), 4 by mowers (28.57%) and 1 by high waters (7.14%). From 291 eggs found in all nests, 127 chicks havehatched (or 43.64%). Hatching percent is lower by over 20% in 2009 as compared to average from three previous years. This may be explained by a fact that in 2009 much more nests were destroyed (56%) than in three previous years (36%), which points that all years are not the same and may not be brought to average. In over 90% cases, pheasant hens have made their nests in meadow grass, in meadow channels, in alfalfa fields, in dense nettle, blackberry and grass, grass and reed, grass and creeping plants, and sometimes in orchards, dense thicket and wheat.

Key words: *nest, pheasant hen, reproduction, hunting grounds*

INTRODUCTION

Since pheasant behavior is different in various habitats, depending on whether they have hatched in nature or in incubators, some authors treat this bird as a semi-domesticated game species, which is not adapted enough to environment conditions and is not able to avoid predators or climate risks. Nevertheless, such statements may be valid only for those pheasants that are artificially bred, since observations of their lifestyle in natural conditions show entirely different results. They are very cautious and timid,

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Original scientific paper / *Originalni naučni rad*

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except in mating period, and because they are able to hide and to run excellently, they have little need for flying which would expose them to foes.

Significant ecological plasticity of pheasant enabled it to acclimatize widely in Western Europe and USA. This bird populates mostly agricultural areas, and its density is sometimes even higher than in their natural habitats. It prefers wet, irrigated terrains if they are rich in food and if there are adequate shelters.

Aim of this investigation was to establish, using reproduction observation in nature, how many pheasant hens brings their eggs to hatching, how many nests are destroyed during egg-laying period and which are causes for nest destruction. Method used in such observations was to find and mark nests, and then to observe until hatching or destruction of laid eggs. Results of the paper were obtained by observation of nests in hunting grounds, with daily notes; in such manner exact period of beginning and the end of pheasant game reproduction was noted, percent of successful hatching in total number of nests observed and percent of chicks hatched in successful nests calculated, or from total number of eggs in such nests.

LITERATURE REVIEW (EGG-LAYING CAPACITY IN NATURAL CONDITIONS)

Day or two after laying the last egg, pheasant hen sits on the nest. At first it is restless, but after 14 days becomes unrelenting and leaves nest only in emergency. Chicks are hatched in 24 days.

A group of Swedish scientists (Grahn M, Goranssen G, van Shantz T, Wittzell H, 1996) have used radiotransmitters to find out where pheasant hens are building their nests, and on the basis of size and weight of eggs they calculated exact date of hatching. This data have no practical importance, since chicks stay in their nest only one single day after hatching (Ristić, 2005).

Apart of weather conditions, bird and mammal predators have large influence on pheasant reproduction in hunting grounds.

According to results of Sekera (1959), in June when daily temperature is below 10°C and precipitation above 80 mm, with minimal insolation, pheasant chicks mortality reaches even up to 80%, and if temperatures are above 20° C and precipitation below 50 mm, even 90% of newly hatched pheasant chicks will probably survive.

In their investigations, Hanuš and Fišer (1975) found that annual natural losses, including hunting, range about 75% from total number of pheasant population.

Natural potential of pheasant reproduction is closely connected to locality and environment factors that have direct or indirect influence on egg-laying capacity, i.e. on number of eggs laid during nesting season. By investigations of Pekić (1969), hens lay 10 - 16 eggs in average during 20 to 30 days. The egg-laying occurs in nature from late March or early April, and every cold spell in this or previous period may cause postponing of laying, which strongly shortens the reproduction period.

According to Allen (1956), pheasant nesting in Midwest in 1950 begun 3 weeks later than in 1949 and 1951, when weather conditions were more favorable (Pekić, 1969). In Pennsylvania, Randall (1940) found the first egg on April 6th, and in Iowa ten days earlier. In Hawaii, pheasant hens begin to lay eggs in late February, just as in Sicily. Early April nests, according to Homerstrom (1936), have 12 to 13 eggs in average, and only 8.5 eggs in second egg-laying in July. Most authors agree that pheasant hens begin

nesting when grass is tall enough to protect them from predators. Therefore, factors preventing normal growth of herbaceous vegetation will considerably influence beginning of egg-laying of this game species (Pekić, 1969).

The lowest breeding potential with 6 eggs in average per hen was noted in 1956 and 1958 (Pekić, 1965). In these years, winters were long and harsh, and snow cover lasted 34 days in 1958 and 49 days in 1956. Late snow melting caused delays in development of herbaceous plants, which is important stimulus for pheasant hens to lay the first eggs and hence for successful nesting. After long winter, rainy spring followed, so that in April, May and June of 1958 there was 28, and in 1956 even 43 rainy days. Minimal temperatures were -4°C between April 8 and 12 in 1956, and about -2°C in the same period in 1958.

Precipitation and low temperatures in breeding season have negative influence not only to game activity, but also on average number of eggs laid in belated nests. By experimental verification (Pekić, 1960-62), it was found that egg-laying capacity of pheasant hens was decreased for about 50% when temperatures were below 10°C. Especially harmful were harsh oscillations between maximal and minimal daily temperatures, so hens made several days' pause between laying one egg and another.

Besides temperatures and precipitation, breeding potential of pheasant game was influenced also by active day length, closely connected to daylight duration and percent of relative insolation. A large number of rainy and cloudy days during nesting periods in 1956 and 1958 caused later getting up and earlier going in of pheasants, which effectively cut down their active daytime when all activities connected to nesting, feeding and other needs occur. According to Dustman (1950), most of pheasant nests were found in areas with 2.5 inch precipitation and 67% insolation. In Dobanovački zabran, during whole breeding season 1956, insolation never reached even 45%, and when in April 1958 it was 85%, 87 mm precipitation fell – mostly in torrential rain showers with daily maximum of 19 mm. Since this was happening at the beginning of breeding season, first eggs were not covered yet so were exposed to devastation. Pheasant hens were forced to start nesting again, so these second, much smaller nests extended reproduction period even to July, when high daily temperatures have negative influence on incubation process. In this way, combined effect of ecological factors as active daytime length, light regime, humidity, temperature and diet determines not only beginning of egg-laying but also the amount of realized natural breeding potential of pheasant game.

Although these five factors always work jointly, their summary effect may differ in certain seasons. If precipitation and cloudiness shorten active daytime, birds will not have time to feed, since their food consists of insects that will also seek shelter, and then to do all activities connected to breeding, nesting and other motions. In the next year, at the same time, higher insolation level will have more favorable effect on temperature, daytime activities and other factors that define egg-laying capacity of pheasant game in breeding season. "Active daytime" here understands day length between morning and landing from trees and up to evening end of activity when birds end their daily movements. Due to diffuse light, pheasant may wake up 1 to 2 hours before sunrise and end their daily activities about 20 minutes after Sun is completely below the horizon. In effect, pheasant activity is much longer than daytime insolation in the same period, so their active day in December and January lasts 10,40 hours, in November and February 11,30 hours, in March and October 13 to 13,20 hours, in May 16 and in June more than 17 hours.

Pheasant is often cited as an example of fast breeding in nature, which is not the case even to those species that live in a certain area for thousands of years.

An experiment has been conducted at a protected island off coast Washington DC, where 6 females and 2 males of ring-necked pheasant were released. This island is rather far from the mainland so the pheasants were completely isolated in natural conditions. In six years, populations grew from 8 to almost 2000 pheasants, so total number was 250 times more than at the beginning. Human influence on the island was prevented, and number of predators was only partially controlled (Ristić, 2005).

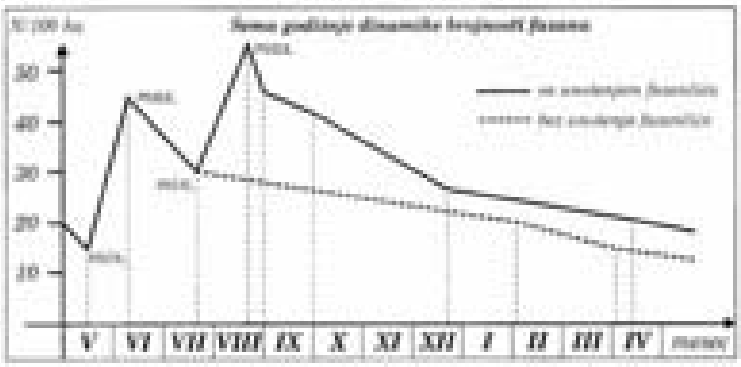
Another experiment has been conducted in Oregon, but there total increase of pheasant population was only 11.7% from growth on an isolated island near Washington DC. This difference was mostly ascribed to human influences.

A similar experiment could have been organized in Bjelopavlićka ravnica near Danilovgrad, in 1952-1958 period. The plain itself is bordered by mountain ranges that are excellent natural barrier, keeping pheasants from dispersing further. In 1952, 60 females and 14 males of ring-necked pheasant were introduced, and in spring of 1958 their number was established ad approximately 5000 specimens. Pheasants were feeding from natural sources in all seasons, and predators were partially reduced. The plain is crisscrossed by underbrush, hedges, fields and orchards (Ristić, 2005).

From all these it may be concluded that we must know ecology and a way of life for every game species (in this case a pheasant) but this is, sadly, not always a case.

In investigations by Pekić (1960), it was established that due to poor whether conditions about 10% of pheasant chicks in experimental nests died before hatching. Lengthy rains prevented mowing valleys and clearings where same belated nests were located. During the mowing, by carelessness, either by scaring off pheasant hen or by crushing eggs, about 8% of growth increase was destroyed. By hale, rains, storms and colds or parasites due to weather conditions another 16% of surviving chicks also died. Birds of prey destroyed 11% of youngsters, and foxes, wild cats, skunks and other mammal predators were responsible for deaths of another 19% of pheasants. Later, during a hunting season (in winter), 9% of pheasants were caught, which means that only 28% of chicks lived longer than a year. Majority of pheasant chicks died before they were 2 months old. In older specimens, percent of mortality is considerably lower.

Annual dynamics of population number in pheasants is influenced by biological (reproductive) traits of the species, and by environment factors (including human) on the population.



Graph 1. Annual dynamics of population number for pheasant with introduction (_____) and without introduction (------) of pheasant chicks

Grafikon 1. Šema godišnje dinamike brojnosti fazana sa unošenjem (_____) i bez unošenja fazana (------)

Pheasant breeding begins in mid-March and in our climate lasts until late May. The female starts to look, usually at early April, for a nesting spot and makes slight hollow in soil, covers it with a grass and starts to lay eggs in it. One pheasant hen usually lays 8 to 16 eggs and two days after laying the last one it starts to sit on them. After 24 days, chicks are hatched. Percent of fertilized eggs and hatched chicks is very high in nature, even up to 96%, so in mid-May, when most hens start bringing out their chicks, number of population abruptly rises to its maximum (until July 15), but it also suddenly plummets since newly hatched chicks are viable to high losses due to a number of causes (much rain, predators etc). Number falls until middle of summer, when artificially bred chicks are introduced to hunting grounds, so the number rises again. Due to losses that are present during introduction of pheasant chicks (ranging between 10 and 60% in the same shelter), and to further losses of chicks hatched in nature, population number steadily decreases. From the beginning of a hunting season, which in most European countries starts in October and lasts until middle or end of January, number suddenly drops because adult specimens are being caught, and also continues to decrease after a hunting season due to harsh winter conditions (winter losses) until the moment when newly hatched chicks appear to surpass total number of lost individuals. Therefore, lower "peak" of population number is in the first third of hatching period, around mid-May (Ristić, 2004).

After investigations by Scott A. Smith, Nancy J. Stewart, J. Edward Gates (1999), in period between January 4th and May 15th 1988, we caught 16 female and 10 male pheasants and equipped them with radiotransmitters. Then we monitored their movements in agricultural fields of about 2002 hectares until they died and radiotransmitters ceased to work. From 16 pheasant hens with radiotransmitters, 8 survived until nesting period. Of 10 male pheasants, 7 survived until nesting period. Predators are responsible for 11 deaths (61%), namely foxes *(Vulpes vulpes, Urocyon cinereoargenteus)* with 5 (28%) and birds of prey, including *Bubo virginianus* with 6 (33%). Foxes attacked most often in bushes (5), and other predators killed pheasants in bush (4), agricultural crops (1) and in forest (1). Vehicles killed 2 (11%) pheasants, 3 (17%) were caught and other 2 (11%) died by unknown causes. Much more pheasants died in high and dense vegetation

than in open terrain.

During a nesting season, females choose habitats as hay or wheat fields, pastures, unkempt roadsides, old agricultural fields. In Great Britain, male territory is most dense in bush along forest borders, bordering to cultivated areas.

According to research by Richard A. Schmitz and William R. Clark Source (1988) hand-reared pheasant hens are more often to be killed by fox than wild ones. Condition differences may not be counted as a cause, since in February and March there is not much difference in weight between those two groups. Hand-reared pheasants are often more disturbed by wild birds and they often do not succeed to take part in breeding. Wild pheasants had more success in this investigation due to their social dominance.

Increased vulnerability of hand-reared pheasants is connected to lack of experience with predators at younger age.

Mortality by mammal predators is 66.7%, mostly by foxes, then bird predators 20.8% and unknown reasons 12.5% (Richard A. Schmitz and William R. Clark Source, 1999).

MATERIAL AND METHODS

Observation of pheasant game reproduction in hunting grounds begins with marking the nests by grounds keeper, and afterwards nests are daily visited and behavior of pheasant hen is observed. Observation encompasses following parameters: date when egg-laying begun, nest location, date of last egg laid, number of eggs in a nest, predator attacks on nest or destruction of nest by mowers and other causes, whether there was any damage to nest, date of hatching, number of pheasant chicks hatched, number of non-hatched eggs, number of nest – first or second one... Grounds keepers duly noted every change and kept notes. Investigations were carried out in hunting grounds of hunters' societies in Bečej, Nova Crnja and Novi Bečej.

RESULTS AND DISCUSSION

Every one who looks deeper into pheasant's way of life, its natural diet and other important factors, especially in connection to pheasant reproduction in nature conditions, may see that this bird may live completely free in all parts where basic conditions are present and where are no humans as predators. It is not the bird of closed pens but a real game, if it has the chance.

Observations of pheasant reproduction in our hunting grounds in 2008-2010 period, by Ristić, had the following results:

- total of 50 nests were observed during three years,
- successful were 32 nests or 64%,
- average egg count per nest observed was 11.52, (in 2008 average egg count per nest was 6 - 14; in 2009 8 - 21 eggs and in 2010 7 - 16 eggs),
- total number of eggs in all nests was 553 (in 2008 - 8 nests and 79 eggs; in 2009 - 25 nests and 291 eggs; in 2010 - 15 nests and 183 eggs),
- total number of chicks hatched was 356 or 64.38% (in 2008 - 67, in 2009 - 127 and in 2010 – 162 chicks),
- 44 first nests were observed, 4 second ones and 2 third ones,

- in first nests in 2008 egg-laying period was between 4 and 30 April, in 2009 between March 18 and April 1, and in 2010 between April 8 and May 12,
- hatching of pheasant chicks in hunting ground was noted in 2008 between May 8 and 15, in 2009 between May 1 and 8, in 2010 between May 15 and June 10.

Based on these observations, d ifferent influence of negative factors was established in destroying nests and eggs in 2009.

From 25 nests observed, 11 were successful (or 44%), and from unsuccessful (destroyed) 14 nests, causes of destruction were:

1) two nests (8,00%) destroyed by unknown doers,
2) in these nests 21 eggs were destroyed (7.22% from 291 eggs evidenced in all nests),
3) magpies destroyed 5 nests or 20% with 53 eggs or 18.21%,
4) badgers destroyed 2 nests or 8% with 29 eggs or 9.97%,
5) mowers destroyed 4 nests or 16% with 48 eggs or 16.49%,
6) high waters destroyed one nest or 4% with 7 eggs or 2.41%,
7) total number of hatched eggs was 127 or 43.64%.

According to investigations by Jovetić (1966), magpies and crows destroy in average 49.30% eggs of pheasant game, and 36.66% of partridge. Investigations of Ristić (for 1983-1987 period) found that magpies and crows destroy 49.95% of pheasant eggs and 50.16% of partridge eggs (Ristić, 1996, 2007).

Based on these facts, it may be concluded that success rate of natural breeding was considerable, even 64%, and that in 50 nests total of 553 eggs was found, with good average egg count per nest of 11.52; minimal number was 6 and maximal 21 eggs per nest. From these eggs, 356 pheasant chicks hatched (64.38%), which is in average 7.12 chicks per nest or 11.13 per successful nest. From all 50 evidenced nests 44 (88%) was the first nest, 4 (8%) the second one and 2 (4%) the third one. For first nest, egg-laying period was between March 18 and May 12. Hatching period in first nest was between May 8 and 15.

In 2009, 14 of 25 nests were destroyed (56%). From those destroyed, 2 were destroyed by unknown doers (14.29%), 5 by magpies (35.71%), 2 by badgers (14.29%), 4 by mowers (28.57%) and 1 by high waters (7.14%). From 291 eggs found in all nests, 127 hatched (43.64%). Hatching percent was for more than 20% lower than in all three years together. This may be explained by the fact that much more nests were destroyed in 2009 (56%) than in all three years together (36%). Pheasant hens have made their nests in meadow grass, in meadow channels, in alfalfa fields, in dense nettle, blackberry and grass, grass and reed, grass and creeping plants, and sometimes in orchards, dense thicket and wheat.

CONCLUSION

In order to enable successful breeding of pheasant game in our hunting grounds, it has to have adequate conditions provided by building annual or permanent shrubbery shelters. In habitats where pheasant is present, it must have peaceful conditions. Number of predators (both bird and mammal) must be restricted, and this is especially important in pheasant breeding period (March-June).

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REPRODUKCIJA FAZANA U OTVORENIM LOVIŠTIMA

ZORAN A. RISTIĆ, BRANKO RISTANOVIĆ, MILOSAVA MATEJEVIĆ, TANJA ARMENSKI, TATJANA JOSIN

Izvod

Procenat uspešnosti prirodne reprodukcije kod fazana iznosi 64%. U 50 osmotrenih gnezda evidentirano je 553 jaja. Prosečan broj jaja po gnezdu iznosi 11,52; s nađenim minimumom od 6 i maksimumom od 21 jajeta po gnezdu. Izleženo je ukupno 356 jednodnevnih fazančića (što je 64,38%), a to je u proseku 7,12 fazančeta po gnezdu, ili 11,13 jednodnevnih fazačića po uspelom gnezdu. Od ukupno 50 evidentiranih gnezda u prvom leglu je bilo 44, šro iznosi 88%, u drugom 4 ili 8%, i u trećem 2 ili 4%. Period nošenja u prvom leglu se odvijao od 18. marta do 12. maja. Period piljenja prvog legla se odvijao između 8. i 15. maja.

U 2009. godini evidentno je da je od 25 gnezda stradalo 14 gnezda (56%). Od 14 stradalih gnezda, 2 su stradala od nepoznatih počinilaca (14,29%), 5 od napada svraka

(35,71%), 2 od napada jazavaca (14,29%), 4 od kosačica (28,57%) i 1 od visokog vodostaja (7,14%). Od 291 jajeta koje je nađeno u svim gnezdima ispililo se 127 pilića (što je 43,64%). Procenat piljenja manji je za preko 20% u 2009. godini, u odnosu na prosek za posmatrane tri godine. Ovo se objašnjava činjenicom što je mnogo veći broj stradalih (56%) gnezda u 2009. godini, nego što je to za tri godine (36%), a to nam dokazuje da se ne može poistovećivati i uprosečavati svaka godina. Fazanske koke su gnezda „pravile" u preko 90% slučajeva u: livadskoj travi, livadskoj travi u škarpi kanala, lucerištima, gustoj koprivi, kupini i travi, travi i tršćaku, travi i puzavici, i jedan manji broj u voćnjaku, gustom šiblju i pšenici.

Ključne reči: gnezdo, fazanka, reprodukcija, lovište.

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THE BLOOD UREA CONTENT IN DAIRY COWS DEPENDING ON BREEDING LOCATION AND NUTRITION

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SUMMARY: Urea is a liver product from ammonia and carbon dioxide. It is a product of catabolism from different compounds. Ammonia created during the amino acid dissemination process in the extra hepatic tissue is transported to the liver in a different form. The most frequent forms are: glutamine and alanine. Urea is created from these compounds in liver cells. The dairy cows were fed extensively and had lower milk yield and better reproductive characteristics than the dairy cows fed intensively with good balanced diet. Blood samples of dairy cows have been taken from two locations: Mrkonjic Grad and Gradiska. Dairy cows from Mrkonic Grad location were fed with the diet based on fodder. The urea blood content of these cows was within the normal physiological range. On the other hand, the urea blood content of the cows from Gradiska location was above the normal physiological limit. According to the phase of dairy cow production, the lowest urea concentration was in the dry period. Urea concentration was above the physiological limit in three phases of lactation. There was a very high urea content in the summer nutrition period. The data showed that there were statistical differences in the urea content depending on the locations. The statistical differences between the dairy cow production phases and the interaction of both the location and production phases were not noticed. In the summer nutrition period there was a higher urea content at the both locations. The differences between the urea blood content depending on location, nutrition period and interaction are both statistically significant.

Key words: *dairy cow, urea, blood, production phase, nutrition period*

INTRODUCTION

It has been known for a long time that amino acid dissemination process occurs during the degradation of the food raw proteins inside the rumino-reticular area, while the ammonia is set free. Paunch microorganisms use ammonia for the synthesis of the amino acids and proteins. It happens only when there are enough digestive carbohy-

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drates, i.e. energy. The excess of the toxic ammonia in the liver turns into the urea, then liver urea goes into the blood and other body liquids (milk, and so on). According to Symonds et al. (1981), cow liver can successfully remove harmful ammonia to the concentration of 12 g of NH_3-N·h^{-1}. Cow poisoning happens at the concentration of 23g of NH_3-N·h^{-1}. This is manifested by shivering, neck attack, high salivation, tetanium, tympanites and breathing problems. If ammonia reaches the concentration of 2-4 mg/100mL in the peripheral circulation, the animal can die. Physiological limits of the blood urea content in dairy cows that give average amount of milk are from 3.00 to 6.00 mmol/l. The aim of this paper was to examine blood urea content in dairy cows from two locations: Mrkonjic Grad and Gradiska. The reason for this is different nutrition in these locations. Cows from Mrkonjic Grad location were fed with the diet based on fodder with the minimal addition of fodder concentrate. Cows from Gradiska location were fed with the diet based on fodder as well, but with the sufficient amount of the fodder concentrate.

MATERIAL AND METHODS

Blood samples were taken randomly from the Simmental dairy cows whose average milk production in lactation is 4.500 kg. Blood samples of ten cows were taken in two locations, in Mrkonjic Grad and Gradiska, during four producing phases of animals: 1/3, 2/3, 3/3 lactations and dry phases which correspond to the winter, spring and autumn nutrition period. Analysis of the blood urea content was carried out by the standard method of the Faculty of the Veterinarian Medicine of the University of Belgrade. Data were processed by the schemata of the factorial experiment 2 × 4, whereas one of the factors was location (a), (Mrkonjic Grad, a_1 and Gradiska, a_2). The second factor was production phase (1/3, b_1; 2/3,b_2; 3/3, b_3; and a dry period b_4) in one schemata of the data processing. On the other one (b), the factor was a nutrition season: winter (b_1), spring (b_2), summer (b_3) and autumn (b_4).

RESULTS AND DISCUSSION

In Table 1 there are the data of the average blood urea content in dairy cows depending on the location of blood sampling and the production phase.

Table 1. The average blood urea content in dairy cows depending on location and production phase, mmol/l

Tabela 1. Prosječan sadržaj ureje u krvi mliječnih krava zavisno od lokaliteta i faze proizvodnje, mmol/l

Location	Production phase	Average value for factor b	Average value for factor a
Mrkonjic Grad	1/3 lactation	4.81	4.57
	2/3 lactation	4.87	
	3/3 lactation	4.68	
	Dry period	3.91	
Gradiska	1/3 lactation	6.20	5.87
	2/3 lactation	6.31	
	3/3 lactation	6.18	
	Dry period	4.71	

Data in the table show that blood urea content in dairy cows is different because of the location and the production phase. In the blood samples of dairy cows in Mrkonjic Grad location, there is a lower urea content than in the blood samples of dairy cows in Gradiska location. Considering production phases, the lowest blood urea content is observed in the dry period at the both locations. The other three production phases are characterized by the balanced urea blood content at the both locations, but there was a higher blood urea content in dairy cows from Gradiska location during these phases. The average blood urea content from Mrkonjic Grad research location, as well as in production phases, is within the acceptable physiological range. Unlike the location of Mrkonjic Grad, the blood urea content in dairy cows from Gradiska location is not within the acceptable physiological range considering the production phases. The samples taken from the first, second and third lactation phase showed higher urea content than it is allowed. The reason for this is the nutrition process. The amount of synthesis of microbiological proteins depends on the amount and speed of synchronizing the fermentation of the consumed organic material and fermentation of the nutrition proteins (Grbesa, 1993). The differences of the blood urea content in dairy cows, depending on location of the blood sampling and production phases, are compared with the variance analysis, the results of which are in Table 2.

Table 2. Variance analysis of urea content in blood of dairy cows depending on location and production phase.
Tabela 2. Analiza varijanse o sadržaju ureje u krvi mliječnih krava zavisno od lokaliteta i fazi proizvodnje

Variation sources	F – cal.	F – tab.	
		0.05	0.01
Treatments	1.34	2.12	2.90
A	5.47*	3.97	7.00
B	1.27	2.73	4.00
AB	0.09	2.73	4.00

Results of the variance analysis indicate that there is a statistically significant difference of blood urea content in dairy cows between the locations where the samples are taken. The nutrition processes in these researched locations can explain the difference. The difference of the urea content on the levels of the production phases is not huge, nor is the interaction of the researched factors, i.e. locations and production phases. According to numerous researches the correlation between urea content in blood and milk is high and positive (Refsdal, 1983; Rook and Thomas, 1985; Roseler et al, 1993; Butler et al, 1996; Broderick and Clayton, 1997; Rodriguez and all, 1997; Campanile and all, 1998; Wittwer and all, 1999). Therefore, urea content in blood, especially in milk, is nowadays used as a practical parameter for following the intake of the proteins and energy with the meals. It can also be used to check efficiency and usage of nitrogen from food. The average value of urea content in blood of dairy cows depending on location and nutrition season is shown in Table 3.

Table 3. The average value of urea content in blood of dairy cows depending on location and nutrition season. mmol/l
Tabela 3. Prosječne vrijednosti sadržaja ureje u krvi mliječnih krava s obzirom na lokalitete i sezone ishrane, mmol/l

Location	Nutrition season	Average value for factor b	Average value for factor a
Mrkonjic Grad	winter	4.00	4.57
	spring	3.78	
	summer	5.63	
	autumn	4.94	
Gradiska	winter	4.53	5.87
	spring	4.20	
	summer	9.85	
	autumn	4.98	

In Table 3, we can notice that the average values of the urea blood content in dairy cows considering the nutrition seasons and location of Mrkonjic Grad are within the acceptable physiological range. Considering the average values according to the nutrition seasons at the same location, urea blood content in dairy cows is the lowest during the spring nutrition season, and the highest during the summer nutrition period. Analyzing the average values of urea blood content in dairy cows in the location of Gradiska, it can be seen that there was not high urea blood content in dairy cows during winter, spring and autumn. It was within the acceptable range. Only the summer nutrition process led to the higher urea blood content in dairy cows, which is much higher than the acceptable value for dairy cows. Content of the total nitrogen and real proteins (casein mostly) in milk is lowering during the summer period, while the content of the non-protein nitrogen, which includes urea, is increasing. (Carlsson et al., 1995; Ferguson et al., 1997). Rajala-Schultz and Savillc (2003) estimated an important interaction between a season and milk production of pasture cows. Urea blood content in dairy cows was much higher during summer. However, it is known that fresh pasture contains digestive proteins and large ratio of energy and proteins (Soriano et al., 2001). It is also emphasized that there is a highly positive correlation between urea blood content and urea milk content which can account for the highest urea blood content during the summer nutrition period in cows from the both locations. Results of the variance analysis of the urea blood content in dairy cows depending on the location and nutrition season are shown in Table 4.

Table 4. Results of the variance analysis of the urea blood content in dairy cows depending on location and nutrition season.
Tabela 4. Rezultati analize varijanse za sadržaj ureje u krvi mliječnih krava vezano za lokalitet i sezonu ishrane

Variant sources	F – cal.	F – tab.	
		0.05	0.01
Treatments	12.77	2.12	2.90
A	11.31**	3.97	7.00
B	19.65**	2.73	4.00
AB	6.38**	2.73	4.00

Results of the variance analysis of the urea blood content in dairy cows depending on the location and nutrition season show that there is a statistically significant difference on all researched factors and their interactions. There is a huge difference in all researched factors and their interactions during summer nutrition period on one hand and the other seasons on the other hand. The difference in urea blood content in dairy

cows during spring, autumn and winter nutrition seasons is not statistically significant. The influence of the location factors interactions (a) inside factors of the nutrition seasons (b) are tested by the F-test and the results are in Table 5.

Table 5. Results of the variance analysis related to the interaction of the location factors (a) inside factors of the nutrition seasons (b)
Tabela 5. Rezultati analize varijanse vezano za interakciju faktora lakocaije (a) unutar faktora sezone ishrane (b)

Variant source	F-cal.	F-tab.		Variant source	F-cal.	F-tab.	
		0.05	0.01			0.05	0.01
a inside b1	0.47	3.97	7.00	A inside b3	29.68**	3.97	7.00
a inside b2	0.29			a inside b4	0.003		

Data in Table 5 give a review in which the only statistically significant difference is the one between the locations during the summer nutrition season. Differences of the urea blood content in dairy cows between locations among the other nutrition seasons are not large. In Table 6 there are data of the variance analysis for the nutrition season for the locations with calculated F-test.

Table 6. Results of the variance analysis of the nutrition seasons (b) inside the location (a)
Tabela 6. Rezultati analize varijanse interakcije sezona ishrane (b) unutar lokaliteta (a)

Variance sources	F-cal.	F-tab.		Variant sources	F-cal.	F-tab.	
		0.05	0.01			0.05	0.01
b inside a1	0.027	2.73	4.07	b inside a2	0.060	2.73	4.07
b inside a1	1.475			b inside a2	15.723**		
b inside a1	0.491			b inside a2	0.112		
b inside a1	1.901			b inside a2	17.734**		
b inside a1	0.747			b inside a2	0.337		
b inside a1	0.264			b inside a2	13.175**		

Variance analysis showed that there is a statistically significant difference in the urea blood content by the combinations on the level of Gradiska location between the summer nutrition process on one hand and the winter, spring and autumn nutrition season on the other hand. The differences between urea blood content in dairy cows during other nutrition seasons inside location were not statistically significant.

Determination of the urea blood content or urea milk content, as a simple method, can have an important role in optimizing nutrition on the farm, because: 1) too high intake of the raw proteins can have a negative effect on the reproduction and milk production; 2) consuming too much raw proteins increases the energy need of cows; 3) protein additives are expensive; 4) too large extraction of the nitrogen has a negative effect on the environment (Broderick and Clayton, 1997). Having too much of protein fermentation in the paunch has the influence on lowering the pH values in the womb by an unknown mechanism during the luteal phase of the embryo development. This can result in low fertility (Elrod and Butler, 1993). The early embryo development demands certain conditions inside the oviduct and womb. The high level of urea or nitrogen in the blood can be toxic for the sperm, ovary or embryo. It can cause destruction of the cells in the ovary (Moore and Varga, 1996). According to Prpic et al. (2005), high urea

milk concentration, which is related to the over-consumption of the proteins, has bad influence on cows health and fertility, environmental pollution and higher food expenses. It has already been mentioned that urea blood content and urea milk content are correlated.

CONCLUSION

According to the research on the urea blood content in dairy cows, the following can be concluded:

- The level of urea blood content in dairy cows is higher in Gradiska location than in Mrkonjic Grad location and the difference is statistically significant;
- The urea blood content in dairy cows in Gradiska location is above the acceptable physiological content during the second and third lactation;
- Summer nutrition period increased the urea blood content in dairy cows in Gradiska location. The difference is statistically significant compared to the content of the same parameter during the other nutrition seasons.

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SADRŽAJ UREJE U KRVI MLIJEČNIH KRAVA ZAVISNO OD LOKALITETA I NAČINA ISHRANE

BOŽO VAŽIĆ, MILANKA DRINIĆ, MILAN KRAJINOVIĆ, BILJANA ROGIĆ

Izvod

Ureja se sintetiše u jetri na račun amonijaka i ugljendioksida, dakle na račun proizvoda katabolizma, nastalih iz raznih jedinjenja. Amonijak nastao u ekstrahepatičnim tkivima procesima dezaminacije aminokiselina prenosi se do jetre u obliku više spojeva, od kojih su najzastupljeniji: glutamin i alanin. Jetra na račun ovako dopremljenog amonijaka, kao i na račun amonijaka osloboljenog deaminacijom aminokiselina u svojim ćelijama, sintetiše ureju. Krave koje se hrane na prirodniji način, ostvaruju manju proizvodnju mlijeka, pokazuju bolje reproduktivne karakteristike u cijelosti u odnosu na krave koje se drže u intenzivnoj proizvodnji, što znači da se hrane sa izblansiranim obrocima, koji u pojedinim slučajevima imaju nešto povećan procenat proteina u svom sastavu. Uzorci krvi krava koji su uzeti sa lokaliteta Mrkonjić Grada, gdje je obrok zasnovan u velikom procentu na kabastoj hrani, prosječno i po fazama proizvodnje, kao i sezonama ishrane, sadržaj ureje se nalazio u granicama fiziološke dozvoljenosti. Sadržaj ureje u krvi krava, gdje su zastupljeni kompletni obroci, na području Gradiške prosječno se nalazio na gornjoj fiziološkoj granici, a posmatrajući faze proizvodnje najmanja koncentracija ureje je zabilježena u krvi krava koje su se nalazile u suhostaju, dok su u ostale tri faze koncentracija ureje nalazila iznad dozvoljenog fiziološkog nivoa. Kod istih krava što se tiče sezona ishrane, zabilježen je enorman sadržaj ureje u krvi za vrijeme ljetnjeg obroka. Obradom podaka utvrđeno je da postoji statistička razlika u sadržaju ureje u krvi između ispitivanih lokaliteta, dok utvrđena razlika između faza proizvodnje i interakacije faza proizvodnje i lokaliteta statistički nisu značajne. Sadržaj ureje u krvi krava, povezan sa sezonama ishrane, na oba lokaliteta je zabilježeno da je ljetnji obrok poveća sadržaj ureje u krvi, a da je najmanja koncentrracija u krvi zabilježena za vrijeme proljetnog obroka. Utvrđene razlike u sadržaju ureje u kravi krava po lokalitetima, sezonama ishrane i interakciji lokaliteti i sezone ishrane su statistički visoko značajni.

Ključne reči: krava, ureja, krv, faza proizvodnje, sezona ishrane

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MINERAL NITROGEN DYNAMIC IN SOIL OF DIFFERENT FERTILITY AS AFFECTED BY AGRONOMIC PRACTICES*

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SUMMARY: The variability and accessibility of NO_3-N in the soil derives from the complex interaction of mineralization and ecological conditions. The results showed that in unfertilized two–year rotation conditions for mineralization had the most dominant effects on distribution and dynamics of NO_3–N. However, in the fertilized rotation NO_3–N dynamic and distribution was a result of mineral N application, plant assimilation and mineralization. In our agro-ecological conditions year to year NO_3–N variations were extensive. However, without the addition of nitrogen potential NO_3–N released from the mineralization is not sufficient for achievement of high yields. Moreover, fertilization must be based on Soil Fertility Control System.

Key words: *nitrogen, fertilization, crop rotation*

INTRODUCTION

The content and availability of mineral nitrogen (N) in the soil, as well as its dynamics and distribution in the soil profile among other macronutrients could be a critical indicator of high–yield potential for particular production year. The required amount of N needed to achieve economically viable yield differ, since numerous factor are involved in nitrogen balance such as climatic conditions, soil properties, crop variety and production technology (Bogdanovic et al., 1998). The processes of N transformation in soil are complex and comprise a part of the N cycle, which in natural ecosystems is closed. However, in the agro-ecosystems, management of agricultural land leads to disruption of N cycle and removal of N coupled in aboveground biomass therefore external application of N is needed in order to preserve or enhance the productivity of the soil

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(Hofman and Van Cleemput, 2004). At the same time, the soil mineralization ability can play an important role in meeting the crop N demand. Though, due to difficult assessment of soil mineralization capacity, that depends on time of sampling and dynamics of NO3–N in the soil, it could result with a rough estimation of readily available N in soil. According to Marinković et al. (2008) good results in agricultural production can be achieved only through a balance between all cultural practices and weather conditions on the one hand and the potential of the growing field and the cultivar/hybrid on the other. Nowadays, growing high–yielding varieties and hybrids could led to increased plant N demand for that reason additional research on mineral nutrition is of great importance to enhance the cropping technology. The reason for this is the fact that the variability of climatic conditions, inadequate cultural practices significantly affects the availability of N from the soil, often reducing availability of the nutrients for plants in conditions of insufficient application of fertilizers (Bogdanovic, 2009). The degree of utilization of mineral fertilizer N seldom exceeds 50% (Rasmussen, and Rhode, 1991) and the most common form of nitrogen loss applied are denitrification, leaching and migration (NO3–N), volatization and gaseous losses of NH3–N by plant organs (Raun et al., 1999). The aim of this study was to determine distribution of NO3–N in soils of contrasting fertility and to evaluate the role of climatic conditions on its dynamic.

MATERIAL AND METHODS

The present study was performed on a long–term crop rotation experiment (LTE) carried out at the Rimski Šančevi Experimental Field of the Institute of Field and Vegetable Crops in Novi Sad (N 45°19`, E 19°50`). The LTE was established on a chernozem soil (subtype: chernozem on loess and loess–like sediments; variety: slightly calcareous) (Milosev, 2000). The study treatments were: a two–year fertilized crop rotation (maize–wheat) (MWF) and a unfertilized two–year crop rotation (maize–wheat) (MW) in 3 year 2007–2009. The treatments are regularly fertilized with mineral nitrogen fertilizers at 120 kg ha^{-1} rate for maize and 100 kg ha^{-1} for winter wheat. Fertilizer with P or K and barnyard manure have been used until 1986, after ward only mineral N was used due to high supply of these nutrients in soil.

Table 1. Content N (%) in Field Crop, and Harvest index
Tabela 1. Sadržaj N (%) u biljkama i žetveni indeks

Cropping system Sistem biljne proizvodnje	Property Svojstvo	Winter wheat Pšenica		Maize Kukuruz	
		Grain Zrno	Straw Slama	Grain Zrno	Straw Slama
Fertilized two-year rotation Đubreno dvopolje	N content (%) Sadržaj N (%)	2*	0.45*	2	1.49
	Harvest index Žetveni indeks	0.45+		0.4§	
Unfertilized two-year rotation Neđubreno dvopolje	N content (%) Sadržaj N (%)	1.7*	0.3*	1.28	0.54
	Harvest index Žetveni indeks	0.48+		0.45§	

*(Malešević, 1989); +(Milošev, 2000); §(Boćanski, 1988)

The prevailing climate in the investigated area is continental, with an average an-

nual precipitation of 611 mm, and annual temperature is 11,1°C. For the calculation of N uptake by crops content of N in wheat and maize grain and straw was either measured or adopted from the literature (Table 1). Soil sampling was conducted in different stages of plant growth from March to October. From November to February NO_3–N content was approximated as 0. Determination of available NO_3–N in soil was conducted according to Scharpf and Wehreman (1975).

RESULTS AND DISSCUSION

For the observed period soil reaction remains alkaline with an increase in pH compared to the obtained results in 1988 (Table 2). The lower pH in fertilized rotation could be attributed to the long–term application of the N–fertilizer. Humus content in the fertilized rotation was preserved by proper crop rotation and rational mineral fertilization with plowing in crop residues. Omitting the application of mineral fertilizer and removal of crop residues for a long period of time (> 41 years for the unfertilized rotation), the productive capacity of the chernozem has changed and the total concentration of humus in the soil was reduced (–0.25%) in relation to the concentration at the time of establishing the experiment. Similar finding for chenozem soil were presented in studies of Jovanovic (1995), Starcevic (1986), Molnar et al. (1999).

Table 2. Soil chemical properties of the investigated treatments
Tabela 2. Hemijska svojstva zemljišta proučavanih sistema biljne proizvodnje

Soil chemical properties Hemijska svojstva zemljišta	Treatments/Tretmani					
	Fertilized two–year rotation Đubreno dvopolje			Unfertilized two–year rotation Neđubreno dvopolje		
	1988	2009	Difference Razlika	1988	2009	Difference Razlika
pH H2O	7.69	8.09	+0.4	7.94	8.27	+0.33
pH KCl	7.21	7.28	+0.7	7.37	7.41	+0.03
CaCO3 (%)	4.68	3.21	-1.47	9.41	12.84	+3.34
Humus (%)	2.57	2.59	+0.02	2.47	2.22	-0.25
Total N (%)	0.17	0.17	0	0.16	0.15	-0.1
C/N	9.67	*	-	8.03	8.58	+0.55
mg P2O5 100 g-1	90.80	67.96	-22.84	6.10	7.37	+1.27
mg K2O 100 g-1	40.7	38.43	-2.27	17.10	16.21	– 1.11

In this study, measured C/N ratio in the soil is slightly wider at the unfertilized rotation compared to fertilized. Readily available phosphorus in fertilized rotation experiment in relation to the unfertilized rotation was significantly higher (Table 2). Similar values for P concentration was found in previous investigation on the same experiment (Šeremešić, 2005). The result was expected after a longer period fertilized with manure until 1991. According to research Bogdanovic et al. (1984) high concentrations of readily available P in soils that are medium or even low provided microelements can cause their lack of plants. Hence the soils with > 50 mg P_2O_5 100 g^{-1} in control systems for soil fertility and use of fertilizers recommended the omission of P fertilization in next 3–4 years.

Readily available potassium in the unfertilized rotation after 42 years of cropping is in the class of soil of optimal availability in this element (table 2). The explanation derives from natural fertility since the prevailing chernozem clay minerals are illit and

montmorillonite type which has associated K+, and potential for K release in the soil solution. A very high content of readily available potassium found in the fertilized rotation is a result of long–term application of mineral fertilizers and manure and plowing under a large mass of wheat straw and maize residue.

Table 3. Average uptake of total N in grain and above ground plant remains (kg ha^{-1})
Tabela 3.Prosečno iznošenje N zrnom i ndzemnom masom biljaka (kg ha^{-1})

Cropping system *Sistem proizvodenje*		Grain *Zrno*	Straw *Slama*	Total *Ukupno*
Fertilized Two–year rotation *Đubreno dvopoljw*	Winter wheat *Pšenica*	103.31±23.87	25.38±5.96	128.69
	Maize *Kukuruz*	153.66±45.72	171.78±51.09	325.44
	Total *Ukupno*			454.13
Unfertilized Two–year rotation *Neđubreno dvopolje*	Winter wheat *Pšenica*	17.58±6.61	3.36±1.26	20.94
	Maize *Kukuruz*	31.61±16.77	16.3±8.64	47.91
	Total *Ukupno*			68.85

In the agricultural system N is considerably dynamic and depends on plant–soil interrelation. The amount of total N (kg ha^{-1}) in soils of the two contrasting cropping systems showed considerable difference in quantity of N remain after harvest (Table 3). At the unfertilized two–year crop rotation on the same experiment Bogdanović et al (2008) calculated negative N–balance is soil, and anticipated that new state of equilibrium of N and other biotic element was established. The amount of N remains in the straw of maize (25.38 kg N ha^{-1}) and winter wheat (3.36 kg N ha^{-1}) cannot be considered as an adequate source for the plant nutrition (Table 3). With 120 kg N ha^{-1} application in maize production the considerable amount of N must be provided from soil mineralization. Removal of N in the winter wheat is lesser and could be compensated from soil to some extent. The amount of the total N in the fertilized soil was 6 times higher compared with the unfertilized (454.13 to 68.85). Panković and Malešević (2006) in the 2000–2005 period estimated that removal of N with NS winter wheat cultivars reached 211 kg ha^{-1} (148 grain + 63 straw). In maize production N content in grain and crop residue is strongly influenced by climatic condition and hybrid characteristics. Therefore year–to–year variation can significantly after the N takeout. Starčević at al. (1999), suggest that content and removal of N very in relation to different N fertilization scheme. N content in grain increased respectively with application of higher N doses from 1.15% to 1.51% and in crop residues from 0.45% do 0.67%. Same authors found that removal of the N in maize production with different levels of fertilization fits the quadratic equation with maximum of 153 N ha^{-1} for grain, and 208 N ha^{-1} for crop residue. Similar results for six maize inbred lines were presented by Latković (2009).

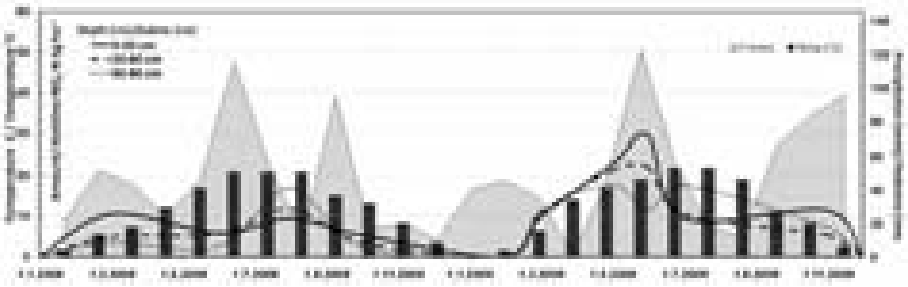

Figure 1. Distribution and dynamics of NO_3–N in different soil layers in unfertilized two–year wheat rotation (kg ha^{-1})

Grafikon 1.Distribucija i dinamika NO_3–N u različitim slojevima zemljišta na neđubrenom dvopolju pšenice (kg ha^{-1})

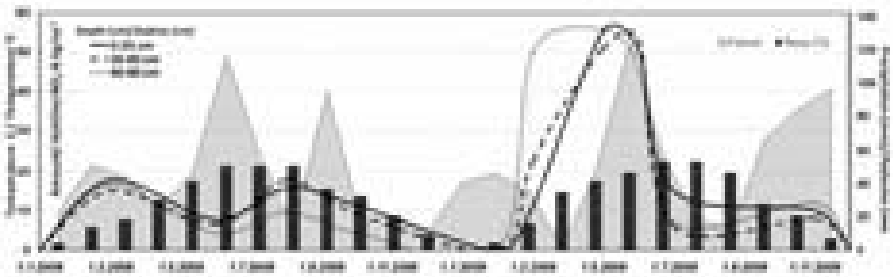

Figure 2. Distribution and dynamics of NO_3–N in different soil layers in the fertilized two–year wheat rotation

Grafikon 2.Distribucija i dinamika NO_3–N u različitim slojevima zemljišta na đubrenom dvopolju pšenice (kg ha^{-1})

Based on the obtained results content of the NO_3 in three depth had a different pattern in the observed cropping system under winter wheat and maize. This could be explained with the differences in dynamics of the microbiological activity in soil and related processes that supply soil with NO_3 (preferably fertilization). During the vegetation of winter wheat, the amount of NO_3–N increase in the March and decrease due to the plant intake and the partial transfer (under the influence of rainfall) (Fig. 1). Malešević et al. (1991) concluded that allocation of nitrate in the soil profile basically depends on soil moisture and that in the "normal" years higher amount of NO_3–N is in the 30–60 cm soil layer. In the unfertilized plots winter wheat plant development was hampered by soil fertility that resulted with shallow root development and intake NO_3 from upper layer. Unlike this in the fertilized treatment higher amount of NO_3 was observed and gradual decline of available nitrate in three depths until harvest (Fig. 2). Stubble tillage after harvest affected the NO_3–N content as a result of N uptake and mineralization of crop residues. In the unfertilized wheat plot in both years higher content of NO_3–N after harvest was found in the 60–90 cm. Subsequently, the amount of NO_3–N in the unfertilized treatment was 20 kg ha^{-1} until winter, whereas content of NO_3–N in 30–90 cm decreased. The application of N in the October at the fertilized plots was followed with higher content of NO_3–N in November before lower temperature occurred.

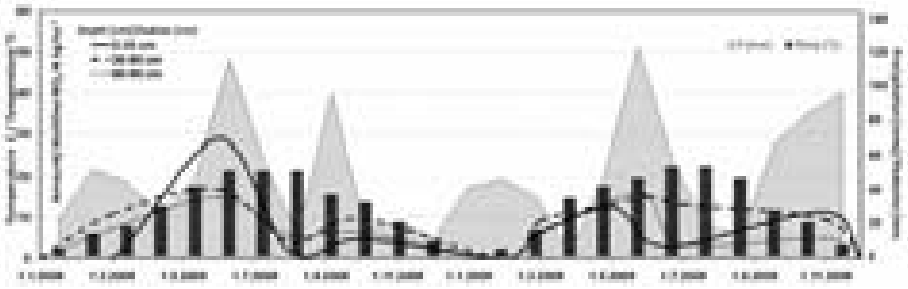

Figure 3. Distribution and dynamics of NO_3–N in different soil layers in the unfertilized two–year maize rotation (kg ha^{-1})
Grafikon 3. Distribucija i dinamika NO_3–N u različitim slojevima zemljišta na neđubrenom dvopolju kukuruza (kg ha^{-1})

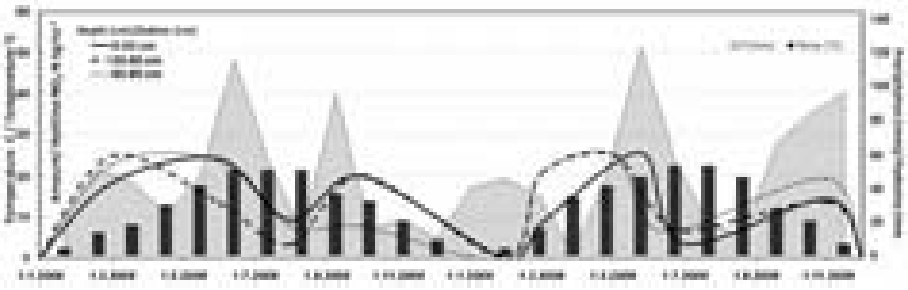

Figure 4. Distribution and dynamics of NO_3–N in different soil layers in the fertilized two–year maize rotation *(kg ha^{-1})*
Grafikon 4.Distribucija i dinamika NO_3–N u različitim slojevima zemljišta na đubrenom dvopolju kukuruza (kg ha^{-1})

In the maize cropping NO_3–N dynamic was influenced by the conditions for mineralization, and the amount of NO_3–N in all layers of the soil increases in May and early June, reached a maximum, then decrease at the begging of July. Similar dynamics of NO_3–N in plow layer after adding N fertilizer under maize crop was found in Ma et al. (1999) study. In the unfertilized plots NO_3–N intake was more pronounced in the 0–30 cm due to shallow root development (Fig. 3). Conversely, for the fertilized maize 30–60 cm soil layer showed higher importance when N nutrition of maize is considered (Fig. 4). Most of the NO_3–N in layer 30–60 cm was recorded earlier during early spring, while in the deepest layer (60–90 cm) achieved the maximum NO_3–N in May. Bundy and Malone (1988) proposed that if > 150 kg N ha^{-1} found in the soil profile (0–90 cm) it is sufficient for attained the high maize yield. In our study the amount of NO_3–N is less than that, and since complex agroecological conditions are involved in its dynamic yield fluctuation is occurred. The intake of NO_3–N by the plants, based on nitrate dynamic in soil is expected to reach maximum in Jun–July. Subsequently, from September (maize harvest) until winter, content of NO_3–N in the unfertilized maize did not exceed 20–30 kg ha^{-1} and 50–60 kg ha^{-1} at the fertilized rotation. Available amounts of NO_3–N in the soil profile had influenced its intake by plants and hence higher yield of grown crops,

compared with the unfertilized plots (Yang et al., 2004). Evaluating the nitrate dynamic in soil it is observed that maize have had a higher amount of the accessible NO_3–N compared with winter wheat. This could be explained with N fertilization (100 kg ha^{-1} N for wheat and 120 kg ha^{-1} for maize) in the fertilized plots, but in some years it could be compensated with favorable soil condition for mineralization under winter wheat.

CONCLUSION

Obtained result showed that in the unfertilized two–year rotation conditions for mineralization had the most dominant effects on distribution and dynamics of NO_3–N in soil. However, in the fertilized rotation NO_3–N dynamic and distribution was a result of the mineral N application, plant assimilation and mineralization. Mineralization intensity was higher in April and May. Without addition of nitrogen potential NO_3–N released from the mineralization is not sufficient for achievement of high yields. In our agro-ecological conditions NO_3–N variations were significant, and therefore not predictable. To achieve yield potential of growing crop it is necessary to judiciously apply fertilizer based on the Soil Fertility Control System.

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UTICAJ TEHNOLOGIJE GAJENJA NA DINAMIKU MINERALNOG AZOTA U ZEMLJIŠTU RAZLIČITOG NIVOA PLODNOSTI

DARINKA BOGDANOVIĆ, DRAGIŠA MILOŠEV,
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Izvod

Varijabilnost pristupačnog NO_3-N u zemljištu posledica je interakcije mineralizacije i faktora spoljne sredine. Dobijeni rezultati pokazuju da na neđubrenom dvopolju dominatnu ulogu na distribuciju i dinamku NO_3-N imaju uslovi za mineralizaciju. Na đubrenom dvopoljnom plodoredu N-đubrenje i brzina mineralizacije utiču na mobilnost azota i njegovo usvajanje biljkama. U našim agroekološkim uslovima najveća količina mineralnog azota je pristupačna u proleće. Međutim bez dodavanja planiranih količina mineralnih đubriva nije moguće realizovati potencijal prinosa gajanih biljaka. Pored toga neophodno je da se đubrenje obavlja prema principima Sistema kontrole plodnosti zemljišta.

Ključne reči: Azot, đubrenje, plodored

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LIVESTOCK INSURANCE AS A FACTOR OF ECONOMIC STABILITY IN THE AGRICULTURE

TODOR MARKOVIĆ, MILENKO JOVANOVIĆ[1]

SUMMARY: The need for livestock insurance has increased in the last few years due to the emergence of new animal diseases. The research of livestock insurance in Europe has been long actualized, while in Serbia a small number of papers are dedicated to this subject. In this paper the claims (liquidated and authoritative) in the animal husbandry, as well as technical premium (invoiced and authoritative) used for the payment of claims are being analyzed for the period from 2004 to 2008 in Serbia. Loss ratio as ratio between the claims and the premiums is also being analyzed. Standard statistical methods are used for the analysis, such as: calculation of average values, calculation of the variation ratio and calculation of the change rates. The application of the mentioned methods showed that the average damages are higher than the average premiums paid, therefore the negative loss ratio occurs. Also, there are large fluctuations in the movement of claims and technical premium and both indicators have expressed change rate. All these points show that the farmers, the insurance companies and the state should be more connected, which would create an integrated system of risk management in animal husbandry.

Key words: *livestock insurance, gross premium, technical premium, damages, loss ratio*

INTRODUCTION

Livestock insurance is a special branch of insurance based on the general principles of the insurance science, but it has a number of specificities, which primarily relate to the subject of insurance (animals) that function under specific biological laws. In comparison to the plant production, in the animal husbandry the realization of risk is more frequent and the occurred damages can significantly disrupt the livestock production. Through the history the livestock insurance in Serbia went through different stages of development (the capitalism, the socialism, the transition period), while today it is on a very low level (Marković, 2007). On the other hand, the research of livestock

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insurance in Europe has been long actualized, but in relation to the crop insurance only a small number of papers is devoted to this subject (Bielza et al., 2007).

The aim of this paper is to examine in the observed period the relationship between the damages in animal husbandry and available funds for their compensation (insurance premium), thus getting the loss ration. Also, the share of livestock insurance premium in GDP of agriculture is being examined. Based on this, the new possibilities for development of livestock insurance in Serbia are being pointed to.

MATERIAL AND METHODS

The research of this problem was carried out using different methods. The basic source of data is the documentation of the Statistical Office of Serbia and National Bank of Serbia, where the data about gross domestic product (GDP), gross insurance premium and technical insurance premium, as well as the damages in animal husbandry are taken from.

In this paper the standard statistical methods, such as calculation of average values, calculation of the variation ratio and calculation of the change rates are being used.

RESULTS AND DISCUSSION

If farmers want to insure their animals (subject of insurance) against various risks,[2] they have an obligation to pay the appropriate compensation for risk-taking. The price of risk (insurance) is a premium, which exists in all types of insurance. Farmers set aside significant funds for the livestock insurance (crop insurance) and they pay the full amount of premium (gross premium). It is interesting to relate gross insurance premium to gross domestic product (GDP), since the cost of insurance is covered from the added value of GDP (Marko, 1989). The participation of livestock insurance premium and the participation of total insurance premium in the GDP of agriculture is given in the table 1.

It is characteristic that agriculture has a share of over 90 % in the GDP of agriculture and fisheries activities. There are not the large fluctuations in GDP of agriculture (coefficient of variation 4.74 %), but there is a slight decrease (change rate -1.28 %). In the reporting period, achieved gross premiums of livestock insurance participates on average with 31.62 % in the total gross premiums of agriculture, with high annual fluctuations (coefficient of variation 27.54 %) and significant change rate (11.45 %). Participation of gross premiums of livestock insurance in the GDP was the largest in 2007 (0.41 %), while in all other years of the observed period it was below 0.40 %. On the other hand, the share of gross premiums of the agriculture in GDP was the largest in 2008 (1.19 %), while the participation was only 0.58 % in 2004.

It is certain that in agriculture only a small part of added value is being directed to the insurance funds, so that the insurance costs do not have a significant place in the GDP. Since the livestock insurance is not mandatory, a relatively small number of farm-

[2]The main risks are death, murder or forced slaughter of animals due to illness or accident, and there is the possibility of insurance of many additional risks (loss at birth, loss of breeding capacity, disappearance and theft).

ers insure their animals, and therefore the allocation for insurance premium is modest.

Table 1: GDP in agriculture, gross premium in agriculture (livestock) insurance and their part in GDP in agriculture for the period from 2004 to 2008 in Serbia
Tabela 1: BDP u poljoprivredi, bruto premija osiguranja u osiguranju poljoprivrede (životinja) i njihovo učešće u BDP poljoprivrede u periodu 2004-2008. godine u Srbiji

Years / Godine	GDP in agriculture and fishery (000 RSD)/ BDP u poljo-privredi i ribarstvu (000 din)	Gross premium in livestock insurance (000 RSD)/ Bruto premija kod osiguranja životinja (000 din)	Gross premium in agriculture (000 RSD)/ Ukupna bruto premija u poljoprivredi (000 din)	Gross premium of livestock insurance in GDP (%)/ Učešće bruto premije kod osiguranja životinja u BDP (000 din)	Gross premium of agriculture in GDP (%)/ Učešće bruto premije u poljoprivredi u BDP (000 din)
2004	143,545,800	259,748	838,010	0.18	0.58
2005	136,500,200	328,554	940,287	0.24	0.69
2006	136,205,300	409,737	1,021,428	0.30	0.75
2007	125,576,600	516,619	1,268,080	0.41	1.01
2008	136,316,200	511,247	1,616,455	0.38	1.19
Average / Prosek	135,628,820	405,181	1,136,852	0.30	0.84
Variation ratio (%)/ Koeficijent varijacije (%)	4.74	27.79	27.41	31.62	29.59
Change rate (%)/ Stopa promene (%)	-1.28	18.45	17.85	20.54	19.68

A significant part of earned gross premiums is diverted to cover the loss suffered by farmers. The amount of this premium is called the technical premium. In order to reflect the performance of an insurance company it is necessary to establish a loss ratio. This ratio is calculated as a relation between the damages and the insurance premium (Bennett, 1992). Instead of the gross premium, the technical premium it is usually taken in the calculation as part of the gross premium for the payment of damages. It can be seen in broad and narrow sense.[3]

It is certain that there is some difference between the authoritative and the technical premiums in the reporting period. This is due to the fact that there are deviations between the transmission technical premium in the previous and the current period. The largest share of authoritative technical premiums in gross premiums was recorded in 2004 (77.92 %), and the same year the largest share of invoiced technical premium in gross premium was recorded (75.55 %). The previous results show that a large part of the gross premium is allocated in the form of technical premium to compensate for the damages.

[3]In the narrow sense the technical premium is invoiced technical premium in the current year, while in the broader sense it is marked as authoritative technical premium and it consists of the sum of unearned premium from the previous year and the invoiced premium in the current year, and of that amount is taken away the unearned premium which is passed in the next year.

Table 2: Invoiced and authoritative technical premium, liquidated and authoritative claims, current and authoritative loss ratio in livestock insurance for the period from 2004 to 2008 in Serbia

Tabela 2: Fakturisana i merodavna tehnička premija, likvidirane i merodavne štete, tekući i merodavni tehnički rezultat kod osiguranja životinja u periodu 2004-2008. godine u Srbiji

Years / Godine	Invoiced premium (000 RSD)/ Fakturisana tehnička premija (000 din)	Authoritative premium (000 RSD)/ Merodavna tehnička premija (000 din)	Liquidated claims (000 RSD / Likvidirane štete (000 din)	Authoritative claims (000 RSD)/ Merodavne štete (000 din)	Current loss ratio (%)/ Tekući tehnički rezultat (%)	Authoritati-ve loss ratio (%)/ Merodavni tehnički rezultat (%)
2004	196,250	202,405	253,970	256,978	129.41	126.96
2005	233,842	207,865	285,655	285,826	122.16	137.51
2006	288,152	276,069	376,068	375,018	130.51	135.84
2007	364,233	343,131	470,998	469,490	129.31	136.83
2008	362,138	346,222	451,801	450,478	124.76	130.11
Average / Prosek	288,923	275,138	367,698	367,558	127.23	133.45
Variation ratio (%)/ Koeficijent varijacije (%)	26.05	25.37	26.33	25.89	2.86	3.61
Change rate (%)/ Stopa promene (%)	16.65	14.36	15.49	15.07	-0.78	0.59

The damages in animal husbandry can be seen in the narrow and broad sense, or as liquidated and authoritative damages.[4] There are obvious differences in the amounts of damages for particular years, but on average they are approximately equal (table 2). It has already been noted that the amount of incurred damages is compensated from one part of collected gross premium (technical premium). If the damages are compared with the technical premium, it leads to a very important indicator of success in the insurance, and this is a technical result. As noted, this indicator can be called a loss ratio and it can be calculated in practice as current or authoritative loss ratio.[5] If the amount of loss ratio is less than 100 %, then it is better. The operating performance of individual insurance companies can be estimated based on the loss ratio. The technical result can be calculated for one year, and also for many years together.

[4]In a narrow sense it represents the liquidated damages in the current year, while in the broad sense the authoritative damages represent the sum of reserved damages transferred from the previous year and the liquidated damages from the current year, and out of that amount the amount of unliquidated damages in the current year which is reserved to the end of the year is taken away.

[5]The current loss ratio is quotient between the liquidated damages and the invoiced technical premium in the current year. Thus, the resulting amount is multiplied by 100 in order to get the appropriate percentage. The authoritative loss ratio is obtained when the amount of authoritative damages is divided by the amount of authoritative technical premiums and multiplied by 100.

To analyse the current loss ratio in the selected period it is necessary to observe the movement of invoiced technical premiums and liquidated damages in that period (table 2). The average invoiced technical premium is on average close to 290 million RSD, with the obvious fluctuations (coefficient of variation 26.05 %) and significant change rate (16.65 %). As opposed to that, the average liquidated damages reach almost 370 million RSD. And for them considerable fluctuations (coefficient of variation 26.33 %) and evident change rate (15.49 %) are characteristic. By observing the relationship between the liquidated damages and the invoiced technical premiums it comes to the current loss ratio (table 2). The lowest loss ratio was achieved in 2005 (122.16 %), and followed by 2008 (124.76 %). In other years the current technical result is around 130 %. There is a negative loss ratio in all years, because the collected technical premium is well below the damages.

On the other hand, in order to determine the authoritative loss ratio, the authoritative damages and authoritative technical premium in the selected period (table 2) must be considered. The average authoritative damages are around 370 million RSD, with pronounced fluctuations (coefficient of variation 25.89 %) and evident change rate of 15.07 %. On the other hand, the average authoritative premium is 275 million RSD. As with the authoritative damages, here is also the coefficient of variation (25.37 %) and less pronounced change rate (14.36 %) apparent. By comparing the amount of authoritative damages and the authoritative technical premium, it comes to the authoritative loss ratio (table 2). The highest loss ratio was achieved in 2005 (137.51 %), while in 2007 it was slightly lower (136.83 %). In these two years, but also in the other years, the established authoritative premium is not sufficient to cover the authoritative damages.

If you compare current and authoritative loss ratio it can be noted that there are differences from year to year. This is due to the fact that there were deviations between the technical and the authoritative premium or liquidated and authoritative damages. Finally, it is evident that the loss ratio (current and authoritative) is significantly fluctuated in the reporting period. This is primarily caused by damages, which have the significant influence on this indicator in the reporting period. On the other hand, in some years farmers have insured more, so they have extracted more money for payment of gross premium.

CONCLUSION

In the agricultural insurance more attention is paid to crop insurance than to livestock insurance. The whole issue of livestock insurance is intended to enable faster development of livestock and to reduce damages in animal husbandry to the minimum. In the selected period technical premium is not sufficient to cover the damages and it is necessary to try to increase it in the future. This can be achieved with better education of farmers, who could use certain preventive measures to partially mitigate the development of specific damages, and on the other hand with appropriate livestock insurance packages to try to encourage more farmers to insure their animals.

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OSIGURANJE STOKE KAO FAKTOR EKONOMSKE STABILNOSTI U POLJOPRIVREDI

TODOR MARKOVIĆ, MILENKO JOVANOVIĆ

Izvod

Poslednjih godina, usled pojave novih bolesti kod životinja, raste potreba za osiguranjem stoke. Istraživanje problematike osiguranja životinja u Evropi je već odavno aktuelizovano, dok je kod nas mali broj radova posvećen ovoj temi. U radu su, na teritoriji Srbije u izbabranom periodu (2004-2008), ispitivane štete (likvidirane i merodavne) nastale od različitih rizika u stočarstvu. Takođe, posmatrana je i tehnička premija (fakturisana i merodavna), koja se koristi za isplatu nastalih šteta. Ispitivan je i odnos između nastalih šteta i tehničke premije (tehnički rezultat). Za analizu su korišćene standardne statističke metode, kao što su: prosečna vrednost, koeficijent varijacije i stopa promene. Primenom ovih metoda uočeno je da su, u posmatranom periodu, prosečne nastale štete iznad prosečne naplaćene premije osiguranja, a samim tim je i negativan tehnički rezultat. Takođe, postoje velike oscilacije u kretanju nastalih šteta i ostvarene tehničke premije, a oba pokazatelja imaju izraženu stopu rasta. Sve ovo ukazuje na potrebu čvršće povezanosti poljoprivrednika, osiguravajućih kuća i države, čime bi se stvorio jedan integrisani sistem upravljanja rizikom u stočarstvu.

Ključne reči: osiguranje životinja, bruto premija, tehnička premija, nastale štete, tehnički rezultat

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IMPACT OF WASTEWATERS FROM PIG BREEDING FARM TO CHANNEL SEDIMENTS PROPERTIES

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SUMMARY: Wastewaters from pig breeding farm near of Vrbas settlement are discharging into lagoon and thereafter are released into melioration drainage cannel network. Detected concentrations of analyzed nutrients and trace elements in channel sediment samples taken at upstream locations (before discharge point from lagoon) and downstream (after discharge point from lagoon) are significantly different. At downstream (vs. upstream) locations nutrients content was increased by 2-fold (for N, S and K_2O), namely by 4.5-fold (for P_2O_5), whereas trace elements content was increased by 10-30%. The most significant increment of trace elements was observed for zinc (>9-fold) and for copper (4.6-fold). At the same time, present copper concentration of 185 mg/kg, and zink concentration of 651 mg/kg are above maximal permitted values in sediments (1.8-fold, namely by almost 2.2-fold respectively) what could implicate detrimental effects to overall environmental resources.

Key words: *channel sediments, nutrients, trace elements, pig breeding farm*

INTRODUCTION

The elemental purposes of melioration cannel network are collecting and draining of excessive surface and ground water resources and/or enabling sufficient water supplies for agricultural irrigation. Mentioned functions have to ensure beneficial preconditions for undisrupted agricultural crop production and related water management activities at certain drainage basin area. At dominantly lowland areas of Vojvodina, mostly characterised with arable and highly productive soils, functionality of relatively dense network of melioration channels (totally length of around 20000 km) is of grate importance from ecological standpoint.

Furthermore, melioration channels are simultaneously exposed to different impact of diffuse/point-source contaminants, such as direct discharge of unrefined sewage and

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industrial wastewaters, leaching and run-off of different agro-chemical substances from surrounding arable land area, etc. In such conditions, certain sections of drainage channel network are very susceptible to sedimentation and precipitation processes of sludge materials, which may significantly impair theirs primary designed agro-hydrotechnic properties, as well as served as "buffer" for different kinds of contaminants. In accordance with recent approaches and concepts of sustainable agricultural development and management, increasing importance is given to quality of melioration cannel's water and sediment materials. Potentially increased concentrations of nutrients and persistent an/organic contaminant elements, as well as their interaction processes (e.g. geochemical reactions, solution transport, etc) within cannel's (water, solid and/or dissolved) medium, may cause adverse and harmful environmental consequences Channel sediment management is very complex issues in melioration systems of Vojvodina due to total length of drainage channel network and potentially large amount of increasing sediment material with inappropriate quality (Pantelić et al., 2008; Savić et al., 2005; 2006; 2007; 2008). One of significant contaminant of certain recipient channel sections at melioration systems are agricultural animal farms too. In this study was analysed the influence of wastewater discharge from a pig breeding farm's lagoon on sediments quality deposited at the bottom of melioration channels.

MATERIAL AND METHODS

The objective of study was to investigate the quality of sediment's material in melioration channel KC-III which at the same time serves as wastewaters recipient from a pig breeding farm. KC-III was designed in trapezoid shape and initially projected with next hydraulic properties: water flow velocity up to 5 m^3/s, water depth 1.0 - 1.5 m, width of the channel's bottom 2 m and side slope 1:1.5. KC-III pertains to drainage melioration system Kula – Crvenka whose totally land area is around 12.650 ha, and the major portion is under arable land (11.000 ha) on dominantly Chernozem soil type. KC-III channel is overspread across the central part of river drainage basin and instils to magisterial channel of Primary channel network of hydro-system Dunav-Tisa-Dunav. In the Vrbas urban zone channel recipes unrefined waste waters from several point-source contaminants including and those from observed pig breeding farms. Analysed sediment samples were taken from the bottom of KC-III channel, at 2 locations: upstream (45°35'07.6" N; 19°35'12.8" E) and downstream (45°35'10.5" N; 19°35'35.8" E) from wastewater discharge point of pig breeding farm's lagoon (Figure 1).

By conducted analyses were detected a certain physical and chemical properties of channel's sediment that a relevant in evaluation process for sediment quality. In this paper an accent was put on study of concentrations of primary nutrients and trace elements as one the most important and frequent harmful and critical materials in drainage channels sediments. Detected concentrations of elements were compared in up/downstream samples, whereas the content of trace elements was compared with maximally permitted concentration (MPC) for soil (Regulation on permissible quantity of toxic and hazardous substances in the soil (Official Gazette R.S., no. 23, 1994.).

By proposed procedure is possible conclude about eventually increment (over MPC) of analysed parameters in downstream (vs. upstream) sediments samples, as a consequence of discharge unrefined wastewaters from pig breeding farm. Furthermore, it is possible to identify importance of observed elements and their influences to overall

environment.

Figure 1. Sampling locations of channel's sediments and discharge point from pig breeding farm to KC-III channel near of Vrbas (Vojvodina, R. of Serbia)
Slika 1. Lokacije zahvatanja uzoraka sedimenata i mesto ispusta laguna svinjogojske farme u kanal KC-III u okolini Vrbasa

Sediment samples were taken in destroyed state from the bottom of drainage channel. Sediment analyses were performed in soil laboratory of the Institute of Field and Vegetable Crops, Novi Sad by standard procedures. Total nitrogen (N) content was detected by CHNS automatic analyser. Concentrations of readily available forms of phosphorous (P_2O_5) and potassium (K_2O) were detected by spectrophotometry and by atomic flame spectrometry respectively. Trace elements determinations (Pb, Cd, Cr, Ni, Cu, Zn, Co, Mn and Fe) were made using inductively coupled plasma - optical emission spectrometry (ICP-OES) (Varian Vista Pro).

RESULTS AND DISCUSSION

Potential treat to water resources from different agricultural pollutants, such as an/organic fertilisers and/or pesticides are recognised and clearly stated in environmental legislation, acts and recommendations of many European countries. For instance, Water Framework Directive (2000/60/EC), Nitrates Directive (91/676/EEC), principles of Good agriculture practice (GAP) and Good farming practice (GFP), as well as national legislation (e.g. Water Act, Environment Protection Act, Agricultural Land Act) are dealing with above mentioned issues and indicate to not only potential, then also and real risk of contamination of river basin area from agricultural sector, especially from intensive livestock farming (Belić et al., 2005; www.drepr.org; www.istocar.bg.ac.rs; www.hzpss.hr). Melioration channel network in Vojvodina is under the highly risk from such contamination processes, due to of its relatively low water flows, velocity, slopes, sediment transportability and self-filtration ability. Furthermore, at certain locations some of melioration channel sections dominantly served as wastewater recipients, whereas their drainage/irrigation purposes are minimised, as was confirmed in presented study.

Due to the fact that average nutrients content in fresh pig manure is relatively high (e.g. N - 0.6%; S - 0.1%; P2O5 - 0.5%; K2O - 0.4%) it significantly impacts to increment

of their concentrations in downstream (after release of farm's effluents) sediment samples compared to upstream (before release of farm's effluents) samples.

Concentration increase index, defined as ratio of detected downstream concentration and upstream concentration (D/U) for certain element, varying between 2 (for nitrogen, sulphurous and potassium) and 4.5 (for phosphorous); Table 1. Significantly increment of nutrient elements in KC-III channel sediments, due to potentially intensive geochemical interactions between water medium and sediment material, may accelerate eutrophication processes, as well as many other negative related environmental consequences.

Table 1. Nutrients content in channel' sediments taken at up/downstream locations in comparison to discharge point from pig breeding farm
Tabela 1. Sadržaj nutrijenata u kanalskim sedimentima uzvodno i nizvodno od ispusta otpadnih voda svinjogojske farme

Canal cross section Profil kanala	Nutrients / Nutrijenti			
	Total N (%) Ukupni N (%)	Total S (%) Ukupni S (%)	P2O5 (mg/100g)	K2O (mg/100g)
Upstream Uzvodno	0.27	0.23	14.7	22.7
Downstream Nizvodno	0.61	0.46	65.5	44.5
Concentr. increase index Indeks povećanja	2.26	2.00	4.46	1.96

One of the most important and frequent group of potentially toxic substances in aquatorial sludge medium are trace elements. Their presence in sediment material is very potential threat to overall environmental resources, especially to surrounding coastal arable land area, then primary/secondary channel network etc. Adverse ecological impacts caused by excessive presence of trace elements can be manifested currently (e.g. leaching to ground water resources), and/or subsequently (cumulatively), i.e. after translocation and accumulation into the edible plant tissues and forward to human food chain. Many of trace elements (e.g. Pb, Cd etc) have relatively high ability to be persistent in environmental resources, and are potentially highly toxic, carcinogenic, mutagenic, teratogenic etc.

Comparison of trace elements content in up/downstream sediment samples clearly indicates to their increased concentration and contamination impact along analysed channel network (Figure 2). For most observed elements (Pb, Cd, Cr, Ni, Co, Mn, Fe) in downstream samples detected concentrations were higher by 10-30% compared to those at upstream locations. The most significant alteration in trace elements of KC-III channel sediments were observed in cuprum and zinc concentrations, which content was increased by 4.6-fold (Cu; from 40.17 to 184.79 mg/kg), namely by >9-fold (Zn; from 72.13 to 651.13 mg/kg). Otherwise, MPC for cuprum (100 mg Cu/kg) and zinc (300 mg Zn/kg) in sediment sample at downstream location was exceeded by almost 185%, namely by almost 220% respectively. Achieved results for Cu and Zn alert to possibility of different adverse impact to environment.

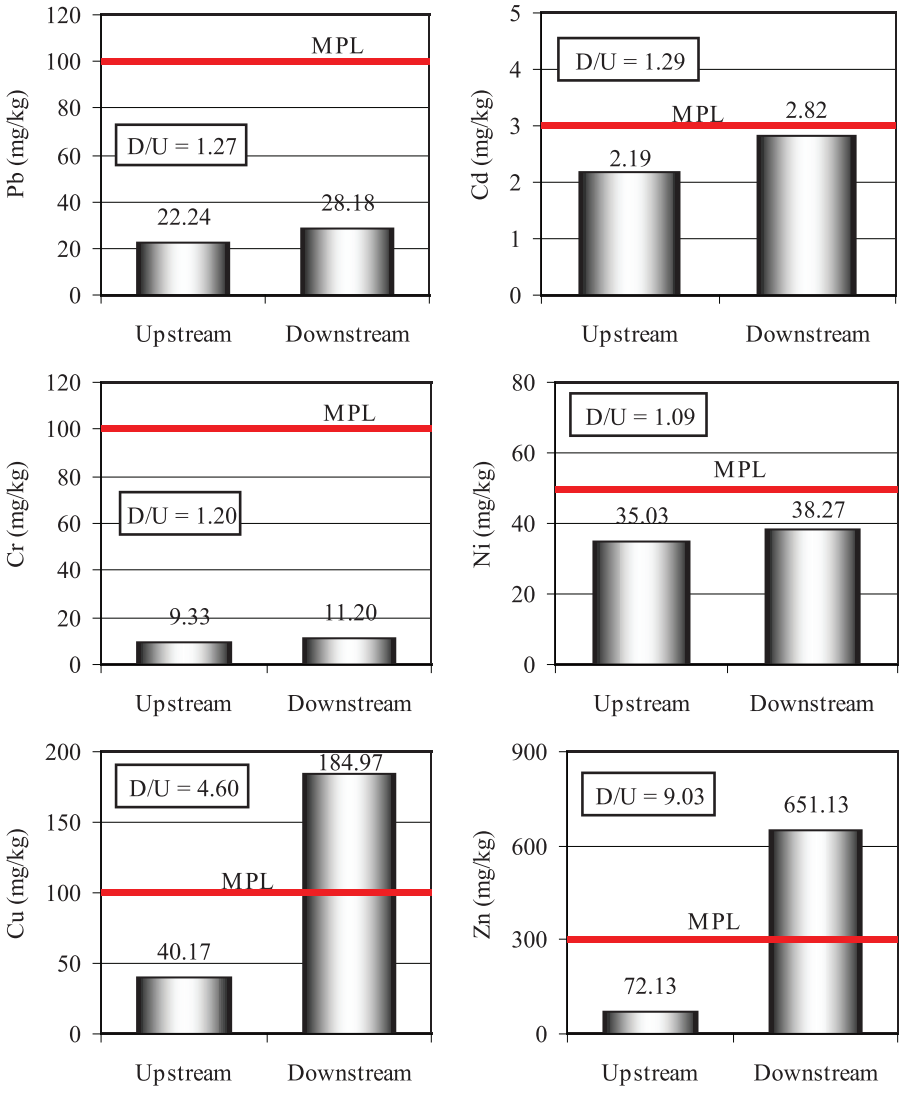

Figure 2. Trace elements content, Concentration increase coefficient (D/U) and Maximally permitted level (MPL) in channel' sediments taken at up/downstream locations in comparison to discharge point from pig breeding farm

Slika 2. Sadržaj teških metala u uzorcima sedimenata iz melioracionog kanala pre (uzvodno) i nakon izliva (nizvodno) otpadnih voda iz lagune svinjogojske farme, maksimalno dozvoljene koncentracije (MPL) i indeks povećanja sadržaja u nizvodnom uzorku u odnosu na uzvodni (D/U)

Excessive presence of analysed elements in channel sediments is the most probably due to the facts that Cu and Zn are using as supplements in animal feed for better fattening results. Namely, although Cu and Zn concentrations in animal food composite are mostly in consistence with permitted and regular levels as stated in Regulation book for maximally levels of contaminants and supplements in animal food, and in Regula-

tion book of animal food quality (Ćupić et al., 1997), nevertheless it was confirmed their excessive presence in the analysed sediment samples. Observed results indicate on the possibility of inappropriate animal feeding management (e.g. low nutrient use efficiency, excessive supplement usage etc) at animal farms. Since observed elements are potentially highly toxic and harmful substances, their excessive presence in channel sediments represent tremendous ballast in sustainable water and land management.

Although many legislation and regulation documents, acts and/or directives from environment protection area clearly indicate to potentially highly risk of endanger under/ground water sources by different nutrient and contaminant elements originating from agriculture, in effect there is still considerably different situation which is not in accordance with decelerated intentions and attentions for protection of drainage basin area zones.

CONCLUSION

By presented study was confirmed the influence of pig breeding farm to properties of sediment material in recipient KC-III channel near of Vrbas.

- Nutrients and trace elements content in downstream sediments (after discharge point of waste water from farm) was by 2-4.5-fold higher compared to upstream sediment (before discharge point)
- Content of the most observed trace elements (Pb, Cd, Cr, Ni, Co, Mn, Fe) was increased (10-30%) in downstream (vs. upstream) sediment samples, however their concentrations were not exceed MPC for certain element
- The most significant increment was observed at Cu (4.6-fold) and Zn (9-fold), whose concentration exceeded MPC by 2-fold (Cu), namely by 2.5-fold (Zn)
- Cu and Zn are very important animal supplements that by inappropriate feeding management at farms can pose deleterious environmental impacts
- It is strongly recommended that in accordance by actual legislation and regulation in environment protection area, discharge of waters with inappropriate quality (e.g. waste water from farms) to melioration recipient channel network should be restrained and strongly controlled. Modern environmental concepts imply that melioration channel network not served as hydrotechnical objects only (especially not as recipients for unrefined waste water), but also should have and important surrounding ecological functions and values.

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UTICAJ OTPADNIH VODA SVINJOGOJSKE FARME NA SVOJSTVA SEDIMENATA U KANALU RECIPIJENTU

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Izvod

U kanal za odvodnjavanje u okolini naselja Vrbas upuštaju se otpadne vode iz lagune svinjogojske farme. Razmatran je njihov uticaj na svojstva sedimenata, mulja, na dnu ovog kanala - recipijenta otpadnih voda. Analizirani parametri kvaliteta sedimenata zahvaćenih uzvodno (pre izliva) i nizvodno (nakon prijema otpadnih voda) značajno se međusobno razlikuju. Pored povećanja sadržaja svih nutrijenata (2 do 4,5 puta) uticaj se manifestuje i na povećano prisustvo teških metala i mikroelemenata u nizvodnom uzorku mulja za oko 10-30%. Izdvajaju se visok porast koncentracija bakra (4,6 puta) i cinka (preko 9 puta) u nizvodnim uzorcima. Pri tome konstatovane koncentracije bakra od 185 mg/kg, odnosno cinka od 651 mg/kg, premašuju maksimalno dozvoljene vrednosti u sedimentima (1,8 puta, odnosno 2,2 puta, redom za bakar i cink) što ukazuje na moguće nepovoljne posledice po stanje životne sredine u samom kanalu, njegovom priobalju i širem okruženju.

Ključne reči: kanalski sedimenti, nutrijenti, teški metali, svinjogojska farma.

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DIURNAL VARIATION OF BLOOD METABOLITE IN DAIRY COWS DURING HEAT STRESS*

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SUMMARY: Climate change, defined as global warming, has implication on our geographical area. THI>72 manage dairy cows to heat stress and over heat. We hypothesized that diurnal short term variation of THI (moderate heat stress in province of Vojvodina) can alter energy balance in dairy cows. It was founded significant increase of NEFA and urea concentration in stress-period of day. Also was detected significant decrease of glucosa and total cholesterol concentration. Protein concentration were increased in hotter period of day, but not significantly. Moderate heat stress in Vojvodina has potency to alter energy balance in dairy cows, which agree with our previously results about negative impact of heat stress on milk production, general health and reproduction.

Key words: *heat stress, dairy cows, NEFA, glucose, urea, protein, total cholesterol, diurnal variation.*

INTRODUCTION

Climate change, defined as global warming, has implication on our geographical area. Data about average temperature in Serbia showed increase for more then 1.2°C. Also, number of tropical day showed increase from 10 to 30 day/year in differential region (HMZS). Current climate models indicate a 0.28C increase per decade for the next two decades and predict the increase in global average surface temperature by 2100 may be between 1.88C and 4.08C (IPCC, 2007). Combination of ambient temperature and humidity is the most important sign of heat stress presence in dairy cows. Heat stress in dairy cows occurs when THI index is higher then 72 (Dikmen and Hansen, 2009).

Original scientific paper / *Originalni naučni rad*

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Heat stress is a important financial burden, because his typical consequence is decreased milk production (Cincović and Belić, 2009). Endocrine acclimatoin to hot environment including: reduced glucocorticoid, aldosteron, somatotropin, thyroxine and sex hormone secretion an increased epinephrine, leptine and prolactin secretion. Metabolic adaptation is characterised by: negative energy balance (NEBAL), with decrease of glucose concentration and increase of non-esterified fatty acids (NEFA) and BHB in dairy cows. And last but not least hematological change implies increase in RBC number, decrease in hemoglobin concentration and change in neutrophile:lymphocyte ratio (Cincović et al, 2010; Belić et al, 2010; Bernabucci et al, 2010). In Vojvodina's geographical area days with THI>72 can be detected during late spring, whole summer and early autumn. In summer average THI is about 68, but during sunny day THI is out of thermoneutral zone. Duration of time in which THI is higher then 72 is from four to seven hour per one sunny day. THI is often in thermoneutral zone during the night. That imply diurnal variation of THI, which is important risk factor to negative response to heat stress (Cincović et al, 2010).

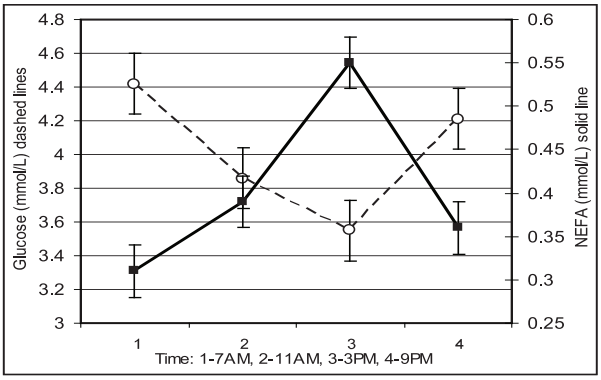
The aim of this study is to investigate diurnal variation of metabolite, because we hypothesized that diurnal short term variation of THI can alter energy balance in dairy cows.

MATERIAL AND METHOD

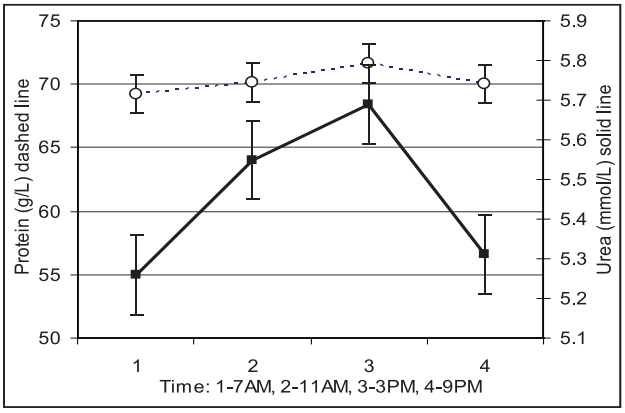
In experiment were included 15 cows which showed decreased milk production and increased rectal temperature and respiration rate during heat stress. Experiment was performed during july and august. Cows are in second and third parity and in middle lactation. All cows were fed a total mixed ration (TMR) with premix for high yield cows.

Blood samples were colected at 6:00 AM, 11:00AM, 3:00PM and 9:00PM. Blood was centrifuged and analysed immediately after sampling. In each plasma sample was measured: glucose, NEFA, protein, urea and cholesterol concentration. Measurements of metabolites were performed using commercial photometric assays in semiautomatic analyser Rayto-RT 1904C. The statistical analysis involves Z-test for mean value.

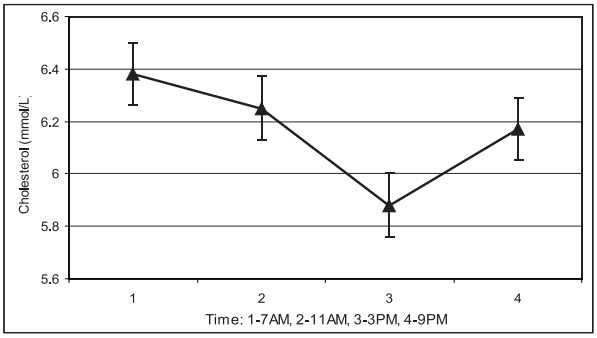
RESULTS



Graph 1: Diurnal variation of glucose and NEFA concentration
Grafik 1: Diurnalna varijacija koncentracije glukoze i neesterifikovanih masnih kiselina



Graph 2: Diurnal variation of protein and urea concentration
Grafik 2: Diurnalna varijacija koncentracije uree i proteina



Graph 3: Diurnal variation of cholesterol concentration
Grafik 3: Diurnalna varijacija koncentracije holesterola

DISCUSSION

Short term heat stress with diurnal variation of THI leads dairy cows to negative energy balance. This fact was confirmed by our results, which shoved decrease of blood glucose and increase of NEFA concentration (Graph 1) ($p<0,01$). In previously research was found decreased glucose concentration during heat stres compare with thermoneutral zone (Cincović et al, 2010). Glucosa and NEFA are in negative corelation. Reasons for development of NEBAL are reduced in dry matter intake and change in anabolic axis (Rhoads et al, 2009). Second important reason for increasing of NEFA is increased concentration of circulating cortisol, norepinephrine and epinephrine during acute heat stress (Alvarez and Johnson, 1973). Diurnal variation of THI from neutral to stress level precluding dairy cows to good acclimation and adaptation, which sign is elevated level of mentioned hormones. This is according to standard concept of stress with alarm reaction. NEFA is product of adipose mobilization, and activation of catabolic axis may indicate that sign of negative energy balance can exist without change in dry matter intake.

Heat stressed cows showed higher urea concentration (Graph 2) ($p<0,05$). This result agree with previous findings (Shwartz et al, 2009). Plasma urea nitrogen can originate from at least two sources: inefficient rumen ammonia incorporation into microbial proteins or from hepatic deamination of amino acids mobilized from the skeletal muscle (Kaneko et al, 2008). Decrase cholesterol concentraton (Graph 3) ($p<0,05$) could be in contact with decreased dry matter intake in heat-stressed cows (Moore et al, 2005). Diurnal variations of urea and TC are mostly related to feed time and intake (Nikkhah et al, 2008).

CONCLUSION

Diurnal variation of THI during summer day expose dairy cows to heat stress. In heat-stressed cows were found diurnal variation of metabolite. Increasing concentration of NEFA and urea with decrease concentracion of glucose and cholesterol were significant change in metabolic profile during stress-period of day. This picture suggest negative energy balance in dairy cows during heat stress, despite thermoneutral night in our geographic area.

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DIURNALNA VARIJACIJA METABOLITA KRVI KOD KRAVA TOKOM TOPLOTNOG STRESA

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Izvod

Klimatske promene, definisane kao globalno zagrevanje, sve više pokazuju uticaj i na naš geografski region. THI>72 dovodi mlečne krave u stanje toplotnog stresa i pregrejavanja. Pretpostavili smo da kratkotrajna diurnalna varijacija vrednosti THI indeksa (što je karakteristika umerenog toplotnog stresa u Vojvodini) može izmeniti energetski bilans u organizmu mlečnih krava. Nađeno je da koncentracija NEFA i uree signifikantno raste tokom stresnog perioda dana, dok koncentracija glukoze i ukupnog holesterola opada. Koncentracija proteina je bila povišena tokom toploijeg dela dana, ali ne signifikantno. Srednje jak toplotni stres na teritoriji Vojvodine ima moć da ošteti

energetski status krava, što se slaže sa našimpredhodno dobijenim rezultatima o negativnom učinku toplotnog stresa na proizvodnju mleka, opšte zdravlje i reprodukciju mlečnih krava.

Ključne reči: toplotni stres, mlečne krave, NEFA, glukoza, urea, proteini, ukupni holesterol, diurnalne varijacije.

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MORPHOLOGY OF ERYTHROCYTES AND KETOSIS IN DAIRY COWS WITH DIFFERENT BODY CONDITION

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SUMMARY: Ketosis of dairy cows is a disease that occurs as a result of negative energy balance. It is characterized by increasing concentration of ketone body. Ketone bodies cause the state of oxidative stress in the body. Oxidative stress is more pronounced in cows with high body condition score. The aim of this study was to examine the difference in the values of erythrocyte parameters in healthy cows and cows suffering from subclinical ketosis in the function of body condition. Proved a statistically significant difference in the concentration of hemoglobin in healthy and diseased cows ($p<0.01$), and hemoglobin concentration was lower in cows with high body condition score ($p<0.05$). It was found a higher hematocrit value in cows with ketosis, but not significantly. MCV, MCH and MCHC have been modified in the function of the previously mentioned changes, but not statistically significant. Clinical analysis showed that over 75% cows with ketosis show decrease of the concentration of hemoglobin, while the 15% value of hemoglobin remained similar and in 10% of the cows is slightly higher than the average of healthy controls. Microscopic examination of blood smear determined an increase in the number of morphologically altered red blood cells in cows with ketosis (5:9.5%, $p<0.05$), as well as an increase in red blood cells whose diameter was outside of physiological values (3,77:6,54, $p<0.05$). Poikilocytosis is significant frequent in obese cows ($p<0.05$). Anizocytosis and poikilocytosis are negatively and linearly dependent on the concentration of hemoglobin ($p<0.01$). These results may be related to the state of oxidative stress and altered liver function of dairy cows during ketosis.

Key words: *dairy cows, ketosis, erythrocyte morphology, body condition.*

INTRODUCTION

Ketosis occurs when cows are in negative energy balance. This most commonly happens in the last 2 weeks of pregnancy or in early lactation. In the last weeks of gesta-

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tion hormonal factors and decreased rumen capacity may cause a decrease in nutrient intake and/or an increase in lipolysis. Although the volume of milk production and lactose formation is the predominant demand for energy, there is also a secondary (or possibly primary in some cows) lipid demand for milk fat synthesis. Elevated concentration of non-esterified fatty acids in plasma, beta-oxidation of fatty acids for energy production and hyper-ketonemia are the major biochemical changes recorded in ketosis (Dann et al, 2005). The free fatty acids and elevated ketone bodies induce state of oxidative stress (Bouwstra et al, 2008; Jain and McVia, 1999). Oxidative stress is characteristic of periparturient period and it is depend from body condition score in dairy cows (Bernabucci et al, 2005). Erythrocytes are very sensitive to oxidative stress, because of specific lipide bilayer and hemoglobin and iron physiology. Markers of oxidative status in erythrocytes are very usable for oxidative stress assessment (Armstrong, 2010). Latest research shows that incorporation of antioxidants, vitamin E and selenium in conventional treatment with hypertonic dextrose saline solution and corticosteroid of cows suffering from subclinical ketosis reduces erythrocyte lipid peroxides level, and oxidative stress associated with the disease (Sahoo et al, 2009).

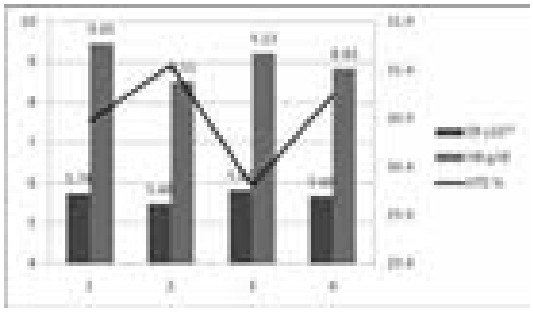
The aim of this paper was to investigate influence of ketosis on erythrocyte morphologic characteristic.

MATERIAL AND METHOD

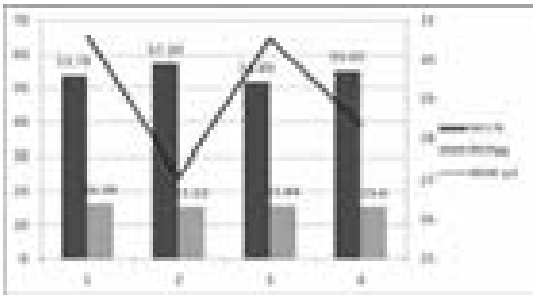
In experiment were included 30 health and 30 cows with subclinical ketosis (15 thin and 15 obese cows in group). There were 4 experimental groups of cows: obese and healthy (group 1), obese with ketosis (group 2), thin and healthy (group 3) and thin with ketosis (group 4). Cows are the same age and production categories and grown under same environmental conditions. Milk was tested for ketone bodies with Rothera test powder, consisting of sodium nitroprusside 50 mg, anhydrous sodium carbonate 10 g, and ammonium sulphate 20 g. Results were graded from 0 (negative) to 3 (maximum).

Blood samples were collected by jugular venipuncture, with the use of anticoagulant heparin. After collection, the blood sample was immediately transferred to the laboratory for estimation of hematological parameters. Hematological parameters like hemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) were determined as per the standard procedure. Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated using Hb, PCV and TEC. Also, was analyzed red blood cell distribution width (RDW), and change in erythrocyte shape in blood smear.

RESULTS



Graph 1: Erythtocyte number, hematocrite and henoglobin concentration in 4 examined groups
Grafik 1: Broj eritrocita, hematokrit i koncentracija hemoglobina u 4 ispitivane grupe

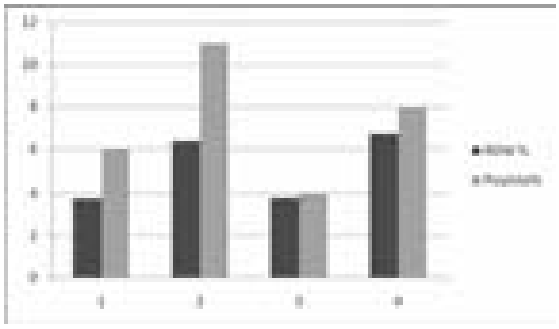


Graph 2: MCV, MCH and MCHC in 4 examined groups
Grafik 2: MCV, MCH i MCHC u 4 ispitivane grupe

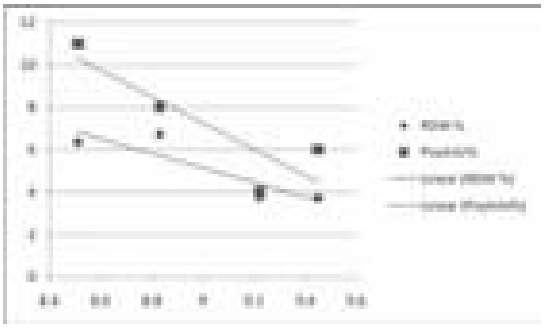
Table1: Clinical examination - cows with changed hemoglobin concentration
Tabela1: Kliničko ispitivanje - krave sa promenama u koncentraciji hemoglobina

	Group/ Grupa 2			Group/ Grupa 4		
	a*	b	c	a	b	c
HB g/dl	13 (86,6%)	1 (6,7%)	1 (6,7%)	10 (66%)	3 (20%)	2 (14%)

*a-decreased, b-in level with healtly cow, c-increased



Graph 3: % of erythrocytes with changed shape and size
Grafik 3: % eritrocita izmenjenog oblika i veličine



Graph 4: Linear dependence between hemoglobin concentration and value of RDW and % of changed erythrocyte
Grafik 4: Linearna zavisnost koncentracije hemoglobina sa vrednošću RDW i % izmenjenih eritrocita

DISCUSSION

The subclinical ketosis in dairy cows is a significant health and economic problem. Negative energy balance and lipomobilization introduced during ketosis the body into a state of oxidative stress, and that is in relationship with body condition (Bernabucci, 2005). Recent research showed that antioxidant activity of red blood cells changed during ketosis (Sahoo et al, 2009). Our hypothesis is that subclinical ketosis might change erythrocyte morphology, and change is in function of body condition. Hematologic parameters during subclinical ketosis, in this study, are in the normal physiological range (Moris, 2009), which coincides with earlier results (Kumar et al, 2001). However, we faund significant differences in erythrocyte morphology in healthy cows and cows with subclinical ketosis. Proved a statistically significant difference in the concentration of hemoglobin in healthy and cows with ketosis ($p<0.01$), and hemoglobin concentration was lower in cows with high body condition score ($p<0.05$) (Graph 1). Clinical analysis showed that over 75% of cows with ketosis shows a decrease of hemoglobin concentration, while in 15% of the value of hemoglobin remained similar, and in 10% of the cows is slightly higher than the average of healthy controls (Table 1). Decrease of hemoglobin concentration might be metabolic adaptation to stress. Hemoglobin concentration decrease during periparturient period (Gavan et al, 2010), and during heat stress (Belić et al, 2010). Both physiological conditions are characterized by negative energy balance, similar to ketosis (Bernabucci et al, 2010). Ketosis is characterized by fatty infiltration of liver (Đoković et al, 2008), which has important role in iron and hemoglobin metabolism (Kaneko et al, 2008). Obese cows showed lower concentration of hemoglobin ($p<0.05$). Difference between MCV, MCH and MCHC index were not statistic significant.

Anisocytosis is mild to moderate in ruminants. Red blood cell shape is relatively uniform, but poikilocytosis is not unusual in blood smears from apparently healthy calves (Raskin and Wardrop, 2010). Microscopic examination of blood smear cow with subclinic ketosis determined an increase in the number of morphologically altered red blood cells (5:9.5%, $p<0.05$). The number of red blood cells whose diameter is outside of physiological values was increased (RDW 3.77:6.54, $p<0.05$) (Graph 3). Poikilocytosis

is significant frequent in obese cows ($p<0.05$). Poikilocytosis is physiological response to oxidative stress, which were investigated by hyperbaric oxygen in controlled condition (Belić i Cincović, 2009). Anisocytosis and poikilocytosis are in linear function with hemoglobin concentration ($p<0.01$) (Graph 4).

CONCLUSION

Cows suffering from subclinical ketosis show changes in haemogram. The changes are reflected in the reduced hemoglobin concentration and changes in shape and size of erythrocytes. These changes were more pronounced in obese cows. These changes have no diagnostic significance, but further research could examine the significance of erythrocyte indicators in the assessment of ketosis.

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MORFOLOGIJA ERITROCITA I KETOZA KRAVA RAZLIČITE TELESNE KONDICIJE

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Izvod

Ketoza je bolest mlečih krava koja nastaje kao posledica negativnog energetskog bilansa. Ona se odlikuje porastom koncentracije ketonskih tela u krvi. Ketonska tela izazivaju stanje oksidativnog stresa u organizmu. Oksidativni stres je izraženiji kod krava sa višim skorom telesne kondicije. Cilj našeg istraživanja bio je da ispitamo da li postoji razlika u vrednosti eritrocitnih parameta kod zdravih krava i krava obolelih od ketoze u funkciji telesne kondicije. Dokazana je statistički značajna razlika u koncentraciji hemoglobina kod zdravih i obolelih krava ($p<0,01$), a koncentracija hemoglobina je niža kod krava sa visokim skorom telesne kondicije ($p<0,05$). Pronađena je viša vrednost hematokrita kod krava sa ketozom, ali nije signifikantno. Eritrocitni indeksi su izmenjeni u funkciji predhodno navedenih promena, ali nisu statistički signifikantni. Kliničkom analizom je utvrđeno da preko 75% ketoznih krava pokazuje pad koncentracije hemoglobina, dok je kod 15% vrednost hemoglobina ostala slična, a kod 10% krava je nešto viša od proseka zdrave kontrole. Mikroskopskim pregledom razmaza krvi krava sa subkliničkom ketozom utvrđen je porast broja morfološki izmenjenih eritrocita (5:9,5%, $p<0,05$), a raste i broj eritrocita čiji je dijametar van fiziološke vrednosti (RDW 3,77:6,54, $p<0,05$). Pojkilocitoza je izraženija kod gojaznih krava ($p<0,05$). Anizocitoza i pojkilocitoza su u linearnoj funkciji sa koncentracijom hemoglobina ($p<0,01$). Ovakvi rezultati mogu biti u vezi sa stanjem oksidativnog stresa i izmenjenom funkcijom jetre tokom ketoze mlečnih krava.

Ključne reči: mlečne krave, ketoza, morfologija eritrocita, telesna kondicija.

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THE METEOROLOGICAL AND HYDROLOGICAL DATA RELATED TO THE IRRIGATION IN THE PROVINCE OF VOJVODINA

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SUMMARY: This paper deals with the role and the state of meteorological and hydrological data required for the planning and conducting of irrigation in the Vojvodina Province. Also, the overview of relevant meteorological and hydrological measures in the Vojvodina Province is given. The paper deals with the given conditions for determining required irrigation water quantities in the mentioned area. The attention is directed to the fact that there is a lack of water in the periods of droughts. The analysis of basic climatic data revealed that the dry years were more common for the period after 1981. The calculated water balance indicates that the total water amount used by evapotranspiration during growing season is larger than the total amount of precipitation in this region.

Key words: *meteorological and hydrological data, drought, water balance, irrigation*

INTRODUCTION

The Vojvodina Province is the northern part of Serbia and it is predominantly an agricultural region. This area is characterized by a continental climate with accentuated annual variability of air temperatures, as well as the others climatic elements on which the soil water regime depends. There is a serious lack of water in the periods of droughts. The droughts that have occurred in the Vojvodina Province in recent years were of catastrophic proportions in respect of agricultural production. They were mainly a consequence of the shortage in rainfalls and/or their unfavorable time distribution. Drought is manifested as a shortage of moisture in the soil, especially in the summer months, when evapotranspiration is most intensive. The fact is that in the agroecological condition of the Vojvodina region, the quantity of precipitation without irrigation

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is not sufficient for crop water requirements. Due to favourable natural conditions, the region of the Vojvodina Province has great potentials for agriculture with irrigation. The planning and conducting of irrigation is impossible without meteorological and hydrological issues. Meteorological and hydrological data could be obtained in the Republic Hydrometeorological Service of Serbia, via measurements and observations obtained at meteorological and hydrological stations. The Service performs a continuous monitoring of weather conditions through measurements and observations at numerous meteorological stations defined by the World Meteorological Organization (WMO). Systematic observations and measurements of surface and ground water at hydrological stations are carried out by the Department of Hydrological Measurement and Observation. The surface water station network of the regional hydrological station Novi Sad consists of 39 stations. The necessary meteorological and hydrological data for solving irrigation problems depend on the type of task which should be solved. Generally, there are two tasks. The first is concerned with the determination of irrigation water requirements as well as irrigation regime. The second task is aimed at establishing and ensuring the water supply for compensation crop water requirements. This paper deals with the given conditions for determining irrigation water requirements in the Vojvodina Province.

RESEARCH RESULTS

The review research of climatic elements, droughts and water balance is given in this paper.

Climatic data: The climatic conditions of the Vojvodina Province display the features of moderate continental climate (Katic et al., 1979). The amount of precipitation (Table 1) often varies, especially during growing period. The coefficient of variation (C_v) ranges from 49.1 % for June to 80.1 % for October. June is usually a month with the highest amount of precipitation in this area. The air temperature is a stable climatic element with a higher coefficient of variation during the winter due to low temperatures. It is important to consider that it ranges during growing period from only 5.6 % in July to 14.4 % in April. The coefficient of variation (C_v) for the sum of sunshine hours is the smallest for August (12.9 %). The values of relative air humidity are relatively stable during the analyzing period of 39 years. The coefficient of variation (C_v) ranges from 3.3 % for December to 11.1 % for August. The relative air humidity is a climatic element with the smallest values of the coefficient of variation. The highest values of wind speed are in March and the smallest during the summer. The coefficient of variation (C_v) ranges from 16.5 % for April to 31.7 % for January. The reference crop evapotranspiration for each month is determined by using the Penman-Monteith Method (Rajic and Rajic, 2005). The values of reference crop evapotranspiration are very changeable from year to year. It is concluded on the basis of calculated values for every year during the observation period. The maximum values of reference crop evapotranspiration are approximately 30% greater than corresponding minimum values. Average values of reference crop evapotranspiration during growing season are approximately 80% of the corresponding yearly values. The values of reference crop evapotranspiration increase from the beginning of growing season to July and decrease from July to September. The coefficient of variation (Cv) ranges from 10.3 % for July to 14.5 % for September dur-

ing growing period. The biggest value for coefficient of variation (Cv) during the whole year is 23.2 % for March.

Table 1. Average monthly precipitation, P (mm), air temperatures, t (°C), sunshine hours, N (hour), relative air humidity, RH (%),wind speed, u (m/s) and reference evapotranspiration, ET_o (mm**),** as well as average annual values and values for growing period for Meteorological station Rimski Sancevi, Vojvodina Province (Rajic et al., 2006).
Tabela 1. Srednje mesečne vrednosti sume padavina P (mm), temperature vazduha t (°C), osunčavanja N (časova), relativne vlažnosti vazduha RH (%), brzine vetra u (m/s) i referentne evapotranspiracije ET_o (mm), kao i srednje godišnje vrednosti i vrednosti za vegetacioni period za meteorološku stanicu Rimski Šančevi (Rajić i sar., 2006)

Periods Periodi	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Year God.	G.P. Veg.p.
P (mm)	35.2	32.2	37.1	51.2	58.9	87.4	69.2	57.3	44.9	54.4	49.9	46.2	624	369
T (oC)	-0.3	2.0	6.4	11.3	16.9	19.7	21.4	21.0	16.7	11.5	5.8	1.4	11.2	17.9
N (hour)	66.8	99.7	154	181	234	259	287	276	202	164	86.1	59.7	2069	1439
RH (%)	86	79	71	68	67	70	68	69	73	76	83	86	75	69
U (m/s)	2.9	3.2	3.5	3.1	2.6	2.2	2.1	2.0	2.1	2.6	2.9	2.9	2.7	2.4
ETo(mm)	14	25	53	80	116	128	139	122	76	47	22	13	835	661

An analysis of probability of occurrence of minimal precipitation on the territory of Vojvodina (Stojsic, 1974) found that all precipitation below the Vojvodina average which occur once in five years or rare, causing a drought in hydrologic year or in its seasons.

On the basis of obtained results of the precipitation analysis on the territory of Vojvodina (Putaric and Josimov-Dundjerski, 1998) it is concluded that the investigated period 1950/51 – 1991/92 is characterized by a decrease of annual precipitation.

Drought: Drought is a phenomenon of very complex properties. Either single component or a number of components can describe it. It can be determined by either one or more components such as periods of no rainfall, insufficient rainfall, high temperature, low relative air humidity, high evapotranspiration, etc. Although many definitions and indicators of droughts exist, the fact is that the main cause of drought is insufficiency of rainfall. Also, the definition of drought depends on the goal of the analysis. For example, in hydrology, drought is defined as a period of time during which the natural river discharge is lower than needed for water supply, navigation, or some other economic purpose. In meteorology it could be defined as extreme rainless periods or periods with the sum of precipitation considerably below the average sum. From the agricultural point of view, the term of drought is referred to periods during which the soil moisture is significantly below the average and insufficient for plant growth and development in the growing season. Drought can produce wide spread undesirable impact to the environment, what lead to the health problems of population. The vulnerability of region to drought depends on the climate, topography, soil, vegetation, and the case in which mentioned resources are used (Jones, 1987). The danger of drought depends on many factors, which are different from one region, country, or year to another. Despite of improved knowledge in this field, there are still many uncertainties at the global, regional and national level about the causes, the extent, and the seriousness of droughts (Rajic et al., 2003). However, in drought prone areas, agriculture is virtually impossible without an irrigation system.

The drought analyses in the Vojvodina Province were investigated by many authors. The results of complete analysis of the stochastic process of extreme dry weather intervals during growing season at 22 meteorological stations in the Vojvodina Province show that the part of the growing season with the highest probability of having the longest extreme rainless period refer to the period of the second half of August and September. Periods of no rainfalls have tendency of increasing from south-west to north-east. The analysis included all available data on extreme dry weather intervals, which are defined as the upper extremes of intervals of no rainfall longer than 15 days (Beric i sar., 1987), (Beric et al., 1988), (Beric et al., 1990), (Beric and Neskovic-Zdravic, 1995), (Beric and Salvai, 1995). In the period of 41 years taken for the analysis, even in 90% of them there was smaller or greater water shortage, i.e. a drought of the different degree was observed (Dragovic et al., 1989). Also, 32% drought years are obtained by analysing annual precipitation sum in Vojvodina during multiyear period and 53% by analysing precipitation sum during growing period (Dragovic, 1989). These past few years, droughts have been a common occurrence in the Panonian Basin. The values of PAI ("drought index") indicated that after the dry 1980's, 1990 was also a drought year (Palfai, 1989), (Palfai & Darnai, 1990). In order to determine dry years, following criteria have been used: the hydrotermic coefficient by Seljaninov, the index of aridity by De Martone, the rain factor by Lang and the coefficient and ratio between precipitation and evapotranspiration for the growing period and for whole year (Rajic et al., 2006). Three dry years are separated during period of 39 years for the meteorological station of Rimski Sancevi, according to ratio between precipitation and evapotranspiration for the whole year. During these years, the ratio is extremely unfavorable and it was less than 0.5. According to the ratio between precipitation and evapotranspiration for the growing period 15 years are separated. In these years their ratio was unsuitable for agriculture production. There were 15, 23 and 14 dry years according to the index of aridity by De Martone, rain factor by Lang and hydrotermic coefficient by Seljaninov. These analyses and analyses of precipitation and other climatic elements showed more frequency of dry years after 1981.

Water balance: Also, drought is manifested as a shortage of moisture in the soil, especially in the summer months (July, August), when evapotranspiration is most intensive. The amount and distribution of precipitation were analyzed and the results have shown that the hydrological 1992 year was extremely dry and because of that the yield of field and vegetable crops were reduced (Dragovic and Labat, 1993). The water deficit in respect to crop water requirements was approximately 300-450 mm. The detailed investigation of soil water balance for the whole territory of the Vojvodina Province was made by Rajic (2000). The amount of water needed for irrigation represents the difference between the total amount of water required by crop for its growth and development and the amount of water available to the crop from rainfalls (effective precipitation). At the basis of calculated water balance for maize, sugar beet and soybean, for 11 meteorological stations, it was concluded that the total water amount used by evapotranspiration during growing season is bigger than total amount of precipitation in Vojvodina. The values of crop water requirements in the Vojvodina Province increase from the beginning of growing season to July and decrease from July to September for all analyzed meteorological stations during growing season. The calculated water deficits during growing season for each of analyzed years are subjected to statistical analyses in order to get the most suitable theoretical distribution function (Rajic and Rajic, 2005). So,

the theoretical and observed distribution function of net water deficit, which should be covered by irrigation, is obtained. The obtained results of water balance for different soil types indicate water deficit in every growing season. The values of water deficit are very changeable during years. According to the maps showing the contours of average water deficit for maize, sugar beet and soybean in the Vojvodina Province (Rajic, 2000) it could be concluded that values of water deficit for all three crops increase from the west, south-west and south-east in the direction of north and north-east. The analyses of water balance indicate that the maximum values of water deficit are approximately 2.5 times bigger than corresponding minimum values for analyzed meteorological stations in Vojvodina Province (Rajic, 2003). Consequently, under the present climatic conditions in Vojvodina an effective and stable agricultural production is possible only by applying crop irrigation (Rajic, 2007).

CONCLUSION

The research conducted in this paper shows that under the agroecological condition of the Vojvodina region, quantity of precipitation without irrigation is not sufficient for water requirements of crops. Although the Vojvodina Province has large water richness there is a lack of water in periods of droughts. The central challenge facing irrigated agriculture today and in the foreseeable future, not only in our country but in world too is how to produce more food with less water. Water is essential for irrigation purpose, but its indiscriminate use can lead not only to shortages, but also to the deterioration of crop yields and soils. It is hence vital to ensure that it is applied as effectively as possible. Irrigation system management should be careful organized because of rational exploitation of water-air regime of agricultural soil according to requirements of optimal crop development. It is necessary to apply only such amount of water that the crops need. At the basis of obtained results, suggestions and solutions for minimizing water deficit problems could be given. As agricultural production is a big user of natural recourses, the solutions of this problem should be at the same time economical, ecological and optimal for the resources. New solutions in crop production technology should be based on the choice of plant sort and determination of irrigation systems priority.

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METEOROLOŠKE I HIDROLOŠKE PODLOGE POTREBNE ZA NAVODNJAVANJE U VOJVODINI

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Izvod

U ovom radu govori se o podlogama koje su potrebne za određivanje potrebnih količina vode za navodnjavanje. U tu svrhu prikazani su rezultati analize osnovnih klimatskih elemenata, padavina, temperature vazduha, relativne vlažnosti vazduha, osunčavanja, brzine vetra i evapotranspiracije, kao i rezultati obračuna vodnog bilansa. Za ocenu suše na području Vojvodine prikazani su rezultati dobijeni analizom ekstremnih beskišnih perioda, indeksa suše De Martonne-a, kišnog faktora Lang-a, hidrotermičkog koeficijenta Seljaninova kao i koeficijenti odnosa sume padavina i evapotranspiracije za vegetacioni period i za godinu. Analiza osnovnih klimatskih elemenata ukazuje da su sušne godine mnogo češće u periodu posle 1981. godine. Na osnovu obračuna vodnog bilansa može se zaključiti da je ukupna količina vode koja se gubi evapotranspiracijom u toku vegetacionog perioda, veća od ukupne količine padavina u Vojvodini.

Ključne reči: meteorološki i hidrološki podaci, vodni bilans, suša, navodnjavanje

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PEDOGEOCHEMICAL MAPPING OF Cr, Hg, Ni AND Zn IN SOILS OF EASTERN SERBIA*

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SUMMARY: This study shows data about main parameters of soil fertility and total content of some harmful elements in three districts in Eastern Serbia. In most of the surface soil samples content of harmful elements does not exceed maximal allowed concentration (MAC). There are elevated concentrations of Ni and Cr in samples from Fluvisol of Velika Morava, and in Leptosol of Deli Jovan mountain, composed mainly from ultramafic and mafic rocks. Higher Hg content is in soils formed on carbonated substrates, in Arenosol of Ramsko-Golubacka sand pit and Fluvisol of the river Pek. The highest Zn content is in soil with pH interval from 6.0 to 6.5. Observed diferences are statistically proven by nonparametric inferential analysis of trace elements per districts, pH and types of soils.

Keywords: *Contamination, trace elements, pedogeochemical mapps.*

INTRODUCTION

Soil contamination is form of land degradation and major threat to sustainable development. Soil surveys and monitoring are performed by national programmes in most of European (Jones et al., 2005) and world countries (Oldeman et al., 1991). Survey on global level in Serbia started in 1991 and it still continues (Protić et al., 2005). These investigations enable identification of contamination sites and their "sphere of influence" and initiate measures for remediation and conservation of soil and other resources (Antić-Mladenović et al., 2009; Mihaljev et al., 2010).

Objective of this investigation is assesing the content of Cr, Ni, Hg and Zn in soils

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of Eastern Serbia, which are of very different characteristics, in order to identify polluted areas, source and nature of contamination.

MATERIAL AND METHODS

Soil sampling and laboratory methods

Soils of three districts, Zajecar, Branicevo and Bor, in Eastern Serbia were investigated. These districts are located between 43º21′- 44º40′ N and 21º 5′- 22º 45′ E. They occupy the area of 10 672 km^2. Total 979 surface soil samples were taken (0-25cm), by grid system at each 3.3 x 3.3 km. Field work was done in 2005-2006.

The basic characteristics of soil samples were determined by methods: soil pH - in a 1:2.5 1M KCl suspension (pH_{KCl}), potentiometrically; humus - by oxidation with solution $KMnO_4$ (after Kotzman); soil texture - combined method of sieving and pipette method. "Pseudototals", hot acid extractable forms of Ni, Cr, Hg and Zn were determined with an SensAA Dual atomic absorption spectrophotometer, after the soils were digested with concentrated HNO_3. All chemical analyses were performed in two replications. The MERCK standards were used for the determination on SEnsAA Dual. Referent soil sample (NCS ZC 73005 soil, CNAC for Iron and Steel) was used for the verification of the results for all studied elements.

Statistical analyses and mapping

SPSS 10.0 was used for statistical analysis of data. We used the Kruskal-Wallis and Mann-Whitney tests to compare the mean of Ni, Cr, Zn and Hg contents in different data sub-sets organized according to districts, soil type, pH. The software used for mapping was ESRI′ Arc View 8.3.

RESULTS AND DISCUSSION

Soil properties and trace elements - content, distribution and corellations

Soils which are suitable for agricultural production (with pH interquartile range from 4.2 to 6.15) are dominant and they are well supplied with humus and potasssium, and poorly suplied with phosphorus (tab.1).

The values of selected trace elements have asymetrical distributions, esspecially Hg. In most of the samples content of trace elements does not exceed MAC.

There are statistically significant corelations among investigated soils parameters, although they are very weak. Correlation coefficient between Ni and Cr is 0.309 (higher content of these elements in soils on ultramafic rocks and in Fluvisols of Velika Morava), and between Zn and Hg it is 0.382 (tab.2). There are positive correlations among Hg and Zn and the content of humus (higher content of these elements in some humic mauntain soils), and among Cr, Ni and Zn with pH (due to higher content of these elements in weakly acid, neutral and carbonated soils like Fluvisol, Leptosols on Serpentine, Folic Histosol, Calcic Cambisol, Rendzic Leptosol).

Table 1. Statistical summary of selected soil properties in Eastern Serbia topsoils
Tabela 1. Statistički parametri osobina površinskog sloja zemljišta Istočne Srbije

Parametri *Parameters*	Srednja vrednost *Mean*	St.. devijacija *St. Deviation*	Koef. varijacije *Coeff. variation* CV %	Skewness	Min	Max	Procenti *Percentages*			% uzoraka iznad MDK *% samples above MAC*
							25	50	75	
pH in KCl	5.15	1.13	1.28	0.18	3.00	7.60	4.20	5.00	6.15	-
Humus %	4.79	1.84	38.47	1.21	0.56	14.03	3.56	4.40	5.64	-
P_2O_5 mg 100g $^{-1}$	10.63	13.54	127.45	2.93	0.00	101.48	3.07	5.67	12.24	-
K_2O mg 100g $^{-1}$	26.27	10.37	39.49	0.00	3.60	41.00	17.60	25.00	38.00	-
$CaCO_3$ %	1.16	4.08	352.35	5.10	0.00	37.76	0.00	0.00	0.00	-
Cr mg kg $^{-1}$	23.75	19.20	80.84	2.81	0.20	185.79	11.83	20.96	31.08	1.30
Hg mg kg $^{-1}$	0.09	0.13	143.07	6.13	0.00	1.67	0.04	0.06	0.10	0.00
Ni mg kg $^{-1}$	24.34	22.60	92.86	3.06	0.06	208.19	9.80	19.80	31.59	7.40
Zn mg kg $^{-1}$	46.53	32.54	69.93	2.95	0.10	330.00	29.50	39.33	54.91	0.20

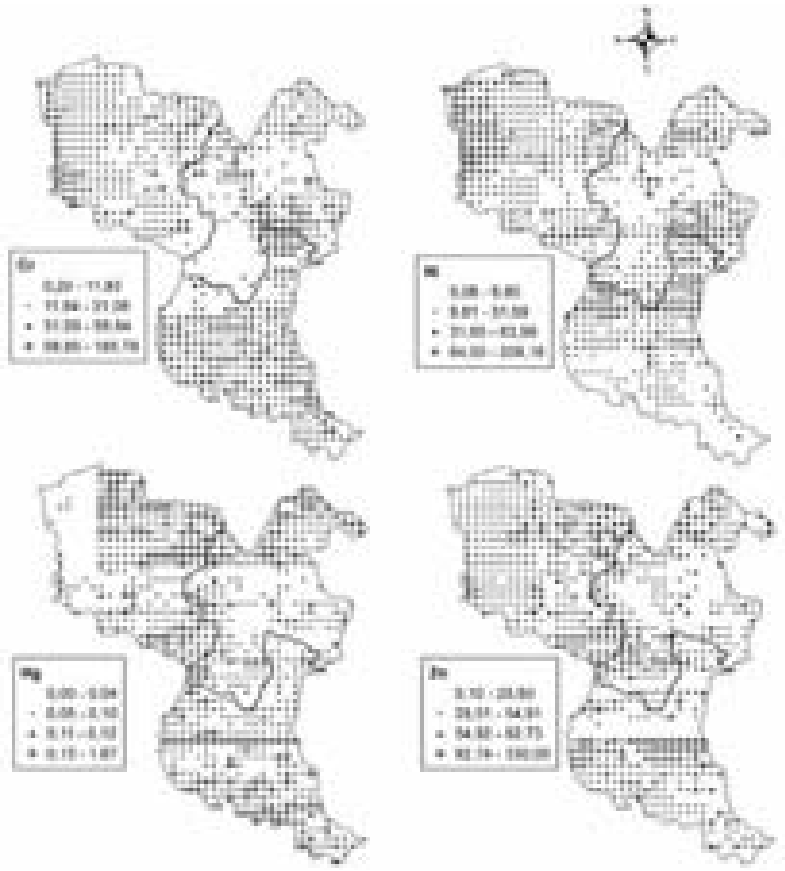
Table 2. Sperman´s correlations coefficient between the studied elements and soil properties
Tabela 2. Spermanov koeficijent korelacije između ispitivanih elemenata i osobina zemljišta

	Cr	Hg	Ni	Zn	pH	humus	P2O5	K2O	CaCO3
Cr	1.00	0.00	0.31**	0.17**	0.25**	-0.05	0.12**	0.13**	0.07*
Hg		1.00	0.00	0.38**	-0.01	0.31**	-0.03	0.03	0.08**
Ni			1.00	0.24**	0.19**	-0.01	0.16**	0.12**	0.08*
Zn				1.00	0.13**	0.28**	0.16**	0.18**	0.11**

***Korelacija je značajna na nivou 0.01.* (**Correlation is significant at the .01 level)
*** Korelacija je značajna na nivou 0.05.* (*Correlation is significant at the .05 level).

Maps. Spatial distributions of trace elements in three districts in Eastern Serbia are given on following maps (graph. 1-4). Classes were determined according to box-plot limits. Analyses of Kruskal -Wallis test among Cr, Ni, Zn and Hg and soil types, districts and pH values, show that there are very significant differences in content of these elements for all three parameters.

The results of Mann-Witman test show that content of **Cr** is significantlly different in all three districts (p=0.000). The smalest value is in Bor (median = 14.96 mg kg^{-1}), and the highest one is in Zajecar (28.50 mg kg^{-1}). In Zajecar district, there are elevated concentrations of Cr on Deli Jovan mountain, which is composed of piroxenic gabbro, harisite and dunites, and serpentines (Kalenic et al., 1976), which are naturally enriched with these elements (as well as with Ni, Co, Mg and Fe). Elevated Cr content is also in some samples located eastern from Knjazevac, which is related to chromites in smaller serpentinite bodies at the verge of granitic massif of Stara Planina mountain (Krstic et al., 1970). In Branicevo district elevated Cr content is in Fluvisol along Velika Morava river.



Graph. 1-4. Spatial distributions of Cr, Ni, Hg and Zn in Eastern Serbia topsoils
Graf. 1-4. Raspored Cr, Ni, Hg i Zn u površinskom sloju zemljišta Istočne Srbije

According to **Ni** content differences between Bor and Zajecar are not statistically significant (mediana 17.49 and 14.52 mg kg^{-1}). The highest content is in Branicevo (mediana 23.95 mg kg^{-1}). Similar to Cr, highest concentrations of Ni are in the alluvial deposits of the Velika Morava river (Branicevo district), deposited during holocen, which are partially composed of material brought by western river flows from surounding terrains that are composed of mafic and ultramafic rock. Previous investigation show that content of Ni and Cr is of natural origin, and that their content decline with soil`s aging (Jakovljević et al., 1997; Mrvić et al., 2009). Trace elemens have relatively small solubility, due to hard mechanical composition, and to neutral soil pH reactions (Antić-Mladenović et al., 2005). Besides Fluvisol of the Velika Morava, Ni is present in Feozem, near Pozarevac. In Zajecar district there are elevetad concentration of Ni on Deli Jovan mountain and on slopes of Kucaj mountain. Sources of Ni and Cr, besides geochemical, may also be the particle emissions form mine-smelter complex Bor, which is located in eastern district (Antonović et al., 1974; Adriano, 2001). The results of Mann-Witman test confirm that soils which are weakly acid and neutral (Fluvisol and Leptosol on ultramafic rock), are significantlly richer with Ni and Cr ($p<0.019$).

Bor district has significantlly smaller **Hg** content (mediana 0.058 mg kg^{-1}) in rela-

tion to Zajecar (mediana 0.061 mg kg^{-1}; p= 0.003) and Branicevo (mediana 0.068 mg kg^{-1}; p = 0.019). Within the studied area there are elevated concentrations of Hg in solis on carbonate substrate, in carbonate Arenosol of Ramsko Golubacka sand pit and in Fluvisol of the Pek valley. These results are comfirmed by Mann-Witman test among subgroups by pH, where soils with pH above 7.0 (p<=0.031), can be clearly distinguished. Significantlly higher **Zn** content is present in Branicevo district (mediana 42.81 mg kg^{-1}) in relation to Bor district (37.35 mg kg^{-1}) and Zajecar district (35.70 mg kg^{-1}). The highest concentrations are in Colluvail soils and Eutric Cambisol, and in Folic Histosol of Beljanica mauntain, i.e. in soils within pH interval from 6.0 to 6.5.

CONCLUSIONS

In Eastern Serbia soils with favourable reaction for agricultural production are dominant, well suplied with humus and potassium, and poorly with phosphorus. Most of the soil samples are not polluted with selected elements. Higher Ni and Cr content are in Fluvisol of the Velika Morava valley and in Leptosol on Deli Jovan mountain, which is mainly composed of mafic and ultramafic rocks. Elevated Hg content is present in soils on carbonates substrate, Arenosol of Ramsko-Golubačka sand pit and Fluvisol of the river Pek. Zink content is the highest in soils within pH interval from 6.0 to 6.5. Observed diferences are statistically proven by nonparametric inferential analysis of trace elements per districts, pH and types of soils.

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PEDOGEOHEMIJSKO KARTIRANJE Cr, Hg, Ni AND Zn U ZEMLJIŠTU ISTOČNE SRBIJE

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Izvod

U radu su prikazani osnovni parametri plodnosti zemljišta i sadržaj štetnih elemenata (Cr, Hg, Ni, Zn) u tri okruga Istočne Srbije. Sadržaj elemenata u najvećem broju površinskih uzoraka ne prelazi MDK. U uzorcima na aluvijumu Velike Morave povećane su koncentracije Ni i Cr, kao i u rankerima planine Deli Jovan, koju čine ultrabazične i bazične stene. Živa je u većim koncentracijama u zemljištima na karbonatnim supstratima, pesku Ramsko-Golubačke peščare u aluvijumu reke Pek. Sadžaj cinka je najveći u zemljištima pH intervala od 6.0-6.5. Primenom neparametarskih analiza elemenata u tragovima po okruzima, pH vrednosti i tipu zemljišta, statistički su potvrđene nađene razlike.

Ključne reči: zagađenost, elementi u tragovima, pedogeohemijske karte.

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EFFECT OF PROBIOTICS ON PRODUCTION AND QUALITY OF EGGS IN EARLY PHASE OF LAYING CYCLE

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SUMMARY: This paper presents the effects of dietary addition of probiotic Biomin® IMBO on production and table egg quality. 360 Hy Line Brown hens were used in experiment, which was conducted from 16 to 28 week of hen's age. Every week average body weight, egg production, percentage of second grade egg, average egg weight, feed consumption and feed conversion ratio were determined. Egg quality was evaluated at 21, 24, 26 and 28 week of hens age, and included the following parameters: egg weight, shell cleanness, shell weight, shell thickness, shell breaking force, albumen height and yolk color. The addition of dietary probiotic resulted in increased egg production and reduced percentage of second grade eggs. Feed conversion ratio for the entire period was significantly better in hens receiving probiotic, while feed intake and analyzed parameters of egg quality were not affected by added probiotic.

Key words: *probiotics, laying hens, egg production, egg quality*

INTRODUCTION

In the new economic circumstances, legislation about food safety and consumer affinity, poultry production has focused on the use of additives that will harmlessly maintain and improve production performance of poultry. With these intentions, commonly are used probiotics, phytogenic additives, prebiotics, organic acids and enzymes. Probiotics are additives which contain one species or mixture of several different species of microorganisms which added in feed or water enter the digestive system of the host and positively affect host benefit (Tellez et al., 2006). Some of the effects of probiotics are antagonistic action by producing substances with a bactericidal effect on

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pathogenic bacteria, competitive exclusion and the competition for nutrient by creating conditions for inhibition of pathogenic bacteria. The effects of probiotics on production performance manifest on reduction of disease risk and improved immunity and intestinal morphofunctional characteristics (Perić et al., 2009). Other authors reported better retention of calcium and phosphorus in laying hens (Nahashon et al., 1994) reduced levels of cholesterol in egg yolk and blood serum (Kurtoglu et al., 2004) increased egg production and better feed conversion ratio (Gallazzi et al., 2008; Xu et al., 2006). The aim of this study was to present results of egg production, nutrition and certain egg quality parameters, accomplished by dietary addition of probiotic Biomin IMBO®.

MATERIALS AND METHODS

The study was conducted in the experimental farm "Pustara" of Faculty of Agriculture in Novi Sad.

Table 1. Composition and nutrient content of laying hens diet
Tabela 1. Sastav i hranljivi sadržaj smeša u ishrani kokoši nosilja

Components, % *Komponente, %*	Pre-lay feed *Smeša pred pronošenje*	Layer feed *Smeša za nosilje*
Corn /*Kukuruz*	57.32	44.46
Wheat middlings /*Pšenično stočno brašno*	6.00	7.00
Oil /*Ulje*	/	2.00
Full fat soya (extruded) /*Punomasna soja (ekstrudirana)*	6.51	15.63
Soyameal (44% CP)/ *Sojina sačma (44% CP)*	11.64	9.54
Sunflower meal (33% CP) /*Suncokretova sačma (33% CP)*	9.5	9.00
Limestone /*Kreda*	6.26	3.25
Ca particles/ *Grit*	/	6.13
MCP /*Monokalcijum fosfat*	1.91	1.40
Salt /*So*	0.25	0.28
Sodium bicarbonate /*Natrijum bikarbonat*	0.15	0.10
L-lizin/*L-lizin*	0.07	0.05
DL Methionine /*DL metionin*	0.09	0.16
Premix /*Premiks*	1.00	1.00
Nutrient content, calculated /*Hranljivi sadržaj, kalkulativno*		
Crude protein /*Sirovi protein*	16.5	18.00
ME MJ/kg /*ME MJ/kg*	11.52	11.8
Lysine /*Lizin*	0.82	0.93
Methionine /*Metionin*	0.41	0.46
Calcium /*Kalcijum*	2,75	4,00
Avaliable P /*Raspoloživ P*	0,40	0,44

Experimental animals were housed in three-floor battery cages. A total of 360 commercial Hy Line Brown hens were divided into control and experimental group with six repetitions of 30 hens each. Hens from both groups consumed *ad libitum* standard diet for pre-laying period (16-18. weeks of age) and diet for laying period (Table 1). Hens in experimental group consumed diet with added probiotic Biomin® IMBO (BIOMIN GmbH, Herzogenburg, Austria) in the amount of 0.5 kg/T kg feed, while diet for control group was without probiotic. Probiotic contains probiotic bacteria *Enterococcus faecium* with fructooligosaccharides, cell wall fragments and phycophytic substances.

During the experimental period average body weight, egg production, percentage of second grade eggs, average egg weight, feed consumption and feed conversion ratio were determined every week. Egg quality was determined at 21, 24, 26 and 28 week. Monitored egg quality parameters were: egg weight, shell cleanness, shell weight, shell thickness, shell breaking force, albumen height and yolk colour. Shell cleanness was assessed by points on a scale from 1 (very dirty) to 5 (completely clean). Shell breaking force was determined by instrument Egg Force Reader (Orka Food Technology Ltd, Israel). Yolk colour was determined using the Roche fan. Albumen height was measured with a tripod micrometer. On the basis of egg mass (M) and albumen height (H), Haugh units were calculated according to formula $HJ=100\log(H+7{,}57-1{,}7M^{0.37})$. Albumen height in measurements for 26 week was not determined, due to technical reasons, and this parameter and Haugh units are not shown.

Obtained results were statistically analysed using GLM MANOVA and LSD *post hoc* test, with statistical software Statistica 8 (StatSoft, 2009).

RESULTS AND DISCUSSION

During the experimental period, hens of experimental group had higher body weight compared to control, but difference was statistically significant only in the 21 and 22 week of hen's age (table 2). Hens receiving probiotic had slightly higher body weight at the end of 28 week, but not significantly. Similarly, Kurtoglu et al. (2004), Bageridizaj et al. (2006), Yousefi et al. (2007), Gallazzi et al. (2008) found no effect of probiotics on the body weight of laying hens.

Statistical differences in laying percentage were found in the 23 and 24 week in favor of experimental group, but for entire period there were no significant differences. The absence of a significant increase in laying percentage when probiotic was used also reported Yoruk et al. (2004), Mahdavi et al. (2005) and Yousefi et al. (2007). In contrast to these results Kurtoglu et al. (2004), Gallazzi et al. (2008) reported on increased laying percentage. Average number of eggs per average laying hen, also, was not differing at the end of the experimental period.

Table 2. The effect of probiotic on production parameters of laying hens
Tabela 2. Efekat probiotika na proizvodne parametre kokoši nosilja

Parameter *Parametar*	Treatments *Tretmani*		Significance *Značajnost*
	Control *Kontrola*	Probiotic *Probiotik*	
Average body weight of hens at 16 weeks, g *Prosečna masa kokoši u 16. nedelji, g*	1320	1330	NS
Average body weight of hens at 22 weeks, g *Prosečna masa kokoši u 22. nedelji, g*	1695[b]	1756[a]	0,04*
Average body weight of hens at 28th weeks, g *Prosečna masa kokoši u 28. nedelji, g*	1838	1866	NS
Average number of eggs per average laying hen *Broj jaja po prosečnoj nosilji*	48,58	49,07	NS
Percentage of the second grade eggs,% *Udeo jaja druge klase, %*	3,82	2,83	NS
Average egg weight, g *Prosečna masa jaja, g*	59,32	59,65	NS
Average feed consumption, g *Prosečna konzumacija hrane, g*	103,4	102,8	NS
Feed conversion ratio *Konverzija hrane*	2,23[a]	2,15[b]	0,03*

[a b] Values within rows with no common superscript are significantly different
** P<0,05; NS - not significant*

Percentage of second grade eggs in entire period was significantly lower in the experimental group. This result is in agreement with findings of Gallazzi et al. (2008). Effect of added probiotic on egg weight in analyzed laying period could not be established. This result is in agreement with previous reports of Kurtoglu et al. (2004), Gallazzi et al. (2008), Balevi et al. (2001).

The use of probiotics in nutrition of laying hens led to a slight reduction in feed intake compared to the control group (Table 2). A recent study of Kutroglu et al. (2004), Mahdavi et al. (2005) and Yousefi and Karkoodi (2007) showed no effect of probiotics on feed consumption. Gallazzi et al. (2008) found higher feed intake in control group compared to treatment with probiotics. Considering the entire period, experimental group had continuously improved feed conversion ratio ($P < 0.05$), and significant difference ($P < 0.01$) was established in 28 week. According to Mahdavi et al. (2005) there was no effect of probiotics on feed conversion ratio, while Balevi et al. (2001) stated that probiotics improve feed conversion ratio.

Significant effect of probiotic on analyzed parameters of egg quality has not been established (Table 3). There was certain decrease in shell breaking force and shell thickness of eggs from experimental group at 22 weeks, but this effect in other measurements was not repeated. On the contrary, at 28 week, eggs from experimental group had a significantly stronger shell. Similar effect was found on yolk colour, but even than it can not be considered as significant, because at 22 week it was more intense in eggs of control group, while at 28 week was opposite.

Table 3. The egg quality parameters
Tabela 3. Parametri kvaliteta jaja

Week of hens age *Nedelja života*	Treatmants *Tretmani*	Egg weight (g) *Masa jaja (g)*	Shell Cleanliness *Čistoća ljuske*	Shell breaking force (kg) *Sila loma ljuske (kg)*	Shell thickness (0.01 mm) *Debljina ljuske (0.01 mm)*	Shell weight (g) *Masa ljuske (g)*	Shell weight (%) *Masa ljuske (%)*	Albumen height (mm) *Visina belanca (mm)*	Yolk color (Roche) *Boja žumanca (Roche)*	Haugh units *Hogove jedinice*
21	C	52,67	4,72[a]	3,22	34,21	5,66	10,79	10,68	11,97	103,49
	P	53,19	4,92[b]	3,2	33,92	5,73	10,79	10,77	11,85	103,70
22	C	60,62	4,87	3,15[a]	35,25	6,65	10,98	10,46	12,78[a]	101,02
	P	60,29	4,86	2,50[b]	35,95	6,69	11,13	10,50	12,56[b]	101,16
26	C	63,18	4,85	2,82	33,43	6,45	10,25	-	10,17	-
	P	64,63	4,88	2,85	33,35	6,55	10,14	-	10,15	-
28	C	64,26	4,88	2,74[a]	35,38	6,93	10,81	9,85	11,59[a]	97,18
	P	64,60	4,87	3,02[b]	35,33	7,01	10,86	10,02	12,03[b]	97,93

C - control, P - probiotic group
[a b] *Values within columns with no common superscript are significantly different (P<0,05)*

Other authors came to similar conclusions. According to Gallazzi et al. (2008) eggs from treatment with probiotics had higher Haugh units. Yousefi and Karkoodi (2007) have confirmed the effect of probiotics on egg weight and shell thickness. Results published by Mohan et al. (1995) showed that use of probiotics improve shell thickness. Gallazzi et al. (2008) and Mahdavi et al. (2005) recorded no effect of probiotics on shell thickness.

CONCLUSION

Based on the obtained results it can be concluded that positive effect of probiotics was established on body weight of hens at 21 and 22 week. Added dietary probiotics resulted in increase laying performance and reduced proportion of second grade eggs. Considering the entire period feed conversion ratio was significantly better, while effect of probiotic on feed intake was not found. There was no consistent significant effect of probiotics on analyzed parameters of egg quality.

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EFEKAT PROBIOTIKA U HRANI NA PROIZVODNJU I KVALITET KONZUMNIH JAJA U RANOJ FAZI NOSIVOSTI

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Izvod

U radu su predstavljeni rezultati primene probiotika Biomin® IMBO u hrani za nosilje konzumnih jaja. Istraživanje je provedeno u periodu od 16. do 28. nedelje života kokoši nosilja provenijencije Hy Line brown. U navedenom periodu svake nedelje su određeni proizvodni parametri: prosečna telesna masa, proizvodnja jaja, udeo jaja druge klase, prosečna masa jajeta, konzumacija i konverzija hrane. Kvalitet jaja je ispitan u 21, 24, 26. i 28. nedelji života nosilja, a obuhvatio je sledeće parametre: masa jaja, čistoća ljuske, masa ljuske, debljina ljuske, sila loma ljuske, visina belanca, boja

žumanca. Dodavanje probiotika rezultiralo je povećanjem nosivosti i smanjenjem udela jaja druge klase. Konverzija hrane, pomatrana za ceo period, bila je značajno bolja, dok nije zabeležen uticaj na konzumaciju hrane. Nije utvrđen uticaj probiotika na analizirane parametre kvaliteta jaja.

Ključne reči: probiotik, nosilja, proizvodnja jaja, kvalitet jaja

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KERNEL NUMBER PER SPIKE STABILITY OF WHEAT GENOTYPES GROWN ON MELIORATED SOIL (SOLONETZ)*

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SUMMARY: In this work was analyzed number of grains per spike stability for 11 bread wheat varieties, on halomorphic soil, solonetz type, in Banat. Stability have been followed in two vegetation periods on null control, and two melioration levels of 25 t/ha, and 50 t/ha phosphor-gypsum. Genotype by environment interaction was quantified using AMMI model. According the results, wheat varietes in the exam reacted differently to different levels of melioration, depending not only on genotype, but also on environmental conditions.

Key words: *wheat, number of grains per spike, solonetz,G/E interaction, AMMI.*

INTRODUCTION

Wheat is one of the most important food crops for a large part of the world population. Also, it was one of the first plants to be cultivated, grown about more then thousands years ago. The wheat is agricultural crop which requires suitable soil in respect to fertility, physical characteristics and chemical reaction.

In Vojvodina province the most of agricultural soils are with good quality, but there is still a significant part of halomorphic soil, solonetz type, not convenient for agricultural production (Vuković, 2009).

The main problem related with solonetz is Bt,na horizon, which is positioned at 40-60 cm depth, with high clay content, presence of adsorbed sodium and high pH value. Halomorphic soil, solonetz type, could be utilized for wheat growing by using

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ameliorative measures, using phosphor - gypsum (Belić et al., 2003). Stability of grain yield and quality characteristics over different soils and years are important. Wheat shows highly significant genotype by environment interaction (G x E) (Reynolds et al., 2002; Vargas et al., 1999). Good variety should have a high mean yield combined with a low degree of fluctuation, when grown over diverse environments (Tarakanovas and Ruzgas, 2006).

The objectives of this research were to examine the effect of solonetz amelioration, using phosphor-gypsum in amount of 25 and 50 t/ha, on stability grains number per spike of different wheat varieties.

MATHERIAL AND METHODS

The trials were conducted on solonetz type of soil at Kumane, Banat, Northwest part of Serbia, during two consecutive crop years of 2004/2005 and 2005/2006. Ten Serbian wheat varietes: Mina (G1), Sofija (G2), Tiha (G3), Anastazija (G4), Nevesinjka (G5), Evropa 90 (G6), NSR-5 (G7), Dragana (G8), Ljiljana (G9), Simonida (G10) which were obtained from Institute of Field and Vegetable crops in Novi Sad and one variete obtained from Cereal research institute in Szeged, Hungary, GK Zügoly (G11). The experiment was set up according to completely randomized block design with 3 replications and 3 treatments. The sowing was done in rows 2 m long at the inter-grain spacing in a row of 10 cm and inter-row spacing of 20 cm. The seeds in both seasons were planted at 15 Oktobar and plants were harvested at 10-15 July. The first treatment was soil without amelioratin, second treatment was amelioration using phosphor-gypsum in amount of 25 and third treatment was amelioration using phosphor-gypsum in amount of 50 t/ha. The NPK (15:15:15) application was split, 50 kg for each tretman. In this trial each treatmen at the season was analysed like specific agro-ecological environment, so we got 6 different environments (tables 1). Data of number of grains per spike were at full of maturity recorded. Analyze of genotype by environment interaction (G x E) was done by AMMI model (Aditive main effects and multiplicative interaction) developed by Zobel et al. (1988). AMMI analysis was processed using the program GenStat for Windows 8th edition.

Table 1. Labels of 6 environments (phosphor-gypsum melioration/years) on solonjetz soil that 11 wheat varietes were grown up
Tabela 1. Oznake 6 agroekoloških sredina u kojima je gajeno 11 sorti pšenice u ogledu na solonjecu

No. *Red. br.*	Labels *Oznake korišćene u radu*	Growth season *Godina gajenja*	Phosphor-gypsum.treatment *Tretman fosfo-gipsom*
E1	$K_{04/05}$	2004/2005	Control, without treatment (Ø) *Kontrola, bez tretmana*
E2	$T25_{04/05}$	2004/2005	Treatment with 25 t/ha *Tretman sa 25 t/ha*
E3	$T50_{04/05}$	2004/2005	Treatment with 50 t/ha *Tretman sa 50t/ha*
E4	$K_{05/06}$	2005/2006	Control, without treatment (Ø) *Kontrola, bez tretmana*
E5	$T25_{05/06}$	2005/2006	Treatment with 25 t/ha *Tretman sa 25 t/ha*
E6	$T50_{05/06}$	2005/2006	Treatment with50 t/ha *Tretman sa 50t/ha*

RESULTS AND DISCUSSION

The AMMI analyses of variance of wheat number grains per spike of the 11 genotypes tested in six environments showed that environment was highly significant, genotype and G x E interaction were significant. The significant G x E effects demonstrated that genotypes responded differently to the variation in environmental conditions of different meliorated levels. The obtained data showed that 47,7 % of total sum of squares was attributable to environmental effects, only 12,5 % to genotypic effects and 39,9% to G x E interactions effects (Table 2). The environments were diversed and caused the most variation on number grains per spike. Genotype sum of squares was about 3 times less than G x E sum of squares, which determined very important differences in genotypic response across environments. The first PCA accounted for 36,1 % of the G x E sum of squares using 14 degrees of freedom (df) in the interaction and it was significan at $P < 0,05$. This obtained results confirm that AMMI is suitable model and made it possible to construct the biplot and calculated genotypes and enviroments effects (Gauch and Zobel, 1996; Yan and Hunt, 2001; Tarakanovas and Ruzgas, 2006).

Table 2. AMMI analysis of variance for number grains per spike of the 11 wheat varietes grown in 6 environments in two years

Tabela 2. AMMI analiza varijanse za broj zrna po klasu 11 sorti pšenice ispitivanih u 6 eko sredina

Source Izvori varijacije	Degree of freedom Stepeni slobode	Sum of squares Sume kvadrata	Mean square Sredine kvadrata	F Value F Vrednosti	F – table F- tablica		Explaned Objašnjeno %
					0,05	0,01	
Total Ukupno	197	9476	48,1	*			
Treatment Ogled	65	5359	82,4	** 2,78	1	1	
Genotype Genotip	10	668	66,8	* 2,25	1,83	2,32	12,5
Environment Еко-sredina	5	2554	510,7	**11,0	2,21	3,02	47,7
Block/Blok	12	557	46,4	1,57	1,75	2,18	5,8
Interaction Interakcija	50	2138	42,8	* 1,44	1,35	1,52	39,9
IPCA 1	14	773	55,2	* 1,86	1,75	2,18	36,1
Residue Ostatak	36	1365	37,9	1,28	1,46	1,69	14,4
Error Pogreška	120	3560	29,7	*			

The biplot in AMMI is constructed so that the genotype and environment means are plotted on the abcissa and the IPCA scores for the same genotypes and environments on the ordinate. The IPCA scores of genotype in the AMMI analysis are an indication of the stability over environments. If their IPCA scores are close to zero than the more stable the genotype are across their testing environments, which contribute little to the interaction. The biplot shows not only the average of number grains per spike of a variety but also their stability. The graph space of Fig. 1 is divided into 4 quadrants from high number grains per spike environments in quadrants 2 and 3 to lower number grains per spike of wheat in quadrants 1 and 4. The varietes G1, G3, G4, G10 and G11 posed in quadrant 1 show that they have good adaptation in a wide range environments (Fig. 1). In this groups of the varieties the genotype G11 (GK Zügoly) was posed close to zero of IPCA 1 and showed that it is more stable genotype and adapted to lower sites, but also very near of genotype grand means. The varietes G6 (Evropa), G7 (NSR-5) posed in quadrant 3, both with higher genotype means. Genotype G6 was posed on zero of IPCA 1 what confirmed that was the most stable genotypes with the grandest mean value in this trial, therefore, it may be characterised by specific adaptation in favourable environments. The least stabled of all varietes, with the highest G x E inteaction was genotype G7 (NSR-5).

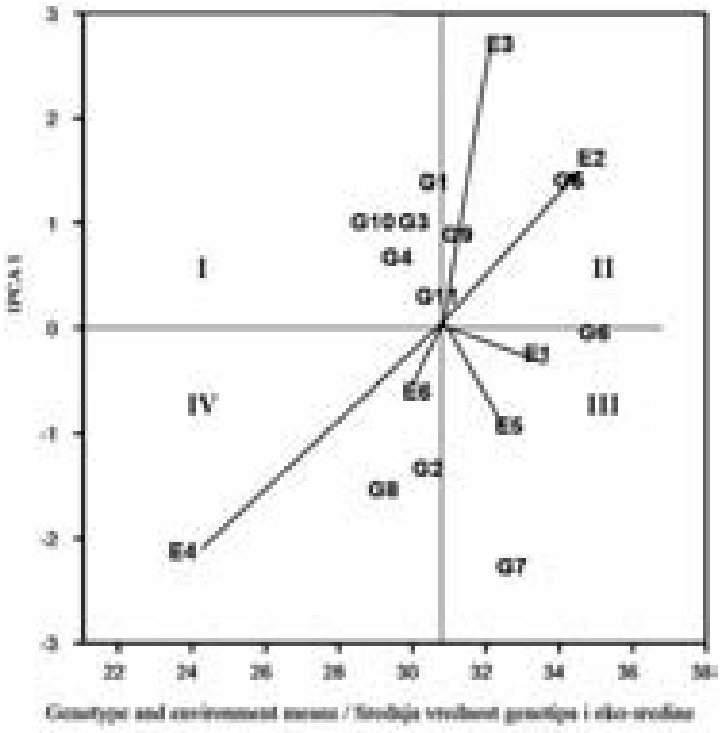

Fig. 1. Biplot of the AMMI model for wheat trial, consisting of 11 varieties grown in 6 environments. Environments codes are given in Matherial and method. Mean values of number grains per spike and the grand mean are labeled on x-axes

Slika 1. Biplot AMMI modela za ogled pšenice, sa 11 sorti u 6 agroekoloških sredina. Oznake su date u Materijalu i metodu. U grafiku je na apscisi data vrednost broja zrna po klasu i označena je srednja vrednost ogleda

The varietes G8 (Dragana) and G2 (Sofija) posed in quadrant 4, were outlying of zero IPCA 1 and showed also G x E interaction, with low grand mean value. The varietes G5 (Nevesinjka) and G9 (Ljiljana) posed in quadrant 2. The genotype G5 (Nevesinjka) was outlying of zero IPCA 1 and showed quite high G x E interaction and showed higher mean value opposed the genotype G9 (Ljiljana) which indicated highly stability with a grand mean value. Considering the point of environment means, we saw that they showed largely depends of climatic conditions in both year. Environmenrs of the first crop year were grouped in qudrants 2 and 3, with higher grains number, except E5 (melioration levels of 50 t/ha in 2004/2005), which is also located in this group. The biplot also accounts the number of grains per spike of genotype at individual site.The genotye G6 (Evropa 90) was the best for high number grains sites in E1 (control, in 2004/2005), also in E2 (melioration levels of 25 t/ha in 2004/2005) and E5 (melioration levels of 25 t/ha in 2005/2006). The greatest interaction and thus showed the lowest stability, were in environments E3 (melioration levels of 50 t/ha in 2004/2005) and E4 (control in 2005/2006). The most of genotypes reacted favorably to the conditions of repair melioration levels of 25 t/ha in both season (Fig. 1).

CONCLUSION

The AMMI analysis of variance of 11 wheat genotypes in 6 environments shows that environment (E) was highly significant, genotype (G) and their interaction (GE) were significant. The first PCA was also significant and participated in the overall variation seen 36%. AMMI model was very effective for studying GE interaction.

Wheat varietes in the exam reacted differently to different levels of melioration, depending not only on genotype, but also on environmental conditions. Most of them have a good performance and good stability and they can be used in commercial production on these soils. The biplot shows that the genotype Evropa 90 (G6) was the most stable and had the highest mean value of the investigated genotypes in wheat trial.

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STABILNOST BROJA ZRNA PO KLASU GENOTIPOVA PŠENICE GAJENIH NA MELIORISANOM ZEMLIŠTU (SOLONJEC)

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Izvod

U radu je analizirana stabilnost broja zrna po klasu 11 genotipova pšenice, na halomorfnom zemljištu, tipa solonjec u Banatu. Stabilnost je praćena u dve vegetacione sezone, na kontroli i dva nivoa popravke zemljišta fosfogipsom. Interakcija genotip/spoljna sredina procenjena je AMMI modelom. Zapažena je različita reakcija sorti pšenice na nivo popravke zemljišta, u odnosu na svaki tretman i vegetacionu sezonu.

Ključne reči: pšenica, broj zrna po klasu, solonjec, G/E interakcija, AMMI.

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EFFECT OF PGPR ON THE EARLY GROWTH OF MAIZE AND MICROBIAL ABUNDANCE IN RHIZOSPHERE

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SUMMARY: The objective of this study was to investigate the microbial abundance in maize rhizosphere depending on the applied bacterial inoculants and the leaf stage of maize as well as to examine the effect of plant growth promoting rhizobacteria (PGPR) on the early growth of maize plants. In this experiment three bacterial species: Azotobacter chroococcum, Bacillus subtilis and Pseudomonas fluorescens applied individually and in mixture were used as inoculants. One hybrid of maize included in the tests: NS 6010 developed at the Institute of Field and Vegetable Crops in Novi Sad. Experiments were established in laboratory and in greenhouse. The number of the investigated microbial groups depended on the applied bacterial inoculants and the leaf stage of maize plants. Bacterial strains used in this study had a statistically significant influence on the number of microorganisms in maize rhizosphere and a positive effect on the early growth of maize. Bacterial inoculants increased plant height and weight of young maize plants.

Key words: *Azotobacter*, *Bacillus*, *maize, PGPR*, *Pseudomonas*.

INTRODUCTION

In agricultural soils, the action of soil microorganisms is a major determinant of efficient nutrient cycling and plant growth. The microbe-plant interaction can be beneficial, neutral, variable, or deleterious for plant growth (Husen, 2003). An important group of these microbial communities that exerts beneficial effects on plant growth were first defined by Joseph Kloepper and Milton Schroth and termed as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). PGPR are the group of bacteria that actively colonize rhizosphere and plant roots and increase plant growth and yield (Adesemoye et al., 2009). Bacteria of diverse genera such as *Azotobacter*,

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Azospirillum, *Alcaligenes*, *Azoarcus*, *Enterobacter*, *Klebsiella*, *Bacillus*, *Pseudomonas*, *Beijerinckia* were identified as PGPR (Berg, 2009). PGPR can directly stimulate plant growth in several different ways. They can: fix atmospheric nitrogen (Mrkovački and Milić, 2001; Jensen and Hauggaard-Nielsen, 2003), synthesize several plant hormones (Dobbelaere et al., 2003), solubilize minerals (Rodrigez and Fraga, 1999; Cakmakci, 2006), synthesize enzymes that can modulate plant hormone levels (Ahmad et al., 2008). The indirect promotion of plant growth include: production siderophores that limit the available iron to the pathogen (Husen, 2003), production antibiotics that kill the pathogen and induction systemic resistance in plant (Ramamoorthy et al., 2001).

The use of PGPR has become a common practice in many regions of the world. Economic and environmental benefits can include increased income from high yields, reduced fertilizer costs and reduced emission of the greenhouse gas (Kennedy et al., 2004). Therefore, the objective of this study was to examine the effect of *Azotobacter chroococcum*, *Bacillus subtilis* and *Pseudomonas fluorescens* and their mixture on the microbial abundance in maize rhizosphere and the early growth of maize plants.

MATERIAL AND METHODS

In this experiment three bacterial species: *Azotobacter chroococcum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were used as inoculants. The effect of four different variants of inoculation has been tested, in three replication: I – *Azotobacter chroococcum*, II – *Bacillus subtilis*, III – *Pseudomonas fluorescens*, IV – *Azotobacter chroococcum* + *Bacillus subtilis* + *Pseudomonas fluorescens*. One hybrid of maize included in the tests: NS 6010 developed at the Institute of Field and Vegetable Crops in Novi Sad. Maize seeds were treated with bacterial inoculants. Inoculation was performed with a liquid culture of mentioned strains and with their mixture, with the concentration of 10^8 cells per ml. *Azotobacter chroococcum* was prepared in the liquid Fiodor medium, *Bacillus subtilis* in the nutrient agar and *Pseudomonas fluorescens* in the King B medium. No treated seeds were designed as control. The effect of PGPR on seed germination and early growth of maize were evaluated in laboratory and in greenhouse. In laboratory ten seeds were placed in Petri dishes on previously moist filter paper with strerile water. In greenhouse ten seeds were sown at 4 to 5 cm depth of soil in each Mitscherlich pot.

The rhizosphere soil for microbiological analysis was sampled at the three- and six leaf stage of maize. At the two stages in greenhouse as well as after 10 days in laboratory conditions the growth parameters like plant height and plant weight were recorded.

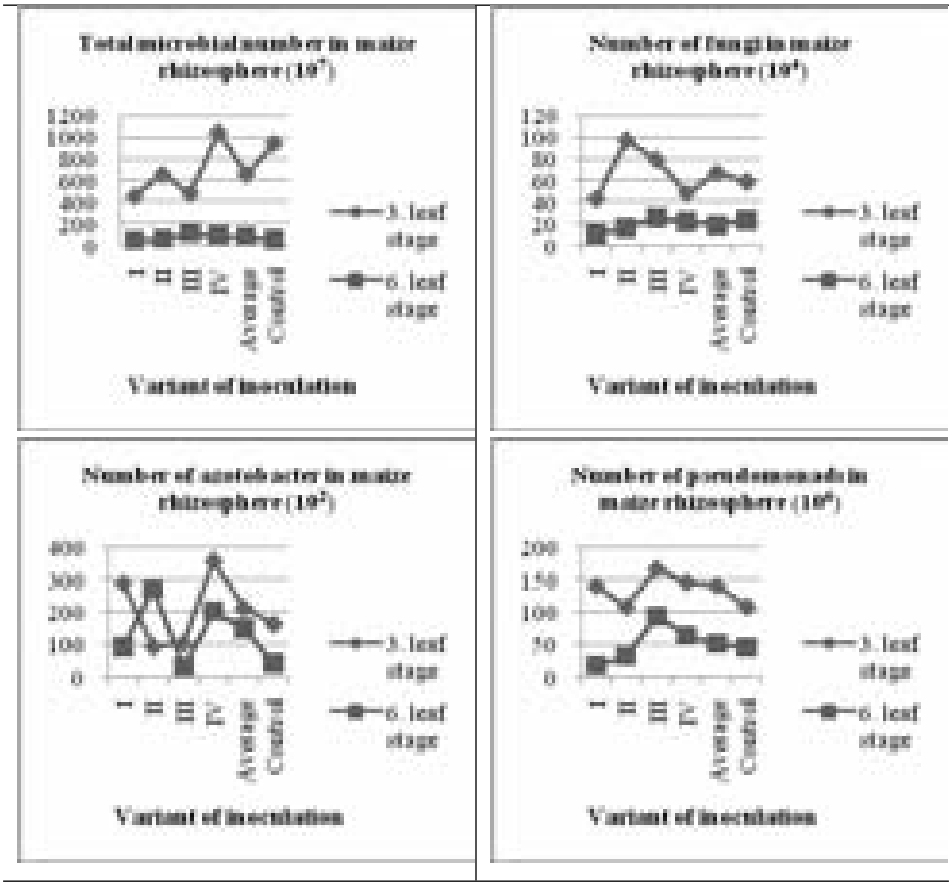
Total number of microorganisms was determined on soil agar (dilution 10^{-7}). Fungi were determined on potato dextrose agar (dilution 10^{-4}), azotobacter on Fiodor substrate (dilution 10^{-2}) (Jarak and Đurić, 2004) and pseudomonads on King B medium (dilution 10^{-6}) (Jošić et al., 2010). Statistical analyses (LSD–test) were performed according Mead et al. (1996).

RESULTS AND DISSCUSION

Effects of the different variants of maize inoculation are presented in Graph 1, Tables 1 and 2. Graph 1 shows the total number of microorganisms, the number of fungi, the number of pseudomonads and the number of azotobacter in maize rhizosphere.

The largest effects of inoculation on the total number of microorganisms, at the three leaf stage, were obtained with mixture of strains (IV) and in the variant with *Pseudomonas fluorescens* (III), at the six leaf stage.

The highest number of fungi was recorded in the variants with *Bacillus subtilis* (II), at the three leaf stage, and with *Pseudomonas fluorescens* (III), at the six leaf stage of maize plants.



Graf. 1. Efekat inokulacije na broj mikroorganizama u rizosferi kukuruza
Graph. 1. Effect of inoculation on the number of microorganisms in maize rhizosphere

The best effect of inoculation on the number of azotobacter, at the three leaf stage, was obtained in the variant with mixture of strains (IV). At the six leaf stage the biggest effect was achieved in the variant with *Bacillus subtilis* (II). At both leaf stages the highest influence on the number of pseudomonads was recorded in the variant with *Pseudomonas fluorescens* (III).

On average for all variants it has been shown that inoculation significantly increased the total microbial number, the number of fungi and azotobacter at the three leaf stage in relation to six leaf stage of maize plants.

The effectiveness of the application of microorganisms in plant production depends on plant species, species of microorganisms, quantities of fertilizers, time and place of sampling (Govedarica, 1986; Cvijanović, 2002). Bacterial stains used in this study had a positive effect on the growth parameters of maize under laboratory conditions as well as in pot experiment. Inoculation was positively affected on the plant height and weight of young maize plants respectively. Results of these studies are presented in Tables 1 and 2.

Tabela 1. Efekat inokulacije na visinu nadzemnog dela kukuruza (cm)
Table 1. Effect of inoculation on the height of maize plants (cm)

Variant of inoculation	Plant height of maize seedlings (cm)	Plant height at the three leaf stage (cm)	Plant height at the six leaf stage (cm)
I	7,36	18,03*	37,68*
II	6,71	17,18	38,64**
III	6,41	18,82**	38,66**
IV	6,41	16,30	39,07**
Average	6,72	17,58	38,51
Control	6,18	15,52	32,89
LSD $_{0.05}$	2.57	2.43	3,71
LSD $_{0.01}$	3.55	3.27	4,92

On average, all three examined strains as well as their mixture (6,72 cm) in relation to the control (6,18 cm) showed an increase in the plant height of maize seedlings. The biggest effect on the plant height under laboratory conditions was obtained in the variant with *Azotobacter chrococcum* (Table 1).

The biggest effect on the height of plants in the pots at the three leaf stage was registrered in the variant with *Pseudomonas fluorescens* (III) and it increase was statistically significant. The greatest effect on the plant height at the six leaf stage was obtained in the variant with mixture of all three strains (IV) (Table 1). On average for the two stage all the investigated variants (17,58 and 38,51 cm) increased plant height in relation to the non-inoculated variants (15,52 and 32,89 cm).

On average, all strains showed a positive influence on the plant weight (0,20 and 0,65 g) of young maize plants in relation to the control (0,15 and 0,48 g) (Table 2). The biggest effect on the plant weight at the two stage was obtained in the variant with all three strains (IV). Inoculation with all applied bacterial inoculants significantly increased the plant weight at the three- and six leaf stage of maize plants.

Tabela 2. Efekat inokulavije na masu suve materije kukuruza (g)
Table 2. Effect of inoculation on the weight of young maize plants (g)

	Variant of inoculation					
Leaf stage	I	II	III	IV	Average	Control
3	0,20**	0,18**	0,19**	0,22**	0,20	0,15
6	0,65**	0,57**	0,58**	0,79**	0,65	0,48
	LSD $_{0.05}$ 0,01 $_{0.01}$ 0,02		LSD $_{0.05}$ 0,02 $_{0.01}$ 0.03			

It has been shown that inoculation with *Azotobacter*, *Bacillus* and *Pseudomonas* strains could increase maize yield, seed germination and seedling growth, root and shoot elongation of maize (Govedarica et al., 2001; Cvijanović et al., 2007; Egamberdiyeva, 2007; Gholami, 2009).

Gholami (2009) examined six bacterial strains PGPR on germination, seedling growth and yield of maize. All bacteria except *Azospirillum lipoferum* increased seed germination up to 18,5% over nontreated control.

Brown (1982) reviewed that use of *Azotobacter spp.* as biofertilizers and concluded that inoculation with these microorganisms occasionally promote growth by mechanism other than biological N fixation. Lalande et al. (1989) suggested that PGPR stimulate plant growth with production of phytohormone. Similarly, Dey et al. (2004); Kloepper et al. (1988) suggested that PGPR enhance the growth and seed emergence. Shaukat et al. (2006) reported that *Azotobacter sp.* inoculation increased the wheat seed germination percentage to about 58,6%. He also reported that *Azotobacter sp.* inoculation significantly enhanced the plant height.

CONCLUSION

The number of the investigated microbial groups depended on the applied bacterial inoculants and the leaf stage of maize plants. On average for all variants it has been shown that inoculation significantly increased the total microbial number, the number of fungi and azotobacter at the three leaf stage in relation to six leaf stage of maize plants.

On average, all three examined strains showed an increase in the height and weight of maize plants in relation to the control. The biggest effect on the plant weight was obtained in the variant with mixture of all three strains.

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EFEKAT RIZOBAKTERIJA (PGPR) NA POČETNI RAST KUKURUZA I BROJNOST MIKROORGANIZAMA U RIZOSFERI

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Izvod

Cilj ovih istraživanja bio je da se odredi brojnost mikroorganizama u rizosferi kukuruza u zavisnosti od primenjenih bakterijskih inokulanata i faze rasta kukuruza kao i da se ispita efekat rizobakterija - promotora biljnog rasta (PGPR) na početni rast kukuruza. Kao inokulanti korišćene su tri vrste bakterija: *Azotobacter chroococcum*, *Bacillus subtilis* i *Pseudomonas fluorescens* primenjene pojedinačno i u smeši. U istraživanjima je korišćen jedan hibrid kukuruza: NS 6010 stvoren u Institutu za ratarstvo i povrtarstvo u Novom Sadu. Ogledi su postavljeni u laboratoriji i u žičari. Broj ispitivanih grupa mikroorganizama zavisio je od primenjenih bakterijskih inokulanata i faze rasta kukuruza. Sojevi bakterija ispoljili su statistički značajan uticaj na brojnost mikroorganizama u rizosferi kukuruza i imali su pozitivan efekat na rast biljaka kukuruza. Inokulacijom je povećana visina i masa biljaka kukuruza.

Ključne reči: Azotobacter, Bacillus, maize, PGPR, Pseudomonas.

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EFFECT OF THE LEVEL OF TRYPSIN INHIBITORS AND THERMAL PROCESSING OF SOYBENAS TO THE SIZE OF BROILERS ORGANS

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SUMMARY: The use of non processed soybeans leads to hypertrophy of the pancreas and other internal organs. The aim of this study was to compare the effects of crude free Kunitz trypsin inhibitor soybean variety "Lana" (SL), standard raw soybeans (SS), extruded soybean free KTI varieties "Lana" (LG) and standard extruded soybean (SG) of the size pancreas and other internal organs of broiler chickens in order to assess the significance of KTI anti nutritive factors in soybean and the importance of heat treatment. The results suggest that extrusion and lower levels of KTI have a significant effect on all tested parameters. Feeding treatment in LG group had a favorable effect on pancreas size to the SS group, but was still significantly worse than feeding treatments in LG and SG groups, where the soybean was heat-treated.

Key words: *broilers, soybean, extruding, organs.*

INTRODUCTION

Soy is the most used nutrient in the diet of broiler chickens to meet the needs of protein and essential amino acids. Full use of high quality vegetable protein in soy beans is only possible with the previous heat treatment, which significantly increases production costs. Therefore, in order to reduce the cost of many tests conducted on the possibility of the use of raw soybeans in the diet of poultry, from which it came to the realization that the main carriers of inhibitory activity of raw soybeans protease inhibitors, primarily: Kunitz trypsin inhibitor (Kunitz, 1945) and Bowman-Birk trypsin inhibitor (Bowman, 1944; Birk, 1961; Tseng Yen et al, 1977). Other factors to taken into account the hem agglutinin or lectins (Douglas et al., 1999), and antivitamin, goitrogene and indigestible olygo-saharide (Parsons et al., 2000). However the inclusion of raw grain in the food does not only have a negative effect on the growth of chickens, but

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also leads to an increase in relative weight of digestive organs, particularly the pancreas (Gertler et al., 1966; Aria et al., 2006, Brenes et al., 2008), which the target organ, and the size and activity changes depending on the level of trypsin inhibitors. Efforts to increase the use of legumes led to the development of a wide range of methode processing, including extrusion. This technology has numerous advantages, including the possibility of wide application, high productivity, energy efficiency and high quality of the resulting product (Brenes at al., 2008).

In an attempt to cut costs and avoid the heat treatment which in inadequate implementation may also have an adverse effect on the production, selection produced varieties with low levels of inhibitors and compatible for broilers. Hymowitz (1986) found soybean seed with low levels of Kunitz trypsin inhibitors. In the world there are varieties of soybean under different commercial names that are free Kunitz, Bovman-Birck trypsin inhibitors or without the lectin. Production results obtained from these varieties of soybean and findings in the research works are contradictory, and they are in connection with properties of soybean varieties. Thus, Palacios et al., (2004) found better production results when the of genetically improved soybean varieties subjected to thermal treatment. On the Serbian market there is domestic variety of Kuniz free trypsin inhibitor soybean with commercial name "Lana" whose effects on the production, physical-morphological and metabolic parameters related to the broilers production have so far not or were only partially tested.

The aim of this study was to compare the effects of raw Kunitz free trypsin inhibitor soybean (SL), standard raw soybeans (SS), extruded Kunitz free trypsin inhibitor soybean (LG) and standard extruded soybean (SG) on broilers organs sizes in order to assess the antinutritive significance of Kunitz trypsin inhibitors in soyabean varieties "Lana" and the importance of extrusion. The usage experimental diet is started from the tenth day of age as in earlier studies found that Kunitz free trypsin inhibitor and lectin free soybean is not desirable to give chickens and piglets of this age without heat treatment (Palacios et al., 2004).

MATERIALS AND METHODS

The trial lasting 42 days was set to determine the effect of different levels of Kunitz trypsin inhibitor and soybean extrusion on the size of the digestive organs. One-day old male broiler chicken Ross-308 hybrids were measured and placed in boxes, so that the average initial weight of chickens was balanced and was 42.5+/-7 g. In the experiment were four groups with four replications. Total numbers of broilers were 64. Food and water were available ad libitum, with a light regime of 24 hours. The experimental mixture includes 30% raw soybean or heat-treated according to the groups: 1) raw soy with no Kunitz trypsin inhibitor (SL), 2) raw standard strain (SS), 3) extruded soybeans without the Kunitz trypsin inhibitor (LG) and 4) standard extruded soybean (SG). The levels of trypsin inhibitors in soya beans are shown in Table 1.

Table 1 Levels of trypsin inhibitors in soybean.
Tabela 1. Nivoi tripsin inhibitora u zrnu soje

Group / *Grupa*	SL	SS	LG	SG
TI (mg/g/min)	15,07	30,21	12,43	12,30

All chickens were fed the same starter mixture based on corn and soybeans during the first 10 days, which contained 23% protein, 12.6 MJ metabolic energy. Grover mixture with 22% crude protein was used from 11 to 24 days, a finisher with 21% crude protein from 25 to 42 days. Mixtures consisting of corn, soybean meal, yeast livestock, livestock chalk, mono-calcium phosphate, salt and premix, showen in Table 2.

Table 2. Composition of diet in experiment
Tabela 2. Sastav smeše u ogledu

Feedstuffs *Hraniva*	Starter *Starter*	Grower / *Grover*				Finisher / *Finišer*			
		Stand. soybean *Standardna. soja*		Low KTI soybean / *Soja nižeg nivoa KTI*		Stand. Soybean *Standardna soja*		Low KTI soybean / *Soja nižeg nivoa KTI*	
Mixture type *Tip smeše*		Raw *Sirova*	Heat *T.Tret*	Raw *Sirova*	Heat *T.Tret*	Raw *Sirova*	Heat *T.Tret*	Raw *Sirova*	Heat *T.Tret*
Maze *Kukuruz*	51,77	53,65	53,35	49,87	49,95	56,56	55,36	52,71	52,82
Soybean meal *Soj. sačma*	24,46	8,22	8,52	12,03	11,95	5,32	6,54	9,16	9,08
Raw low KTI soybean *Sir. soja nižeg nivoa KTI*	-	-	-	30,00	-	-	-	30,00	-
Extruded low KTI soybean *Ekstrud. soja nižieg nivoa KTI*	-	-	-	-	30,00	-	-	-	30,00
Standard raw soybean *Stand. sirova soja*	-	30,00	-	-	-	30,00	-	-	-
Standard extruded soybean *Stand. ekstrud. soja*	15,34	-	30,00	-	-	-	30,00	-	-
Yeast dry *Suvi kvasac*	4,00	4,00	4,00	4,00	4,00	4,00	4,00	4,00	4,00
Limestone Stočna kreda	1,60	1,40	1,40	1,40	1,40	1,40	1,40	1,40	1,40
MCP MKF	1,48	1,37	1,37	1,35	1,35	1,35	1,35	1,38	1,35
Feedstuffs *Hraniva*	Starter *Starter*	Grower *Grover*				Finisher *Finišer*			
		Stand. soybean *Standardna. soja*		Low KTI soybean / *Soja nižeg nivoa KTI*		Stand. Soybean *Standardna soja*		Low KTI soybean / *Soja nižeg nivoa KTI*	
Mixture type *Tip smeše*		Raw *Sirova*	Heat *T.Tret*	Raw *Sirova*	Heat *T.Tret*	Raw *Sirova*	Heat *T.Tret*	Raw *Sirova*	Heat *T.Tret*
Salt *So*	0,35	0,35	0,36	0,35	0,36	0,37	0,35	0,35	0,35
Premix *Premiks*	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Total *Ukupno*	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0

At the end of the experiment, 8 broilers (2 per repetition) were selected from each treatment individually measured and sacrificed cervical dislocations. Broilers are not fed 12 hours before sacrifice. Pancreas, liver, heart, spleen and small intestine were taken from the carcasses, cleaned of surrounding tissue and measured on a digital balance with a precision of ± 0, 01g. In addition, the small intestine and cekum were extracted and determined by their length. The weight and body length is calculated and expressed in relation to body weight of broilers. For statistical data processing software was used Statistica for Windows ver. 8.0 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

The values of relative weight and body length are shown in Table 3.

Table 3. Relative mass and length of digestive organs
Tabela 3. Relativna masa I dužina digestivnih organa

Group *Grupa*	SL	SS	LG	SG
Average body weight before slaughter (g) *Prosečna masa pre klanja (g)*	2332,5	1822,5	2697,5	2778,125
Relative mass of pancreas (%) *Relativna masa pakreasa (%)*	0,38	0,60	0,25	0,24
Relative mass of liver (%) *Relativna masa jetre (%)*	2,09	2,48	1,97	2,12
Relative mass of spleen (%) *Relativna masa slezine (%)*	0,11	0,13	0,14	0,15
Relative mass of heart (%) *Relativna masa srca (%)*	0,46	0,53	0,45	0,45
Relative mass of small intestine (%) *Relativna masa tankih creva (%)*	2,82	3,35	2,52	2,73
Group *Grupa*	SL	SS	LG	SG
Relative length of small intestine (%) *Relativna dužina tankh creva (%)*	10,89	12,57	8,83	7,41
Relative length of cecum (%) *Relativna dužina cekuma (%)*	1,01	1,15	0,94	0,72

The highest average final weight was in the group of SG, but no significant difference with the LG group, the SL group achieved significantly higher final weight ($p < 0.01$) compared to the SS group, but significantly worse ($p < 0, 01$) compared to both groups in which the extruded soybean. Experiments conducted on rats (Friedman et al., 1991), chickens (Anderson-Hafermann et al., 1992, Douglas et al., 1999, Palacios et al., 2004), laying hens (Zhang at al., 1991) and pigs (Palacios et al., 2004) have shown that the inclusion of raw soybeans with reduced levels of Kunitz trypsin inhibitors in the diet has a much better effect on body weight compared with the standard raw soybeans, but significantly less than the thermal processed soy. This indicates the presence of other thermo-labile factors. The importance of lectins as antinutritive factors established by Liener (1953) research on rats that this was confirmed in chickens (Douglas et al., 1999) and Palacios et al. (2004), but it is proven that the negative effect of lectins can be re-

duced with thermal treatment using a standard soybean. Relative pancreas weight was highest in the SS group and the difference was significant ($p < 0.05$) compared to the SL group and highly significant ($p < 0.01$) compared to SG and LG groups, SL group had greater relative weight of the pancreas ($p < 0.01$) compared to SG and LG groups, and between the SG and LG groups there was no difference. The increase can be attributed to the pancreatic effects of trypsin inhibitors and lectins. Inactivation of free trypsin in the intestine stimulates the release of cholecystokinin from neuroendocrine cells in the intestine, thus leading to hyper-secretion of pancreatic digestive enzymes and subsequent enlarge of the pancreas (Lacourse at al., 1999). Cholecystokinin regulate the growth of pancreatic enzyme secretion and contraction of gall bladder (Rehfeld, 1998).

Changes in secretions affect the digestive structure and function, particularly disorders in the digestion and absorption, changes in the passages of content, increase microbial activity in the small intestine and the entire increase in the digestive tract and related organs (Hoerr, 1998). Hypertrophy of the pancreas was detected in chickens and rats in the food that added pure extract of soybean trypsin inhibritor (Gertler et al., 1966), chickens fed raw velvet beans (*Phaseolus vulgaris L.var. Pinto*) (Arija et al., 2006), raw chick pea (Cicero arietinum L.) (Brenes et al., 2008) and smooth beans (Mucuna pruriens) (Carew et al., 2003; Tuleun and Igba, 2008). The effect of these raw legumes on the mass of the pancreas is probably the result of a well-known presence in the grain anti-trypsin factors that interfere with normal function pancreas enzymes, thus forcing the pancreas to enhanced work (Carew et al, 2003). The relative liver weight was higher in the SS group compared to the SL group ($p < 0.05$) in comparison with the SG and LG groups ($p < 0.01$). Between the SL, LG and SG groups were not significant differences. The relative mass of liver influenced the process of extrusion ($p < 0.05$), whereas the relative weight was lower in groups that were fed with extruded soybean, and the impact had a lower level of Kunitz trypsin inhibitor ($p < 0.05$), and the relative weight of liver was less in groups with lower levels of this inhibitor. Observed increase in relative liver weight could be attributed to mobilization of body reserves in order to bear all expenses the need for rapid growth of certain organs which can lead to increase in the liver and cause hypertrophy and poor nutritional status of chicks fed raw soybean (Arija et al., 2006). Carew et al. (2003) have obtained different results in their two experiments, so that in the first experiment observed increase in relative liver weight, whereas in their second experiment, it was not the case, Aria et al. (2006), Brenes et al. (2008) and Tuleun and Igba (2008) have also observed an increase of relative liver weight,. The size of the liver can be increased in response to several factors, especially in the case of deficiency of protein and amino acids, which is linked to the presence of antinutritive factors in raw grains and legumes usually comes to fat accumulation (Carew et al., 2003). Therefore, it is possible that the availability of protein and amino acids differ among the many varieties of soybean and other legumes, which may explain the different results obtained for the impact of legumes in the diet on relative liver weight. Relative spleen weight was highest in the SG group, and lowest in the SS group, but there were no significant differences between treatments. In the work of Arija et al. (2006) and Brenes et al. (2008) extrusion caused the increase in relative spleen weight. Relative heart weight was greater in the SS group compared to the other groups ($p < 0.05$), while among others there were no treatment differences. Carew et al. (2003) have also observed a significantly higher relative heart weight of chickens and found that this increase may represent an additional burden that can lead to stress and

disease. The relative weight of intestine was highest in the SS group compared to the SG and SL groups ($p < 0.05$) and in relation to the LG group ($p < 0.01$), detected a higher weight in the SL group compared to the LG group ($p < 0.05$). The relative length of the thin intestine was highest in the SS group compared to the SL group ($p < 0.05$) and in comparison with the SG and LG groups ($p < 0.01$). Also, the relative length of small intestine was higher in group SL compared to the SG and LG groups ($p < 0.01$), and in LG group compared to the SG group ($p < 0.01$). The process of extrusion was significantly influenced by the smaller length of cekum ($p < 0.01$). The increased relative weight of jejunum is described in the work of Arija et al. (2006) and Brenes et al. (2008). Carew et al. (2003) and Tuleun and Igba (2008) have described the increase in the length of thin tubes and cecum increase in weight and length of thin tubes and cecum may be the result of high levels of carbohydrates complex including resistant starch, and soluble olygo-saharides and non strach polysaccharide present in the raw grain (Parsons et al., 2000) and the presence of lectins (Douglas et al., 1999). Carew et al (2003) show that increasing the size of the segments of the digestive tract of chickens is due icreasing muscular work which appears due to the large quantity of fiber in raw beans. Extrusion significantly improves the performance, size of digestive organs and especially positive effect on the relative mass of pancreas and digestibility of food (Arija et al., 2006; Brenes et al., 2008). These favorable effects may be related to reduction or inactivation of lectins, trypsin, hymotripsin and α amylase inhibitor (Arija et al., 2006; Brenes et al, 2008). Extrusion also leads to gelatination of starch and reduced activity of trypsin inhibitors, which improves the conditions for efficient digestion of protein in the small intestine of pigs (O'Doherty and Keady, 2001) and chickens (Arija et al., 2006). Soybean varieties with Kunitz free inhibitor may have different levels of trypsin inhibitor, depending on the place where the grown (Kumar et al., 2003), the presence of other types of trypsin inhibitor (Tan-Wilson et al., 1987), variations in the level of lipoxygenase (Machado at al.2008) and antinutritive factors such as fitates and tannins (Liener, 1994). Palacios et al. (2004) has results for which soybean with low level of trypsin inhibitors has better quality than the standard soy protein, but Machado at al. (2008), in a biological experiment is not observed significant differences when the two variants of soybean were thermally treated on the same way. This can be explained by different levels of lectins in different varieties of soybean (Friedman et al., 1991). Differences in the quality of proteins are expected due to variations in the quantity of different types of trypsin inhibitors and other antinutritive components in different varieties of soybean (Liener, 1994).

CONCLUSION

Based on the presented results, experiment confirmed that beside Kunitz trypsin inhibitor there are other thermolabile antinutritive factors that negatively affect to the final weight of broilers as well as the size of the digestive organs. Extrusion process remove this negative effects by eliminating anti-nutritional factors which enhance nutritional value of soybeans. It can be concluded that SL did not give satisfactory results and cant fully replace the cooked grain, but the results were much better compared to the SS group, so that future research regarding the use of this sort should be directed toward determining the levels of this species that could be included in the diet without negative consequences.

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EFEKAT NIVOA TRIPSIN INHIBITORA I TERMIČKE OBRADE ZRNA SOJE NA VELIČINU ORGANA BROJLERA

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Izvod

Upotreba termički neobrađenog zrna soje dovodi do hipertrofije pankreasa i drugih unutrašnjih organa. Cilj ovog rada je bio da se uporede efekti sirove soje bez Kunitz tripsin inhibitora (SL) sorte „Lana", standardne sirove soje (SS), ekstrudirane soje bez KTI (LG) sorte „Lana" i ekstrudirane standardne soje (SG) na veličinu organa brojlerskih pilića kao bi se procenio antinutritivni značaj KTI u sojinom zrnu i značaj termičke obrade. Dobijeni rezultati ukazuju da ekstrudiranje i niži nivo KTI imaju značajan efekat na sve ispitivane parametre. Hranidbeni tretman u LG grupi imao je povoljniji efekat na veličnu pnkreasa u odnosu na SS grupu, ali je ipak bio značajno lošiji u odnosu na hranidbene tretmane u LG i SG grupi gde je soja bila termički tretirana. Mogućnost prisustva drugih antinutritivnih faktora pored inhibitora proteaza (KTI) kao što je lektin stvara statistički značajnu razliku između SL grupe i termički tretiranih grupa

tako da se u ovom slučaju ekstrudiranje pokazalo kao efikasniji način za uklanjanje antinutritivnih faktora iz soje, što je u visokoj korelaciji sa veličinom organa na prvom mestu pankreasa.

Ključne reči: brojleri, soja, ekstrudiranje, organi.

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DISTRIBUTION OF NO_3-N WITHIN THE SOIL PROFILE IN ENHANCING CORN YIELD

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*SUMMARY: A four-year study about the distribution of NO_3-N in depth of land profile and its influence on the yield height of corn line was conducted on the long term stationary field at the Institute for Field and Vegetable Crops in Novi Sad. Six inbred corn lines which belong to different FAO groups were researched depending on the variant of nitrogen fertilization (Control; 40 kg Nha^{-1}; 80 kg Nha^{-1} and 120 kg Nha^{-1}). Dependence between NO_3–N content in profile depth, winter precipitation and corn line yield was established through Person's simple correlation coefficients and by the method of Path coefficients analysis. Winter precipitation influenced significantly the rise of content of NO_3-N especially in layers of 30-60 cm (r=0.59**) and 60-90 cm (r=0.41*), and exactly N from these two layers has mostly influenced the yield height.*

Key words: *NO_3-N, winter precipitation, corn, yield, fertilization.*

INTRODUCTION

Vojvodina (Serbia) has favourable conditions for production of mercantile as well as seed corn, whereas yields are sometimes low because of the limited irrigation possibilities and late application of particular cultural practices. Researches in this field are significantly smaller and thus corn seed is usually fertilized with relatively big quantities of NPK nutrients. For that reason it is very important to gain knowledge about the specifications of mineral diet for every important line that is included in the creation of corn hybrids.

Corn yield depends on a large number of factors and their interaction. Key factors are the amount and distribution of rainfall during growing season and crop manage-

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ment practices, especially fertilization system and crop rotation (Tabatabai et al., 1992; Hoffmann et al., 2007; Senwo and Tabatabai, 2005; Overman and Brock, 2003). Besides the quantity of mineral nitrogen in the soil, distribution of nitrogen by the profile depth is also significant when defining total yield variability. The dynamics of NO_3-N is considerably affected by the date and application quantity of fertilizers, both organic and mineral. Most of the yield variation is associated with water availability and its dynamics (Mulla and Schepers, 1997), which affect crop yield both directly and indirectly via the N cycle. The distribution and height of reserves N –min in the profile until the beginning of vegetation depend on the soil type, fertilization of the previous culture, mineralization and nitrogen immobilization, rainfall during the winter, as well as on nitrogen acquirement by crops (Kuhlmann et al., 1983; Starčević et al., 2003; Rosolem et al., 2005; Latković i Starčević, 2006 and Ruffo et al., 2006). Marinković et al. (2008) stated that winter precipitation and soil mineral distribution in the spring, before corn harvest, are the only elements that may serve for determining the annual yield potential of corn which is grown under the climatic conditions of the Vojvodina Province (Serbia). These authors maintained that the previous two elements, if combined with other factors are sufficient to make necessary adjustments of pre-sowing N dose and stand density. In three long-term rotation experiments, Doll (1962) found that 20 – 23% of the deviations of yields from the linear regression during years could be attributed to winter precipitation.

MATERIAL AND METHOD

Long term stationary field experiment involving fertilization of corn lines was set on the experiment field at the Institute of Field and Vegetable Crops in Novi Sad in 1995. The experiment was set as a two-factor split plot experiment with four repetitions. The area of experimental soil was 56 m^2. Researches were conducted in the period from 1996-1999. And the following factors were examined:

The first factor is inbred corn line (L1 –L6) which belongs to different FAO ripening groups: L1 (FAO group 300); lines L2 and L3 (FAO group 400); lines L4, L5 and L6 (FAO group 600). The lines are seeded with the density of 57142 plants (70x25cm), and the sowing was done in these four years of examination in the period from 15 April to 17 April.

The second factor regards the variants of nitrogen fertilization: Control; 40 kg Nha^{l}; 80 kg Nha^{-1} and 120 kg Nha^{-1}. Half of the nitrogen fertilizers (urea 46% N) were taken in autumn during the basic soil cultivation, and the other half of nitrogen (urea 46%N) was applied in spring – before the sowing. Within all variants of nitrogen fertilization in autumn, the same quantity of P and K, 40 $kgha^{-1}$ was applied. In order to determine the mineral nitrogen, the samples of soil were taken in spring (before the sowing) and in autumn (after the corn line harvest). Samples were taken in layers of 30 cm; being deep up to 120 cm. The taken samples were analyzed by the Scharp and Werhmann method (1975). The dependence between the content of mineral nitrogen in the profile depth, winter precipitation and yield of corn lines in different variants of nitrogen fertilization was established with the help of the Pierson's simple correlation coefficients of regression and by the method of Path coefficients analysis ("path coefficients"). The Path analysis was used for separation of relative participation of examined parameters with the use of the standardized partial coefficients of regression, or with a use of spe-

cial correlation coefficients on direct and indirect influences (Ball, et al., 2001).

Weather conditions

Winter precipitation (WP) was calculated on the basis of mean monthly precipitations in the period from October 1 to March 31 and they were compared with the perennial average.

The quantity of winter precipitation in the first two years of the research (Table 1) was on the level of perennial average (247mm). Smaller quantities of WP regarding the perennial average were established in 1998 and 1999 (231mm). The lack of precipitation during the vegetation period regarding the perennial average was the highest in 1998 (30mm), while in other three years the quantity of precipitation was higher regarding the LTA, especially in 1997 (424mm) and in 1999 (558mm).

Table 1. Precipitation (mm) at Srbobran experimental station (N45^0 09' E19^0 50')
Tabela1.Količine padavina (mm) na eksperimentalnoj stanici u Srbobranu (N 45^0 09' E 19^0 50')

	LTA/VGP1	1996	1997	1998	1999
WP/ZP2	247	243	253	231	231
GS/VP3	345	395	424	315	558

[1]LTA – Long Term Averages (1948-1999)/*VGP - Višegodišnji prosek (1948-1999);* [2]WP – Winter Precipitation (X-III)/*ZP - Zimske padavine (X –III),* [3]GS – Growing Season (IV-IX)/*VP - Vegetacioni period (IV-IX).*

RESULTS

On the basis of a simple correlation coefficients (Table 2) it is noticed that WP, in the control and as well as in all nitrogen fertilization variants, had extremely positive influence (r=0.54** and 0.55**) on the height of yield grains on the average for four examined years. Dependency between WP and the quantity of NO_3-N in the layers of the soil was different depending on the examined variation of N fertilization.

On the average, for all variants of the experiment, the effect of WP on the content of NO_3-N in the surface layer was negative, but not statistically significant. Winter precipitation influenced positively the increase of the content of the NO_3-N especially in the layers of 30-60cm (r=0.59**) and 60-90cm (r=0.41*).

In the controlled variant, WP influenced significantly on the increase of the content of NO_3-N especially in the layer of 60-90cm (r=0.46*) and 90-120 cm (r=0.45*). So, with the increase of WP there has been movement of NO_3-N in the soil into deeper layers (60-120 cm), which influenced positively on the yield height. In this variant, the correlation between the yield and the content of NO_3-N by profile is higher with the greater depth. The highest positive correlation (r=0.54**) was established between the content of NO_3-N in the layer of 90-120 cm and the yield, and significant (r=0.50*) was established also between the content of NO_3-N in the layer of 60-90 cm and the grain yield.

In the variant fertilized with 40kg Nha^{-1}, WP influenced mostly on the movement of NO_3-N from the layer of 0-30 cm into the layer of 30-60 cm. The greatest and the most negative influence (r=0.49*) on the yield height had the content of NO_3-N in the surface layer (0-30 cm), while other layers did not have any significant correlations with the yield. Winter precipitation had a great influence (r-0.55*).

Table 2 Simple correlation coefficients among the content of NO_3-N in the soil, winter precipitation (WP) and corn yield, in the different variants of N fertilization.
Tabela 2 Prosti koeficijenti korelacije između sadržaja mineralnog N u zemljištu, zimskih padavina i prinosa linija kukuruza, na različitim varijantama đubrenja N

	Control/Kontrola		40		80		120		Average/Prosek	
	WP	t/ha	WP	t/ha	WP	t/ha	WP	t/ha	WP	t/ha
WP/ZP	1	0.54**	1	0.55**	1	0.55**	1	0.54**	1	0.55**
N 0-30	-0.14	-0.03	-0.31	-0.49*	0.22	0.01	0.05	-0.06	-0.06	-0.21
N 30-60	0.35	0.36	0.32	0.04	0.53**	0.31	0.57**	0.39	0.59**	0.37
N 60-90	0.46*	0.50*	0.07	0.002	0.37	0.31	0.33	0.27	0.41*	0.35
N 90-120	0.45*	0.54**	0.11	-0.03	0.13	0.19	0.32	0.31	0.32	0.32

*significant on the point of significance/*značajno na pragu* α=0.05
**significant on the point of significance/*značajno na pragu* α=0.01

In the variants where bigger doses of N were used (80 and 120 kg Nha^{-1}), WP did not influence significantly the content of NO_3–N in the layer of 0-30 cm, but its quantity increased significantly in the layer of 30-60 cm (r=0.53** in the variant with 80; that is, r=0.57** in the variant with 120 kg Nha^{-1}).The content of NO_3-N in this layer (30-60 cm) has also had the biggest influence on the yield (r=0.31, that is r=0.39), although these influences were not statistically important. As with the previous variant, the greatest influence on the yield height had the winter precipitation: in the variant of 80 kg Nha^{-1} correlation coefficient with the yield was 0.55**, and in the variant of 120 kg Nha^{-1} 0.54**.

In order to determine more thoroughly causes and consequences of the relation between the content of NO_3-N according to the type of soil, WP and yield a Path coefficient analysis was done. In the Table 3, on the basis of the Path coefficients, direct and indirect effects of the sum of WP and the content of NO_3-N were determined. This was done by the soil layers regarding the yield of corn lines.

On the basis of Path coefficients (p) it is noticed that WP had direct, significant positive influence on the yield of lines only in the fertilization variant with 80 kg N ha^{-1} (p=0.68*). Their direct influence on the yield in other variants was positive, but not significant, unlike the simple correlation coefficients which were important in all variants of the Ni. In control and in the variant with 80 kg Nha^{-1} there has been a very high positive indirect effect (p=0.11 that is 0.13) through the nitrogen content in the layer of the soil from 60-90 cm of depth with which it had the biggest direct positive correlation of yield of lines.

In the variant fertilized with 120 kg Nha^{-1} WP had the strongest indirect influence on the yield with the nitrogen content in the layer of 90-120 cm (p=0.08), with which the yield was also in the biggest positive direct correlation (p=0.25). Although these p values are not statistically significant in all cases they indicate that there is a justified analysis of effects of WP and the nitrogen distribution according to layers on the direct and indirect yield effects.

Also, on the basis of Path coefficients, and by comparison with simple correlation coefficients there is a greater direct negative influence on the content of NO_3-N in the surface layer (0-30 cm) on the yield.

If the average values of the Path coefficients are analyzed regardless of the quantity of the nitrogen application, it is noticed that WP had high positive direct effect on

the yield (p=0.44), where the highest indirect effect (p=0.17) of WP was with the movement of NO_3 in the layer of soil from 60-90 cm.

Table 3 Matrix of direct and indirect PATH correlation coefficients (p)
Tabela 3 Matrica direktnih i indirektnih PATH koeficijenata korelacije (p)

Var.	Factor *Faktor*	Direct effect on yield *Direktan uticaj na prinos*	Indirect effect through /*Indirektan efekat preko*					Total *Ukupno* ($r_{x,y}$)
			N 0-30	N 30-60	N 60-90	N 90-120	ZP	Yield
Ø	N 0-30	-0.34	-	0.16	0.13	0.04	-0.03	-0.03
	N 30-60	0.22	-0.24*	-	0.19	0.12	0.07	0.36
	N 60-90	0.24	-0.18	0.18	-	0.16	0.09	0.50*
	N 90-120	0.20	-0.07	0.13	0.20*	-	0.09	0.54**
	WP/*ZP*	0.20	0.05	0.08	0.11	0.09	-	0.54**
40 kg N ha^{-1}	N 0-30	-0.55*	-	0.12	0.11	-0.07	-0.10	-0.49*
	N 30-60	0.20	-0.33*	-	0.16	-0.09	0.11	0.04
	N 60-90	0.35	-0.17	0.09	-	-0.29	0.02	0.00
	N 90-120	-0.33	-0.12	0.05	0.31	-	0.04	-0.03
	WP/*ZP*	0.32	0.17	0.06	0.03	-0.04	-	0.55**
80 kg N ha^{-1}	N 0-30	-0.24	-	-0.20	0.18	0.11	0.16	0.01
	N 30-60	-0.44*	-0.10	-	0.30	0.19	0.37	0.31
	N 60-90	0.35	-0.12	-0.38*	-	0.21	0.26	0.32
	N 90-120	0.24	-0.11	-0.34*	0.31*	-	0.09	0.19
	WP/*ZP*	0.68*	-0.05	-0.24	0.13	0.03	-	0.55**
120 kg N ha^{-1}	N 0-30	-0.15	-	0.00	-0.01	0.07	0.03	-0.06
	N 30-60	-0.02	-0.02*	-	-0.04*	0.19*	0.29*	0.39
	N 60-90	-0.05	-0.04	-0.02	-	0.21*	0.17*	0.27
	N 90-120	0.25	-0.04	-0.02	-0.04	-	0.16	0.31
	WP/*ZP*	0.50	-0.01	-0.01	-0.02	0.08	-	0.54**
Prosek-*Average*	N 0-30	-0.28	-	-0.05	0.15	-0.01	-0.03	-0.21
	N 30-60	-0.12	-0.12*	-	0.37*	-0.02	0.26*	0.37
	N 60-90	0.41*	-0.10	-0.11	-	-0.02	0.18	0.35
	N 90-120	-0.03	-0.08*	-0.10*	0.38*	-	0.14*	0.32
	WP/*ZP*	0.44	0.02	-0.07	0.17	-0.01	-	0.55*

If the average values of the Path coefficients are analyzed regardless of the quantity of the nitrogen application, it is noticed that WP had high positive direct effect on the yield (p=0.44), where the highest indirect effect (p=0.17) of WP was with the movement of NO_3 in the layer of soil from 60-90 cm. Exactly in this layer, the significant positive direct dependency with the yield (p=0.41*) was determined. This also explains the complexity of interaction of WP, quantity and distribution of NO_3 in the soil profile, because the quantity of WP in the period from October to April mostly goes into deeper

layers, often outside the roots, so that their direct influence is insignificant, but indirectly, with the migration of NO_3 into deeper layers from which the root takes its food, their effect is significant.

DISCUSSION

On average, for all the examined lines, fertilization variants and the years of examination on the basis of simple correlation coefficients, it has been established that the effect of WP on the content of NO_3-N in the surface layer (0-30 cm) was negative, although statistically significant. Winter precipitation influenced significantly on the increase of the content of NO_3-N especially in the layers 30-60 cm (r=0.59**) and 60-90 cm (r=0.41*). Simultaneously, on the basis of simple correlation coefficients it has been established that WP (on average for all four years) in all variants of the experiment had great positive influence on the yield height of line grains (r=0.54 ** and r=0.59**). The increase of quantity of WP and the descendent movement of water led to the movement of NO_3-N in the soil profile into deeper layers (30-60 and 60-90 cm) which had positive influence on the yield. Taking into account the average for all lines, fertilization options and years, the greatest influence on the yield height had the content of nitrogen from these two layers (30-60 and 60-90 cm), although these correlation coefficients were not statistically significant. Marinković et al. (2003) and Marinković et al. (2008) claim that winter precipitation (October –April) is not the only factor which influences the yield (by gathering and the content of water in the soil), but its interaction with many other factors, above all the content and the distribution of mineral nitrogen in the soil. Many authors explain that negative influence of bigger quantities of NO_3-N in the surface layer of soil is caused by weak and shallow root system. Where there is enough nitrogen, the root in the initial phases does not have the need to penetrate into deeper layers, which has negative influence on the yield in the late phases, especially if there are droughts (Kastori 2005 and Marinkovć et al. 2008).

CONCLUSION

On the basis of the four year study and the gained results regarding the distribution of the nitrogen depending on the quantity of WP and nitrogen fertilization option it can be concluded:

- On average, for all lines, fertilization options, and years of study (on the basis of simple correlation coefficients) it has been established that the effect of WP on the content of NO_3-N in the surface layer (0-30 cm) was negative, although not statistically insignificant. Winter precipitation had significant influence on the increase of the content of NO_3-N especially in the layers 30-60 cm (r=0.59**) and 60-90 cm (r=0.41*).
- Winter precipitation had great positive influence on the height of the yield of grain lines (r=0.54** and r=0.55**).
- The content of nitrogen from the layers 30-60 cm and 60-90 cm had the greatest influence on the yield height.
- The fertilization with nitrogen has showed a big positive effect on the yield of corn lines.

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DISTRIBUCIJA NO_3-N PO PROFILU ZEMLJIŠTA U FUNKCIJI PRINOSA LINIJA KUKURUZA

DRAGANA LATKOVIĆ, BRANKO MARINKOVIĆ, JOVAN CRNOBARAC, DARINKA BOGDANOVIĆ, FRANC BAVEC, ĐORĐE JOCKOVIĆ

Izvod

Četverogodišnja istraživanja o distribuciji NO_3-N po dubini profila zemljišta i njegovom uticaju na visinu prinosa linija kukuruza obavljena su na višegodišnjem stacionarnom poljskom ogledu Instituta za ratarstvo i povrtarstvo u Novom Sadu. Prvi faktor ispitivanja bilo je šest inbred linija kukuruza koje pripadaju različitin FAO grupama zrenja, a drugi faktor varijante đubrenja azotom (Kontrola, 40 kg Nha^{-1}; 80 kg Nha^{-1} i 120 kg Nha^{-1}). Zavisnost između sadržaja NO_3-N po dubini profila, zimskih padavina i prinosa, utvrđena jr pomoći Pirsonovih prostih koeficijenata korelacije i metodom Path coefficients analisys. Zimske padavine uticale su značajno na povećanje sadržaja NO_3-N u slojevima 30-60 cm ($r=0.59^{**}$) i 60-90 cm ($r=0.41^{*}$), a upravo je azot iz ova dva sloja njviše uticao na visinu prinosa linija kukuruza.

Ključne reči: NO_3-N, zimske padavine, kukuruz, prinos, đubrenje

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APPLICATION OF GEOSTATISTICAL INTERPOLATION METHODS FOR DROUGHT INDICES MAPPING*

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SUMMARY: Spatial maps of drought indices are essential for analysis and risk assessment of agricultural droughts in order to improve drought preparedness and help to reduce drought impacts. This paper presents possibilities of application of geostatistical interpolation methods for drought indices mapping. Ordinary Kriging method was used for interpolation of Standard Precipitation Index (SPI) values and maps were created for the territory of Serbia. Values of SPI for principal meteorological stations in Serbia are calculated for vegetation period, that is, for six month period (SPI6) on the basis of monthly precipitation data for period 1971- 2009.

Key words: *GIS, Ordinary Kriging, SPI.*

INTRODUCTION

Agricultural production in Serbia is often limited by quantity and temporal distribution of precipitation. Over the last decades economy, especially agriculture, has been affected by frequent occurrence of drought periods. Drought is a complex natural disaster that causes serious environmental, social, and economic consequences worldwide (Wilhite et al., 2000). Complex drought phenomenon can be simplified into a drought index, which is a single number assimilating a large amount of water supply data. There are many drought indices and one of the most frequently used is standardized precipitation index – SPI (McKee et al., 1993). Guttman (1997) recommended that the SPI could be used as the primary drought index because it is simple, probability based and spatially invariant in its interpretation, so that it could be used in risk and decision analysis. Although SPI index is not directly related to parameters which determine agricultural

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drought conditions (such as soil water balance) it was recommended also by Commision of Agrometeorology of the World Meteorological Organization to implement SPI worldwide (WMO, 2009.).

Geostatistical interpolation methods are developed for purposes of spatial interpolations of data on investigated area based upon certain or known samples. Known samples are necessary previously to be analyzed in order to determine appropriate model based on which the most probable area represented by the points will be obtained. Geostatistical interpolation assumes that the source data points are a specific statistical sample or realization from some true underlying surface function. This sample must first be analyzed in order to create a suitable model that will provide the best possible estimate of this underlying surface (Smith et al., 2009). One of the most frequent applications of these methods is interpolation of altitude and digital terrain models (DTM) based upon measured altitudes above sea level of characteristic terrain points for a certain area. These methods are frequently used tools in GIS applications. However, the methods could be used for spatial interpolations of other measured values if there are certain values for characteristic points in this area. Application of geostatistics in climatology and meteorology has been recently studied and is widely accepted in meteorological community (Dyras et al., 2005). In this paper geostatistical model is used for spatial interpolation of SPI drought index values. Generation of SPI map for the territory of Serbia enables determining the most probable values of SPI for certain points on the territory, and visually to percept drought index phenomenon on the whole territory of Serbia

MATERIAL AND METHOD

SPI is a relatively simple, but popular and very practical drought index. For its calculation only precipitation data are needed. The SPI has been designed to be spatially invariant indicator of drought that recognizes importance of time scales in analysis of water availability and water use. It can be computed for a total observed precipitation over any duration desired by a user, from less than 1 month to 48 months or more, (Guttman, 1999). Calculation time period depends on user's application. Short-term SPI could be used to detect agricultural drought, and long-term SPI could be used for water supply management. For operational application of SPI for drought monitoring it is essential to calibrate SPI values with drought occurrence, i.e. to find best match of threshold values and accumulation periods with recorded drought impacts.

The SPI is calculated by fitting historical precipitation data to a gamma probability distribution function for a specific time period and location, and transforming a gamma distribution to a normal distribution with a mean of zero and standard deviation of one (McKee et al., 1993). This procedure enables us to simplify interpretation of SPI values as measure of departure of precipitation accumulations from its normal values in units of standard deviation although precipitation accumulations do not follow normal distribution. Negative values of the SPI are showing severity of dryness and positive values of are showing degree of wetness. For the purpose of operative activities and analysis, Republic Hydrometeorological Service of Serbia (RHMSS, 2010) has adopted a ten-category classification for the SPI, table 1.

Monthly precipitation data, period from 1971 to 2009, were used for calculation of the SPI. Data were collected from 28 principal meteorological stations in Serbia. Cali-

bration period from 1971 to 2000 was used to determine parameters of the gamma distribution. The SPI was calculated at 6 month scale - april to september (SPI6), because this temporal scale covers vegetation period which is especially relevant for agricultural production. Besides of values of the SPI for each meteorological station, it is necessary to spatially define station's position. For each station, coordinates are determined in state coordinate system applying Gauss–Krüger projection.

Table 1. Categorization of moisture condition based upon SPI (RHMSS, 2010)
Tabela1. Kategorizacija uslova vlažnosti na osnovu SPI (RHMZS, 2010)

SPI Value Vrednost indeksa	Moisture Conditions Uslovi vlažnosti	Probability Verovatnoća
SPI ≤ -2.326	Exceptional (Catastrophic) drought Izuzetna (katastrofalna) suša	0.010
-2.326 < SPI ≤ -1.645	Extreme drought - Ekstrenma suša	0.040
-1.645 < SPI ≤ -1.282	Severe drought - Jaka suša	0.050
-1.282 < SPI ≤ -0.935	Moderate drought - Umerena suša	0.075
-0.935 < SPI ≤ -0.524	Drought - Sušno	0.125
-0.524 < SPI< +0.524	Near normal - Normalni uslovi vlažnosti	0.400
+0.524 ≤SPI< +0.935	Mildly wet - Malo povećana vlažnost	0.125
+0.935 ≤SPI< +1.282	Moderately wet - Umereno povećana vlažnost	0.075
+1.282 ≤SPI< +1.645	Severely wet - Jako vlažno	0.050
+1.645 ≤SPI< +2.326	Extremely wet - Ekstremno vlažno	0.040
SPI ≥ +2.326	Exceptionally wet - Izuzetno vlažno	0.010

Method used for interpolation is Ordinary Kriging. This method is appropriate for variables without explicit spatial trend or correlation with other external variables. Due to normalization procedure, SPI seems to meet these criteria. Ordinary Kriging is estimating the most probable values of a function in a point x based upon known values (samples) in points x_i. Interpolated value can be formulated as:

$$\hat{Z}(x_0) = \sum_{i=1}^{n} \lambda_i * Z(x_i) \text{ where } \sum_{i=1}^{n} \lambda_i = 1$$

Value $\hat{Z}(x_0)$ is estimated value for point x_0, $Z(x_i)$ is known value of the given point x_i, and w_i is weight coefficient of the given point x_i. During determination of weight coefficients it is assumed that there is spatial relationship between values of given points. For calculation of weight coefficients of Ordinary Kriging it is necessary to produce variogram $\gamma(x, y)$ which will define spatial relationship between points.

Weight coefficients are determined using following expression (Al-Shaery et al, 2010):

$$\begin{bmatrix} \lambda_1 \\ \dots \\ \lambda_n \\ \mu \end{bmatrix} = \begin{bmatrix} \gamma(x_1, x_1) & \dots & \gamma(x_1, x_n) & 1 \\ \dots & \dots & \dots & \dots \\ \gamma(x_n, x_1) & \dots & \gamma(x_n, x_n) & 1 \\ 1 & \dots & 1 & \mu \end{bmatrix}^{-1} \begin{bmatrix} \gamma(x_1, x_0) \\ \dots \\ \gamma(x_n, x_0) \\ 1 \end{bmatrix}$$

More known models could be used for variogram which will best represent spatial dependence. In this research two models were used:

spherical:

$$\gamma(h) = C_0 + C_1\left(\frac{3h}{2a} - \frac{1}{2}\left(\frac{h}{a}\right)^3\right) \text{ when } |h| \le a$$

$$\gamma(h) = C_0 + C_1 \text{ when } |h| > a$$

and exponential:

$$\gamma(h) = C_0 + C_1\left(1 - e^{-\frac{kh}{a}}\right) \text{ when } |h| > 0$$

$$\gamma(h) = 0 \text{ when } |h| = 0$$

(Smith et al., 2009, Al-Shaery et al., 2010).

For both spherical and exponential models of variogram it is necessary to determine tree parameters: C_0 - nugget, a - range and C_0+C_1 - Sill. Choice of model which will define spatial dependence is based upon experimental variogram calculated from given points (sample) shown in fig. 1 and fig. 2.

RESULTS

SPI6 indices are calculated for period 2000 to 2009, and for each year only vegetation periods are assumed. Based upon calculated values of the SPI6 for the principal meteorological stations of the RHMSS spatial dependence was analyzed for each year. An experimental variogram was formed using samples, which were used to assess which model with its parameters fits the best to the experimental variogram. Figure 1 presents variogram for 2002. For this year spherical model with its parameters range a=421000, nugget C_0=0.10 and Sill C_0-C_1= 0.97 better fits, and this model was chosen for interpolation.

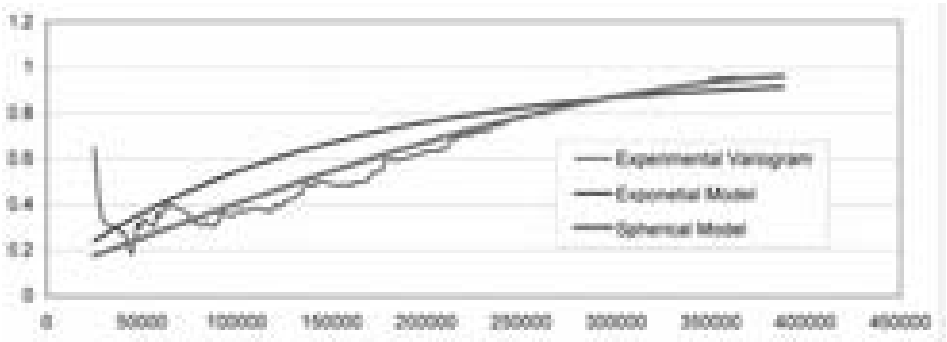

Figure 1: Experimental variogram and adequate models for interpolation of the SPI6 values for 2002, C_0 = 0.10, a = 241000, C_0-C_1 = 0.97

Slika 1: Eksperimentalni variogram i odgovarajući modeli za interpolaciju vrednosti SPI6 za 2002. godinu. C_0=0.10, a=241000, C_0-C_1=0.97

Figure 2 presents variogram for the year 2003, where exponential model with parameters range a=421000, nugget C_0=0.10 and Sill C_0-C_1= 0.59 better fits than spherical model. This model was further used for interpolation with determined parameters.

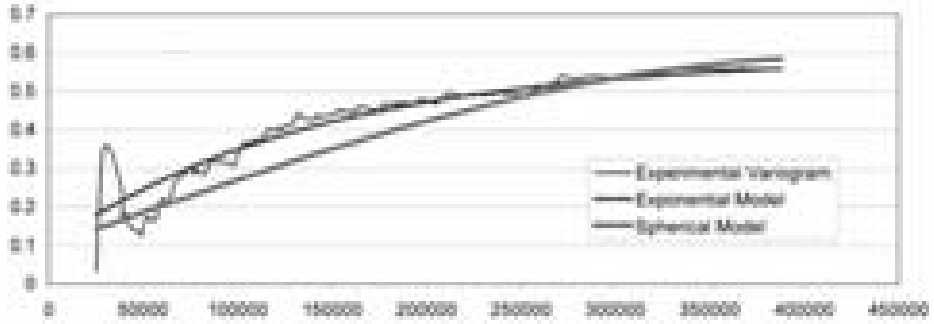

Figure 2. Experimetal variogram and adequate models for interpolation of the SPI6 values for 2003, C_0=0.10, a=241000, C_0-C_1=0.59

Slika 2. Eksperimentalni variogram i odgovarajući modeli za interpolaciju vrednosti SPI6 za 2003. godinu. C_0=0.10, a=241000, C_0-C_1=0.59

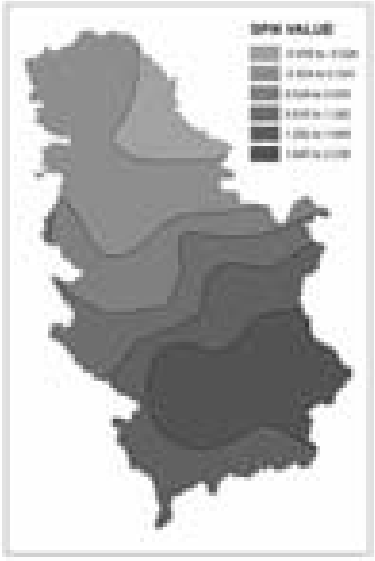

Figure 3. Territory of Serbia, map of the SPI6 for 2002

Slika 3. Teritorija Srbije, karta SPI6 za 2002. godinu

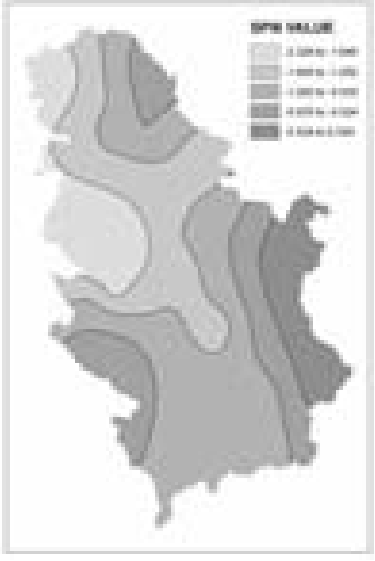

Figure 4. Territory of Serbia, map of the SPI6 for 2003

Slika 4. Teritorija Srbije, karta SPI6 za 2003. godinu

Interpolation of the SPI6 values based upon determined models of spatial distribution for the whole territory of Serbia for period 2000-2009 was presented in a form of maps. The SPI6 maps were created according to range of values given in the table 1. The paper presents map for vegetation period for wet 2002 (fig. 3), and for vegetation period of dry 2003 year (fig. 4). The maps are presenting spatial distribution of the SPI6 values as indicator of moisture on the territory of Serbia for the given years.

DISCUSSION

Although geostatistical models are developed for spatial interpolations in "geo" sciences, these models could be successfully used for spatial distribution of other data which are spatially arranged. Recent examples of meteorological data presentation are creating climatological air temperature maps using geostatistical models for Poland (Ustrnul, Czekierda, 2005), and SPI maps for the territory of Hungary (Szentimrey, Bihari, 2010*)* and Greece (Chortaria, et al. 2010). In this investigation geostatistical methods are used for presentation of spatial variation of drought index values in vegetation period for the territory of Serbia. The most convenient way of presenting of these data is in a form of drought index maps. Values of the drought index are given for each km^2, and at the same time these are grouped according to value ranges shown in the table 1. Maps are presenting areas with categories of moisture in line with categories used by the RHMSS. Given maps are chosen, because there are more categories presented (for some years there were only one or two categories), so spatial distribution of the SPI6 could be easier observed. Analyzing the SPI6 map for 2002 it can be concluded that moisture conditions were ranging from dry to normal (north part of Serbia), to extremely wet (south part of Serbia). Opposite to the mentioned conditions, analyzing obtained map of the SPI6 for vegetation period in 2003 it can be concluded that on almost all territory of Serbia were predominated extremely dry to dry conditions, except in eastern parts where normal conditions of moisture were persisting. Presented results coincide to results of Rajic et al. (2006), where different methodology was applied for north part of Serbia. Advantage of our approach is possibility of spatial differentiation of drought incidence.

CONCLUSION

The paper presents one of possibilities of application of geostatical models in the field of drought analysis (more specifically, for spatial interpolation of SPI index) which is not the most common field of application of geostatistics. Maps were obtained using Ordinary Kriging method for spatial interpolation, which present spatial distribution of the SPI6 values for vegetation period covering the territory of Serbia. Values of the SPI6 were determined for separate areas of 1 km^2 based upon calculations for 28 principal meteorological stations of the RHMSS. Presentation of moisture conditions on maps was in line with categorization used by the RHMSS, and each category was marked by different color.

Drought mapping provides information needed for analysis and risk assessment of agricultural droughts in order to improve drought preparedness and to reduce drought impacts. Spatial maps of drought indices are essential for drought monitoring and management system, such as the Drought Management Centre for South East Europe - DMCSEE.

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PRIMENA GEOSTATISTIČKIH METODA INTERPOLACIJE ZA IZRADU KARATA INDEKSA SUŠE

PAVEL BENKA, ATILA BEZDAN, JASNA GRABIĆ,
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Izvod

Mapiranjem indeksa suše dobijaju se neophodne podloge za potrebe analiza i procene rizika od poljoprivredne suše u cilju ublažavanja neželjenih posledica. U radu se prikazuju mogućnosti primene geostatističkih metoda interpolacije pri izradi karata indeksa suše (SPI). Za interpolaciju vrednosti SPI korišćen je metod Ordinary Kriging i karte su izrađene za teritoriju Srbije. Vrednosti indeksa suše (SPI) za glavne meteorološke stanice na teritoriji Srbije su sračunate za period vegetacije, odnosno za šestomesečni period (SPI6) na osnovu podataka o mesečnim količinama padavina od 1971. do 2009. godine.

Ključne reči: GIS, Ordinary Kriging, SPI.

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METHOD OF NEONICOTINOIDE DETERMINATION IN WATER BY LIQUID CHROMATOGRAPHY

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SUMMARY: The paper deals with the validation paremeters of the determination method of neonicotinoide inseticides and thier metabolites in tap water by liquid chromatography with the detector of diode array (HPLC/DAD). As the values of the studied parameters are within the range of acceptability such a validated method is applicable in the standard laboratory analyses of neonicotiniodes and their metabolite residues.

Key words: *neonicotinoide, water, validation, HPLC-DAD.*

INTRODUCTION

Neonicotinoides belong to one of the newest gproups of insecticides affecing the nocitine group of acetocholin receptors (nAChR) (Tomizawa and Casida, 2009). Their chemical structure resembles the nicotine structure to a great extent, hence the name neonicotinoide. In contrast to the neonicotinoides, the nicotines have a poor insecticide effect which is why they are not used in agriculture. One of the reasons for neonicotinoides being on the top in insecticide application is their quality of being more selectively toxic to insects than to mammals (Fishel, 2005). The insecticides from the neonicotinoide group which are licensed for distribution in our country are the following: acetamiprid, imidacloprid, thiamethoxam and thiacloprid (Janjić and Elezović, 2008). Their significant metabolites are clothianidin and 6-chloronicotinic acid.

Since pesticides are applied directly to soil or sprayed over crop fields and hence, released directly to the environment, some of them could cause serious ambient contamination, principally in water, with human health risk (Seccia et al., 2005). Owing to their toxicity the US Environmental Protection Agency (EPA) and European Union (EU) have included pesticides in their list of priority pollutants. The EU Directive on

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drinking water quality established a maximum allowed concentration of 0.1μg/L for individual pesticide and 0.5 μg/L for total pesticides in drinking water – including their metabolites (Council Directive 98/83/EC).

The determination of pesticides in environmental water and water for human consumption may only be evaluated with sensitive methods (Rodrigues et al., 2007) like gas chromatodraphy (GC) after derivatization (Lazić et al., 2005), liquid chromatography (HPLC) (Lazić et al*.,* 2008; Gall et al., 2005), by electroanalytical, fluorometric i immunoanalytical methods, as well as by derivative spectrophotometry (Lazić et al., 2003; Gal et al., 2005; Guszvany et al., 2006; Guszvany et al. 2009).

Figure 1. Chemical structure of neonicotinoide and nicotine
Slika 1. Hemijska struktura neonikotinoida i nikotina

The present work develops a validated multiresidue method for the analysis of environmental water (SANCO, 2006; Lazić et al*.,* 2008; Bursić et al., 2009). Aacetamiprid, imaidakloprid, tiametoksam as well as their metabolites 6-chloronicotinic acid and clotianidin with HPLC-DAD were studied. A method with good reproducibility, accuracy and reduced matrix effect was introduced and developed.

MATERIAL AND METHOD

Chemicals and Apparatus. The analytical standards of acetamiprid (99.0%), thiamethoxam (98.0%), imidacloprid (99.0%), clotianidin (99.5%) and 6-chloronicotinic acid (99.0%) by Dr. Ehrenstorfer GmbH, Germany, were used. The stock standard solutions of each analyt in the concentration of 100 μg/ml were prepared in ACN. The mix (all neonicotinoide insecticides and metabolites) working standard solutions (0.02, 0.03, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25 μg/ml) were obtained by dilution with ACN. The neonicotinic determination was carried out by the use of an Agillent 1100 system equipped with binary solvent pump, and auto-sampler and diode array detector. A LC column Hypersil ODS (5μm) (2x250mm I.D.) (Agilent, USA) was used with a mobile phase consisting of a mixture of 0.2 % H_3PO_4 in water–acetonitrile at a flow-rate of 0.7 ml/min in gradient mode. The gradient elution was as follows: 0-2 min, 100/0; 2-3 min 90/10; 3-10 min 80/20; 10-20 min 70/30; 20-30 min 60/40. The post time was held at 100% A for 3 min, and with run time of 30 min giving a total cycle time 33 min. The injection volume was 10 μl. The data acquisition and processing were performed using ChemStation Software B.04.01.SP1 (Agilent Technology, 2001-2009). The retention time for clotianidin was 13.01 min, thiamethoxam 14.37 min, imidacloprid 15.65 min, 6-chloronicotinic acid was 17.58 min and acetamiprid 20.54 min.

Validation. The detection limit (LOD) was determined as the lowest concentration giving a response of three times the average of baseline (SHI, 2009). The limit of quantification (LOQ) was determined as the lowest amount of a given pesticide giving

a response of ten times the average of baseline. For the determination of LOD and LOQ tap water spiked with 0.02 μg/ml of acetamiprid, imidacloprid and thiamethoxam and 0.03 μg/ml of clotianidin and 6-chloronicotinic acid was used. The linearity in the response was studied with a standard solution of neonicotinoides ranging from 0.1 to 1.25 μg/ml. The recovery assays were performed by adding to 1.0 l of water multicomponent working standards to final concentration of 0.1, 0.25, 0.5 and 0.75 μg/ml. The SPE was done with ENVI-18 DSK (47 mm) (Supelco No. 57171). After passing the recovery sample to the cartridges, the analytes were eluted with 3 ml of ACN. The solvent was evaporated to dryness and re-dissolved in 2 ml of ACN in triplicate and analyzed by chromatography in duplicate.

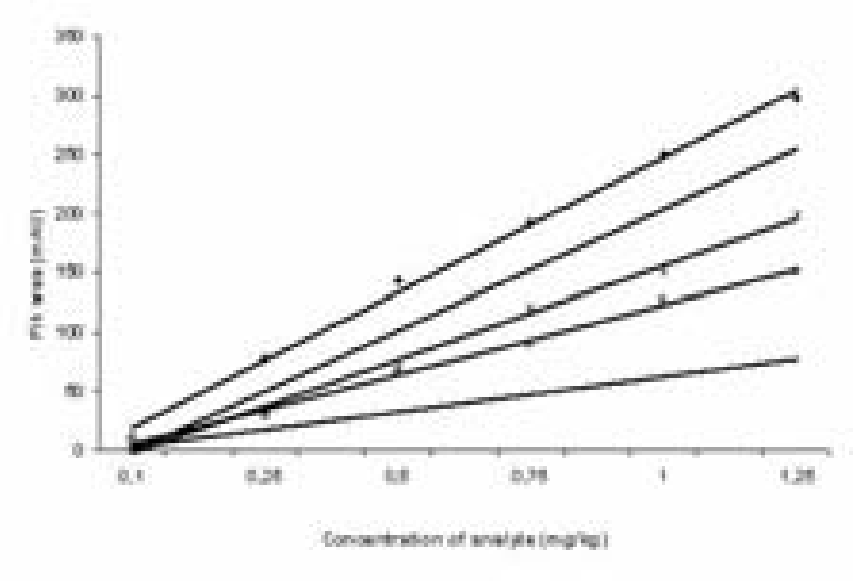
RESULTS

Analytical method. By means of the ANOVA Single Factor and Regression Statistics all the validation parametars were calculated and shown in Tab 1.

Tabe 1: Validation parameters
Tabela 1. Parametri validacije

	LOD (mg/ml)	LOQ (mg/ml)	Average Recovery (%)	RSD (%)
Clotianidin	0.035	0.118	98.0	4.12
Thiametoxam	0.021	0.064	90.0	1.49
Imidacloprid	0.018	0.058	93.0	3.63
6- chloronicotinic acid	0.036	0.118	95.0	4.77
Acetamiprid	0.021	0.061	92.0	3.67

By checking the linearity of the detector response the correlation coefficients of 0.985 for the clotianidin and thiamethoxam, 0.999 for the imidacloprid, 0.993 for the 6-chloronicotinic acid and 0.998 for the acetamiprid were obtained.



Graphic 1. Linearity of detector response (1-thiamethoxam, 2-imidacloprid, 3-acetamiprid, 4-clotianidin, 5-6-chloronicotinic acid)
Grafik 1: Linearnost odziva detektora (1- tiametoksam, 2-imidakloprid, 3-acaetamiprid, 4- klotianidin, 5-6-hloronikotinska kiselina)

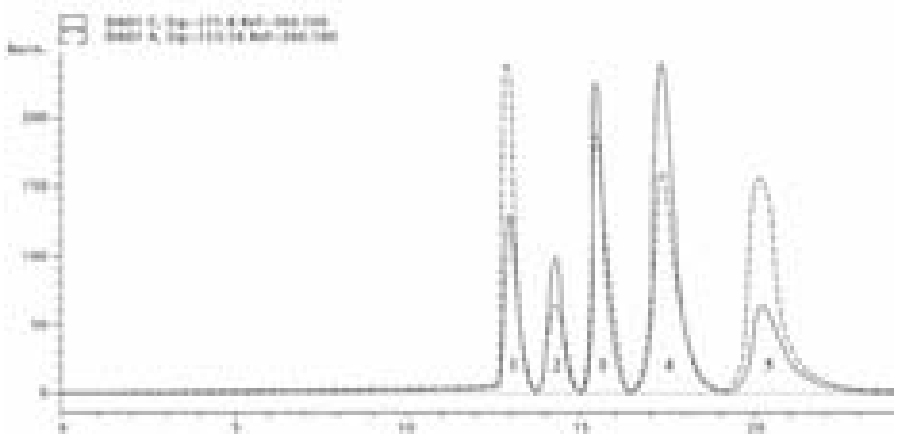


Figure 2. Chromatogram of neonicotinoides mix of amassed concentration 0.50 µg/ml (1-thiamethoxam, 2-imidacloprid, 3-acaetamiprid, 4-clotianidin, 5-6-chloronicotinic acid)
Slika 1. Hromatogram mešavine neonikotinoid amasene koncentracije 0.50 µg/ml (1- tiametoksam, 2-imidakloprid, 3-acaetamiprid, 4- klotianidin, 5-6-hloronikotinska kiselina)

DISCUSSION

By statistical analysis of the studied validation parameters (LOD, LOQ, linearity, replication, recovery) the detection limit below 0.04 mg/l with the quantification limit

lower than 0.12 mg/l was confirmed. The linearity of detector response with the regression coefficient in the range of 0.989-0.999, confirms the linearity. The recovery for the analyzed neonicotinoides and their metabolites ranges from 90.0-98.0% (RSD<5.0%).

By analyzing the obtained chromatograms it is obvious that the wavelength of 253 nm is more favourable for clothianidin and acetamiprid, whereas at the wavelength of 271 nm thiamethoxam, imidacloprid and 6-chloronicotinic acid reach their maximum.

Comparing our results with the accessable reference data the obtained method is applicable in the analyzes of neonicotinoides in water. On the other hand, the lower LOD for the determination of these insectides in the drinking water of 0.01 μg/ml can be obtained with a more sensitive detector – MS (Seccia et al., 2005), or with photochemically induced florescence detection – PIF of 0.8 ng/ml (Vilchez, 2001).

CONCLUSION

The multi-residual method for the determination of neonicotiloides in water, presented in this paper implies the extraction by acetonitril and the determination with HPLC-DAD at the wavelengths of 253 and 271 nm. The method was confirmed through the statistical analyses of valaditation parameters. The obtained method is applicable in the laboratory analyses of neonicotinoides in environmental water, while for their analysis in drinking water the application of more sensitive detectors is necessary because the MRLs is lower than LOD. The LOD for the studied neonicotinoides ranges from 0.021 to 0.636 mg/l.

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METODA ODREĐIVANJA NEONIKOTINOIDA U VODI TEČNOM HROMATOGRAFIJOM

VOJISLAVA BURSIĆ, SANJA LAZIĆ, SLAVICA VUKOVIĆ, DRAGANA ŠUNJKA, DUŠANKA INĐIĆ, GORICA VUKOVIĆKOVIĆ, BOJANA ŠPIROVIĆ

Izvod

U radu su prikazani validacioni parametri metode određivanja neonikotinoidnih insekticida i njihovih metabolita u tap vodi, tečnom hromatografijom sa detektorom sa nizom dioda (HPLC/DAD). Kako se vrednosti ispitanih parametara nalaze u opsegu prihvatljivosti, ovako validizovana metoda se može koristiti u standardnim laboratorijskim ispitivanjima ostataka neonikotinoida i njihovih metabolita.

Ključne reči: neonikotinoidi, voda, validacija, HPLC-DAD.

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TIME - AND DOSE - DEPENDENT TOXICITY OF SULFONAMIDE – CHARACTERISTIC OF METABOLIC PROFILE IN WISTAR RATS

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SUMMARY: The experiment has entered 96 rats Wistar strain. Formed four groups: one control (C) and three experimental which was received 0.066% (O1), 0.2% (O2) and 0.6% (O3) sulfadimidin-sodium in drinking water for 8 weeks. The blood was sampled in which were determined by the following parameters: iron, triglycerides, cholesterol, urea, uric acid, creatinine and alkaline phosphatase. It was done measuring the body mass of rats. Also, it was measured absolute mass of liver, kidney, spleen, heart and tymus. Toxic effects of sulfonamides ware seen in nephrotoxicity and probably hepatotoxicity, taking into account the mass of organs and characteristics of the metabolic profile. Toxicity is expressed depending on the dose. Exposure time was not significant in animals that received the optimal dose or less.

Key words: *sulfonamides, toxicity, organ mass, metabolic profile.*

INTRODUCTION

Sulfonamide is the basis of several groups of drugs. The original antibacterial sulfonamides (sometimes called simply sulfa drugs) are synthetic antimicrobial agents that contain the sulfonamide group. Its molecular structure is similar to p-Aminobenzoic acid (PABA) which is needed in bacteria organisms as a substrate of the enzyme dihydropteroate synthetase for the synthesis of tetrahydrofolic acid (THF). Sulfonamides, derived from chiefly sulfanilamide, are capable of interfering with the metabolic processes in bacteria that require PABA. They act as antimicrobial agents by inhibiting bacterial growth and activity and called sulfa drugs (Riviere and Papich, 2009).

Good susceptibility to sulfonamides shows: Bacillus spp., Brucella spp., Erysipelotrix rhusiopathiae, Listeria monocytogenes, Nocardia spp., pyogenic Streptococcus spp., Chlamydia spp., coccidian, Pneumocystic carinii and Cryptosporidium spp. Sulfonamides antibacterial activity is significant limited by the resistance that has developed after more

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Original scientific paper / *Originalni naučni rad*

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than 50 years of use (Prescott et al, 2000).

It is known that sulfonamides are important residues in the products of livestock production (Wang et al, 2006). Their presence is a consequence of uncritical use of sulfonamide therapy or as growth promoters in animals (Juric, 2001; Stojanović, 2005; Stojanović and Boboš, 2007). These data are important considering that about 3% of the human population shows hypersensitivity to sulfonamides (Tilles, 2001).

The aim of this study was to determine relation between time- and dose-dependent toxicity of sulfonamide.

MATERIAL AND METHOD

The experiment has entered 96 rats Wistar strain. Formed four groups: one control (C) and three experimental which was received 0.066% (O1), 0.2% (O2) and 0.6% (O3) sulfadimidin-sodium in drinking water for 8 weeks.

The blood was sampled in which were determined by the following parameters: iron (Ferrozine method), triglycerides (method by Wahlefeld), cholesterol (method by Trinders), urea (method by March), uric acid (method with phosphor-wolfram acid), creatinine (method by Jaffe method), and alkaline phosphatase (method by Morgenstern). All parameters were determined spectrophotometrically (SMAC analyzer). It was done measuring the body mass of rats. Also, it was measured absolute mass of liver, kidney, spleen, heart and tymus.

It was used a Sartorius precision balance. Samples were taken in the standard way to zero, the second, fourth, sixth and eighth week of experiment. The statistical analysis involves testing the significance of mean differences of parameters examined in relation to the initial and control values.

RESULTS

Table 1: Body and organ mass during experimental period
Tabela 1: Telesna masa i masa organa tokom eksperimentalnog perioda

The mass *Masa (g)*	Time (weeks) *Vreme (nedelje)*	Controle group *Kontrolna grupa*	O1 group *O1 grupa*	O2 group *O2 grupa*	O3 group *O3 grupa*
Body mass *Telesna masa*	2	251.2±29	309±15	287.5±26.3	216.9±28.2
	4	321.1±42.2	302.1±40.3	332.9±32.1	$233.6\pm22^{a,b}$
	6	364.8±52.5	351.9±26.9	369.8±29.2	$262.2\pm26.8^{3a,3b,3c}$
	8	407±50	421±35	413.2±30.5	$274\pm31.4^{3a,3b,3c}$
Liver *Jetra*	2	7.6±1.3	7.4±0.8	8.0±1.2	7.9±1.8
	4	7.5±0.5	9.0±1.8	8.3±1.5	7.2±0.8
	6	8.9±1.0	10.1±1.6	10.0±1.4	$7.5\pm0.9^{a,2b,2c}$
	8	10.4±1.8	10.7±1.4	10.5±1.4	$7.5\pm0.8^{3a,3b,2c}$
Kidney *Bubreg*	2	1.8±0.2	1.7±0.3	1.97±0.1	1.6 ± 0.1^{2c}
	4	1.7±0.3	2.3 ± 0.3^{2a}	2.3 ± 0.5^{a}	1.6 ± 0.2^{c}
	6	2.08±0.2	2.2±0.1	2.5 ± 0.1^{2a}	$1.7\pm0.2^{2a,2b,3c}$
	8	2.5±0.4	2.3±0.2	2.5±0.3	$1.9\pm0.2^{2a,2b,2c}$

Splen *Slezna*	2	0.6±0.2	0.5±0.08	0.6±0.02	0.7±0.2
	4	0.6±0.2	0.5±0.05	0.6±0.2	0.6±0.3
	6	0.6±0.1	0.6±0.2	0.7±0.1	0.4±0.08[2a,2c]
	8	0.5±0.05	0.6±0.2	0.7±0.3	0.3±0.1[2a,2b,c]
Heart *Srce*	2	0.7±0.1	0.8±0.2	0.8±0.12	0.6±0.1[b,c]
	4	0.9±0.08	1.1±0.1	0.9±0.2	0.7±0.09[3a,3b,3c]
	6	0.9±0.3	1.2±0.1	1.6±0.2	0.7±0.2[2b,2c]
	8	1.2±0.1	1.2±0.1	1.2±0.2	0.7±0.1[3a,3b,3c]
Thymus *Timus*	2	0.55±0.1	0.4±0.1	0.4±0.1	0.3±0.08[2a]
	4	0.4±0.1	0.4±0.09	0.5±0.1	0.3±0.04[a,b,c]
	6	0.4±0.09	0.4±0.2	0.4±0.04	0.3±0.1
	8	0.5±0.1	0.5±0.04	0.5±0.08	0.4±0.1[a,b]

[a] – statistically significant difference compared with the control
([a]- p<0.05,[2a]- p<0.01,[3a]- p<0.001),
[b] – statistically significant difference compared with the group O1
([b]- p<0.05,[2b]- p<0.01,[3b]- p<0.001),
[c] – statistically significant difference compared with the group O2
([c]- p<0.05,[2c]- p<0.01,[3c]- p<0.001).

Table 2: Metabolic profile in rats during experimental period
Tabela 2: Metabolički profil pacova tokom eksperimentalnog perioda

Metabolic parameters *Metabolički parametri*	Time (weeks) *Vreme (nedelje)*	Controle group *Kontrolna grupa*	O1 group *O1 grupa*	O2 group *O2 grupa*	O3 group *O3 grupa*
Iron *Gvožđe* (U/l)	2	20.2±11.9	13.5±4.8	20.8±10.6	17.5±5.5
	4	24.5±10.6	27.7±16.4	21.8±3.3	30±4.8[2c]
	6	21±6	39.7±8.4	26.4±2.3[2b]	29.3±10.3[2a]
	8	25±6.5	24.5±6.2	24.7±2.9	35±10[2c]
Triglycerides *Trigliceridi* (mmol/l)	2	1.02±0.37	0.4±0.11[a]	0.35±0.1[a]	0.37±0.13[2a]
	4	0.5±0.2	0.6±0.3	0.4±0.1	0.4±0.06
	6	0.8±0.4	0.6±0.2	0.8±0.3	0.3±0.1[2a,b,c]
	8	0.6±0.2	0.8±0.3	0.6±0.2	0.3±0.06[a,2b,c]
Serum cholesterol *Serumski holesterol* (mmol/l)	2	1.8±0.2	1.7±0.2	1.5±0.3	2.1±0.3[a,b,3c]
	4	1.7±0.3	1.6±0.3	1.5±0.1	2.0±0.4[c]
	6	1.6±0.2	1.1±0.2	1.4±0.06[2b]	2.0±0.2[a,3b,3c]
	8	1.4±0.1	1.4±0.1	1.5±0.2	1.9±0.3[2a,2b,2c]
Urea *Urea* (mmol/l)	2	6.0±1.1	5.6±1.2	6.0±2.1	9.4±1.5[3a,3b,3c]
	4	4.3±0.6	5.8±1.1[2a]	6.7±1.1[3a]	9.4±1.5[2a,3b]
	6	7.1±0.8	7.0±2.3	5.8±0.6[2a]	12.5±1.8[3a,3b,3c]
	8	6.8±1.2	5.5±1.7	6.7±0.7	10.6±1.3[3a,3b,3c]
Uric acid *Mokraćna kiselina* (μmol/l)	2	122.8±44.1	109±26.5	93±13.5	119±32.6
	4	137±24	192.5±33.4[2a]	201.3±32[2a]	172.6±46.6
	6	123±19.2	109±14.5	137±40.9	160.8±22.6[2a,3b]
	8	155±26.8	145±31.4	187.5±56.9	245.2±24.9[3a,3b]

Creatinine *Kreatinin* (μmol/l)	2	51.3±18.8	54.3±21.2	32.5±11.9	39.7±9.1
	4	47±4.6	53±8	48.5±4.9	54.8±7.5
	6	57.3±19.9	55.7±7.1	58.6±10.4	69.5±10.5
	8	62±5.7	44±5.7[3a]	39.5±4.9[3a]	40.4±4.9[3a]
Alkaline phosphatase *Alkalna fosfataza* (U/l)	2	310.5±67.4	293.3±79.5	308±67.4	462.5±126.3[a,b,c]
	4	262.3±130.4	247.7±104.7	264.2±56.1	207.4±89.1
	6	274.3±33.2	247.2±78	220.8±53.4	437.3±100[2a,2b,c]
	8	191±84.6	232.3±141.8	189.3±28	409.8±114[2a,b]

DISCUSSION

Previous results were showed clinical signs of sulfadimidine poisoning including anorexia, decreased growth, alotriophagia, oliguria, diarrhea, ittirability and solmolence (Stojanović et al, 2001, Stojanović et al, 2002).

Sulfonamides are less soluble in acid urine than in alkaline urine, thiterefore, crystal formation is generally greater in the urine of carnivores as compared to herbivores. Dosage and duration of therapy are obvious considerations, since increased concentration increases the probability of crystallization in the urine. Toxicoses often result when the traditional dosage of 70 - 100 mg/lb of body weight is exceeded. Administration of sulfonamides for longer than the customary course of 3 - 5 days may also increase the likelihood of poisoning. Dehydration due to decreased water intake or diarrhea for any reason increases the probability of toxicosis (Prescott et al, 2000). Sulfonamides may have the potential to cause agranulocytosis and thrombocytopenia and forming methemoglobin. Sulfonamides may inhibit carbonic anhydrase and therefore can cause diuresis and acidosis. This drug is response for a peripheral neuritis in chickens, and cattle. Sulfas may also cause acute anaphylactoid reactions, apparently due to a hypersensitivity (immune mediated) reaction (Clipsharm, 1980; Giger et al, 1985). Topical applications of sulfonamides may inhibit wound healing. Sulfonamides may possibly interfere with ruminal flora B-vitamin production or enteric vitamin-K synthesis, although little data exists for the theories. Some sulfonamides, such as sulfaquinoxaline may directly antagonize the action of vitamin K in the liver. Poultry and especially dogs may develop changes in clotting function. Dogs may exhibit increased one stage prothrombin time and increased activated partial thromboplastin time, and clotting time. Because they are highly protein bound to plasma proteins, it may be possible for sulfonamides to displace anticoagulant rodenticides from albumin, making them more available to hepatocytes and hence more toxic (Gupta, 2007; Leitner et al, 2010). Sulfamethazine has been implicated recently as a tumorigen by virtue of the occurrence of thyroid enlargements in laboratory animal studies (Torres et al, 1996). This and the presence of residues in the tissue of animals no longer being given these drugs in their feed intentionally has caused great consternation among regulatory agencies and may result in cessation of the use of this drug as a feed additive on many farms.

CONCLUSION

Toxic effects of sulfonamides ware seen in nephrotoxicity and probably hepatotoxicity, taking into account the mass of organs and characteristics of the metabolic profile. Toxicity is expressed depending on the dose. Time-dependent toxicity was not significant in animals that received the optimal dose or less.

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VREMENSKI I DOZNO ZAVISNA TOKSIČNOST SULFONAMIDA

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VLADIMIR VIDOVIĆ

Izvod

U eksperiment je uključeno 96 pacova Wistar soja. Formirane su 4 grupe: jedna kontrolna (C) i tri eksperimentalne koje su dobijale 0.066% (O1), 0.2% (O2) I 0.6% (O3) sulfadimidin-natrijuma u pijaćoj void tokom 8 nedelja. Uzorkovana je krv i određivani su sledeći parametri: gvožđe, trigliceridi, holesterol, urea, mokraćna kiselina, kreatinin i alkalna fosfataza. Merena je telesna masa i masa jetre, bubrega, srca, slezine i timusa. Toksični efekat sulfonamide ogledao se u nefrotoksičnosti i verovatno hepatotoksičnosti uzimajući u obzir masu organa i karakteristike metaboličkog profila. Toksični efekat zavistan je od doze. Vremenski zavisna toksičnost nije značajna kod životinja koje su dobijale optimalnu ili smanjenu dozu sulfadimidina.

Ključne reči: sulfonamidi, toksičnost, organska masa, metabolički profil.

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GENETIC CHARACTERISATION AND DNA FINGERPRINTING PROFILE OF NEW POPLAR CLONES*

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SUMMARY: The objective of this paper was to determine DNA fingerprinting profile for agronomicaly perspective new ILFE selectioned poplar clones using SSR marker system. Genetic profile of 4 poplar clones belonging to Populus deltoides species (Aigeiros section) were evaluated. Sixteen locuses were considered for genetic evaluation and 5 of them revealed considerably high power of discrimination valued 0,75. Results obtained using SSR data showed in total 62 different alleles and 3, 87 number of alleles per loci Cluster analysis shows the closest connection between 182/81 and B229 clones (0,579 similarity). The PE19/66 clone is genetically more similar to the above clones (0,419) and very distant from B81clone (0,182). When evaluating polymorphisms within genotypes of interest microsatellite DNA markers proved to be a useful tool for distinguishing genetic background of the clones of interests thus grouping them according to their genetic similarity and consequently successfully reveal their DNA fingerprinting profiles. The results presented in this paper were introduce in ILFE poplar breeding programs, and also could be used as a valuable data in new breeding processes as well as in breeders right protection process.

Key words: *poplar, new clones, DNA fingerprinting, SSR markers.*

INTRODUCTION

P. deltoides Marsh. (cottonwood) of section Aigeiros, is one of the most important species for interspecific poplar breeding programs worldwide (Zsuffa 1975, FAO 1979, Rahman and Rajora, 2002). This species are abundant in ILFE nurseries and plantations

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Original scientific paper / *Originalni naučni rad*

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and are widely distributed in forest sector in Serbia. Until now 16 new poplar cultivars created in Institute of Lowland Forestry and Environment (ILFE) were introduced into newly established plantations. Serbian forest breeding programs in ILFE until now were based on conventional clonal identification system, which was based on morphological and phenological traits characterization (Orlović et al., 1997).

The highly polymorphic, consistent and codominant marker, such as SSRs, was excellent tool for clone identification and resolving a unique genetic profile of the new poplar clones under observation which is in accordance to Dayanandan et al. (1998); Rahman et al. (2000), Rajora and Rahman (2003); Van der Shoot et al. (2000), Galović et al. (2004, 2007), Orlovic et al. (2009).

The objective of this paper was to genetically differentiate four, new selected *Populus deltoides* clones, by evaluating their detailed DNA profiles using highly specific allelic polimorphism,

MATERIAL AND METHODS

Plant material

Four newly released poplar clones in ILFE introduced in this study belongs to *Populus deltoides* species, section *Aigeiros Duby*. They originated from ILFE poplar gene pool collection (Vojvodina, Serbia). Their Accession numbers in collection are as following: 1. B81; 2. B229; 3. 182/81; 4. PE19/66 (Tab. 1).

Table 1. Name of poplar clones under investigations and their belonging to species and sections
Tabela 1. Nazivi posmatranih klonova topola, pripadnost vrsti i sekciji

Genotypes under observation *Istraživani genotipovi*	Section *Sekcija*
1.B81 (Populus deltoides)	Aigeiros Duby
2.B229 (Populus deltoides)	Aigeiros Duby
3. Panonija - M1 (P. x euramericana)	Aigeiros Duby
5. PE19/66 (Populus deltoides)	Aigeiros Duby

Plant DNA extraction

Fresh young poplar leaves, 100mg fresh weight, previously treated with liquid nitrogen were ground in tissue lyser (Retsch MM400, www.Retsch.com), 1min at 30 Hz. DNA extraction were according to user manual instructions of Qiagen DNeasy Plant Mini Kit system (www.qiagen.com). DNA concentration was determined by spectrophotometry analyses and its quality and purity evaluated by 0,8% agarose gel. Quantification of the DNA was to 20ng/µl.

SSR markers

Twelve SSR marker loci (PTR1, PTR2, PTR3 PTR4, PTR5, PTR6, PTR7, PTR8, PTR11, PTR12, PTR14, PTR15), originally developed from *P. tremuloides* (Dayanandan et al., 1998; Rahman et al., 2000); and 4 WPMS SSR marker loci (WPMS3, WPMS5, WPMS7 and WPMS12) originally established from *P. nigra* (J. van der Schoot et al., 2000) were used to determine the genetic profile of the clones of interest. PCR protocols followed for SSR analysis were: for WPMS markers according to J. van der Schoot et

al. (2000) and for PTR markers according to Dayanandan et al. (1998) and Rahman et al. (1999).

DNA amplification

Table 2 lists the 16 SSR primer set performed in 25µl PCR reaction mixture containing 20ng gDNA, 0,2 µM each primer, 2,5 mM $MgCl_2$, 0,2mM dNTPs, 0,5 U of Taq DNA polymerase (Fermentas) and 1xTaq buffer as its final concentration. Amplification procedure was performed in Thermal cycler (Eppendorf) at initial denaturation of 94^0C 1min, 5 cycles of denaturation step at 94^0C for 1min, annealing temperature according primer melting temperature for 5min and extention step at 72^0C for 1min. Then 30 cycles of 94^0C for 30 sec., annealing temperature 30 sec., extention step at 72^0C or 30 sec followed by final extention at 72^0C for 7 minutes. For chequing the presence of PCR products the produscts were analysed by electrophoresis in 2% agarose gel in 0,5xTBE buffer, dyed with EtBr and run for half an hour at 80V. Contact prints were made on DIAS system (SERVA Electrophoresis, GmbH, www.serva.de) to obtain permanent images.

Data analysis

PCR products that met the criteria were processed by Bioanalyzer 2100 (Agilent Technologies, www.agilent.com) using high separation fragment chip DNA system analysis software in order to evaluate fragment size and the quantity (Tab.3). Microsatellite alleles at one locus were identified by their molecular sizes in base pairs (bp). Their single and multi-locus genotypes indicated in different allele paterns and ranges were constructed for genetic identification of each individual. Microsatellite DNA variabillity parameters were determined for each of SSR locus as well as over all 16 SSR loci for each *Populus* species: recorded size range, number of alleles, number of alleles/loci and power of discrimination (*Tab 2*).

Statistical analysis

Statistical analysis carried out with the software NTSYS-PC software package, version 2.1 (Rohlf, 2000). Genetic similarity (GS) among accessions was estimated from the number of shared amplified fragments by using the similarity coefficients Dice, GS(*ij*)=2a/(a+b+c) where GS(*ij*) is the measure of GS between the individuals *i* and *j*, *a* is the number of polymorphic fragments that are shared by *i* and *j*, *b* is the number of fragments present in *i* and absent in *j*, and *c* is the number of fragments present in *j* and absent in *i*. The agreement among the Dice's similarity matrix was tested using Mantel's test (Mantel, 1967). The randomization procedure as implemented in NTSYS-pc version 2.1 (Rohlf, 2000) software package included 10^4 random permutations.

The unweighted pair group method with arithmetic mean (UPGMA) analyses was performed based on the similarity matrix using PHYLIP software v.3.63 (Felsenstein, University of Washington, Seattle, WA, USA). In addition, bootstrap analysis was performed on 1000 bootstrap samples to test the reliability of branches (Felsenstein, 1985). UPGMA dendrograms were visualized with the TreeView program (Page, 1996). The power of discrimination (PD) for individual microsatellite DNA loci was determined as follows (Klosterman et al., 1993): $PD=\sum G^2_i$ where *Gi* is the frequency of the i^{th} genotype at a locus.

RESULTS

All out of 16 SSR loci were successfully amplified and all of them showed allele polymorphism. SSR data showed in total 62 different alleles and 3, 87 number of alleles per loci. Among those loci, the individuals that failed to show any amplification product were designated as null genotypes and they were not taking into account in statistical analyses. Eight null alleles were found in 6 loci and in all 4 clones examined. Also alleles under 100 and above 400 haven't taken into consideration for data processing. (Tab 2)

Table 2. Lenght of detected alleles (bp) in all clones under observation
Tabela 2. Dužina detektovanih alela (bp) kod svih posmatranih klonova

Loci *Lokusi*	Genotype*s* *Genotipovi*			
	B81	B229	182/81	PE19/66
PTR1	0	256	258	260
PTR2	214, 224, 295	214, 224, 295	214, 224, 296	213, 224, 296
PTR3	218, 223, 248	0	0	216
PTR4	240	155, 240	240	240, 357
PTR5	148, 269, 276	0	0	0
PTR6	221, 222, 349	223	222	223
PTR7	0	0	236	0
PTR8	143, 149	143, 146	143, 150	143, 162
PTR11	152	64, 152	64, 152	64, 153
PTR12	266	264	264	264
PTR14	159, 164, 194, 205	194, 205	194, 204	156, 194, 205
PTR15	179	185, 309	185	185
WPMS3	281	279	300	0
WPMS5	292, 301, 329	281, 290, 296	290	284, 299, 313, 323
WPMS7	0	0	0	252, 259, 284
WPMS12	173, 184, 235	184	0	168, 187
Total No. of alleles = 62 *Ukupan broj alela = 62*				

Power of discrimination (PD) varied throughout loci. The lowest power of discrimination (0,37) showed four loci: PTR5, PTR7, PTR12 and WPMS7 while the highest PD (0,75) were revealed by loci: PTR1, PTR8, WPMS3, WPMS5 and WPMS12. All other loci showed also high PD but with slightly lower value of 0, 62. (Tab. 3).

Table 3. SSRs size range, no. of detected alleles and power of discrimination (PD) for four new poplar clones investigated.
Tabela 3. Opseg veličine SSR fragmenata, broj detektovanih alela I snaga diskriminacije (PD) za četiri ispitivana klona topole

Loci *Lokusi*	Size range (bp) *Opsegveličine fragmenata (bp)*	No. of allels *Broj alela*	Power of discrimination (PD) *Snaga diskriminacije (PD)*
PTR1	256-260	3	0,75
PTR2	213-296	5	0,625
PTR3	216-248	4	0,625
PTR4	155-357	3	0,625
PTR5	148-276	3	0,375
PTR6	221-349	4	0,625
PTR7	236	1	0,375
PTR8	143-162	5	0,75
PTR11	64-153	3	0,625
PTR12	264-266	2	0,375
PTR14	156-205	5	0,625
PTR15	179-309	3	0,625
WPMS3	279-300	3	0,75
WPMS5	281-329	10	0,75
WPMS7	252-284	3	0,375
WPMS12	168-235	5	0,75
Total No. of alleles/loci = 3,875 *Ukupan broj alela po lokusu = 3,875*			

By screening of all the alleles sizes of all the loci amplified table 4 shows the allele polymorphisms meaning a unique DNA fingerprinting pattern for every genotype under investigation. The most allele polymorphisms were revealed in genotype B81(29), than in PE19/66 (25) and B229 (21) followed with 182/81 (17). All four genotypes have the same allele size amplified for the following loci: PTR2 (224bp), PTR4 (240bp), PTR8 (143bp) and two allele sizes for locus PTR14 (194bp and 205bp). Those were the loci that are the first recognition for the genotypes identity. Here it will be mentioned only the significant alleles that are different for each of the genotypes and represents their recognizable unique DNA fingerprinting pattern: For B81 PTR1, PTR7 and WPMS7 loci doesn't exist; PTR11, PTR12, PTR15 and WPMS3 loci ampilified only one allele sized 152bp, 266bp, 179bp and 281bp respectively. Pattern of genotype B229 showed that PTR3, PTR5, PTR7 and WPMS7 doesn't exist. Only one allele were amplified at loci PTR1 (256bp), PTR6 (223bp), PTR12 (264bp), WPMS3 (279bp), WPMS12 (184bp). The fingerprint of genotype 182/81 are unique for loci PTR7. This loci were amplified only in this genotype. There is no PTR3, PTR5, WPMS7 and WPMS 12 loci amplification in this genotype. Genotype PE19/66 is the one that have amplification in WPMS7 locus vs. there is no amplification at PTR5, PTR7 and WPMS3 loci.

Table 4. A unique DNA fingerprinting profile for genotypes under observation
Tabela 4. Jedinstveni DNK fingerprinting profil posmatranih genotipova

Populus sp *Populus sp.*		Genotypes *Genotipovi*			
Loci *Lokusi*	Alelle size(bp*)* *Veličina alela(bp)*	B81	B229	182/81	PE19/66
PTR1	256	0	256	0	0
PTR1	258	0	0	258	0
PTR1	260	0	0	0	260
PTR2	213	0	0	0	213
PTR2	214	214	214	214	0
PTR2	224	224	224	224	224
PTR2	295	295	295	0	0
PTR2	296	0	0	296	296
PTR3	216	0	0	0	216
PTR3	218	218	0	0	0
PTR3	223	223	0	0	0
PTR3	248	248	0	0	0
PTR4	155	0	155	0	0
PTR4	240	240	240	240	240
PTR4	357	0	0	0	0
PTR5	148	148	0	0	0
PTR5	269	269	0	0	0
PTR5	276	276	0	0	0
PTR6	221	221	0	0	0
PTR6	222	222	0	222	0
PTR6	223	0	223	0	223
PTR6	349	349	0	0	0
PTR7	236	0	0	236	0
PTR8	143	143	143	143	143
PTR8	146	0	146	0	0
PTR8	149	149	0	0	0
PTR8	150	0	0	150	0
PTR8	162	0	0	0	162
PTR11	64	0	64	64	64
PTR11	152	152	152	152	0
PTR11	153	0	0	0	153
PTR12	264	0	264	264	264
PTR12	266	266	0	0	0
PTR14	156	0	0	0	156
PTR14	159	159	0	0	0
PTR14	164	164	0	0	0
PTR14	194	194	194	194	194
PTR14	205	205	205	205	205
PTR15	179	179	0	0	0
PTR15	185	0	185	185	185
PTR15	309	0	309	0	0
WPMS3	279	0	279	0	0
WPMS3	281	281	0	0	0

WPMS3	300	0	0	300	0
WPMS5	281	0	281	0	0
WPMS5	284	0	0	0	284
WPMS5	290	0	290	290	0
WPMS5	292	292	0	0	0
WPMS5	296	0	296	0	0
WPMS5	299	0	0	0	299
WPMS5	301	301	0	0	0
WPMS5	313	0	0	0	313
WPMS5	323	0	0	0	323
WPMS5	329	329	0	0	0
WPMS7	252	0	0	0	252
WPMS7	259	0	0	0	259
WPMS7	284	0	0	0	284
WPMS12	168	0	0	0	168
WPMS12	173	173	0	0	0
WPMS12	184	184	184	0	0
WPMS12	187	0	0	0	187
WPMS12	235	235	0	0	0
Total number of alleles *Ukupan broj alela*		29	21	17	25

Statistical analysis of genetic similarity (table 5) resulting dendrogram that separated three distinct clusters, one included very closely related (0,579 similarity) 182/81 and B229 genotypes, and the other represents PE19/66 genotype that is closer to previous two (0,419 similarity) but highly distant from B81 (0,182 similarity) (Fig. 1).

Table 5. Dice similarity matrix
Tabela 5. Matrica sličnosti po Dice-u

	B81	B229	182/81	PE19/66
B81	1.000	0.360	0.348	0.182
B229	0.360	1.000	0.579	0.383
182/81	0.348	0.579	1.000	0.419
PE19/66	0.182	0.383	0.419	1.000

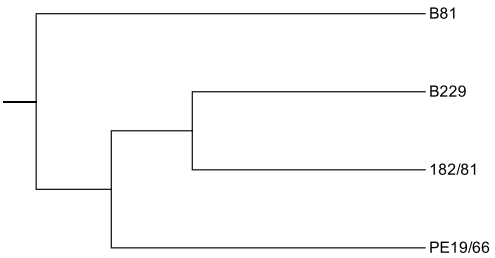

Fig. 1. UPGMA tree of Populus clones constructed from SSR fragment similarities.
Fig. 1. UPGMA dendrogram klonova topola konstruisan na osnovu sličnosti SSR fragmenata

DISCUSSION

Our study revealed the unique DNA fingerprinting patterns for every of the genotypes under observation. Those pattern demonstrates high alleles polymorphisms in each of the genome respectively. However they share some of the genetic material between them indicating the existence of the same allele sizes in some of them making them genetically closer or distinct. Those results showed again that the *PTR* SSR DNA markers developed from *P. tremuloides* (Dayanandan et al., 1998; Rahman et al., 2000) and *WPMS* SSR markers originally established from *P. nigra* (J. van der Schoot et al., 2000) could be successfully used for genetic identification and differentiation of *Populus clones.* Those results are in accordance to findings of Rahman and Rajora (2002); Gomez et al. (2003) and Smulders et al. (1997), Galovic et al. (2006, 2007), Orlovic et al. (2009). They showed very high allele polymorphisms and power of discrimination. The most polymorphic DNA pattern occurred in genotype B81 (with 29 alleles), than PE19/66 (with 25 alleles) followed by B229 (21 alleles) and 182/81 with 17 alleles in total for all loci under investigation. Both markers were reliable for DNA fingerprinting of the new clones investigated in this paper. The SSR markers used showed high genetic simillarity of genotypes B229 and 182/81 and PE19/66 was genetically closer to previous two. Genotype B81 made a separate cluster indicated high genetical distance from the rest of the investigated ones.

Our study is in accordance with the previous findings of high informativeness and discriminativeness of SSR markers applied (Galovic et al. 2007) and also there are findings (Orlovic et al. 2009) that proved the existence of the similar genetic relationship in this four, particular poplar genotypes using AFLP and SSR markers, among other poplar genotypes that were studied. This paper reveals for the first time the DNA fingerprinting of four different and agronomically important new clones of Populus species (B81, B229, 182/81 and PE19/66).

CONCLUSIONS

Our study for the first time revealed DNA fingerprinting for four agronomically valuable poplar genotypes. Using chip technology with Bioanalyzer 2100 for revealing allele polymorphisms, therefore having the exact number and length of the DNA fragments this technology provide us with reliable results that: a) clearly differentiate closely related genotypes e. g. for investigation of genetically specific and interspecific relationships of *Populus* individuals, b) gave us a trustworthy tool for revealing the DNA fingerprinting pattern of the individual using in this study and c) this assertive and undoubtfull results could be used in any expertise proof for breeders rights.

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GENETIČKA KARAKTERIZACIJA I DNK PROFIL LIČNE KARTE NOVIH KLONOVA TOPOLA

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Izvod

Cilj ovog rada je utvrđivanje DNK profila novih agronomski perspektivnih ILFE selekcionisanih klonova topola korišćenjem SSR markera. Determinisani su genetički profili 4 klona topole koji pripadaju vrsti *Populus deltoides* (sekcija *Aigeiros*). Od ukupno šesnaest posmatranih lokusa, pet lokusa je pokazalo visoku vrednost diskriminacije (0,75). SSR analizom dobijeno je ukupno 62 različita alela i 3,87 alela po lokusu. Klaster analiza je pokazala usku povezanost klonova 182/81 i B229 (0,579). Klon PE 19/66 je genetički bliži pomenutim klonovima (0,419) a najviše udaljen od klona B81 (0,182). Sistem mikrosatelitskih DNK markera se pokazao kao pouzdano i informativno sredstvo u determinaciji DNK fingerprinting profila istraživanih klonova. Rezultati prikazani u ovom radu su uvedeni u ILFE program oplemenivanja topola a takođe se mogu koristiti kao dragoceni podaci u novom procesu oplemenjivanja, kao i u procesu zaštite prava oplemenjivača.

Ključne reči: topola, novi klonovi, DNK, SSR markeri.

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ANTIOXIDANTS IN SOYBEAN AND SUNFLOWER GRAIN*

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SUMMARY: The objective of this study was to examine differences in antioxidants content: phytate, phenolics, free thiolics (PSH) and glutathione (GSH) in seeds of 7 soybean varieties and 10 sunflower lines with aim to signify their nutritive quality, valuable for further breeding process. The variations between soybean varieties in phytate content were minor, while el/7 was sunflower line with lowest phytate content, which could be used for program breeding of low phytate grain. The relative high phytate and phenolics content was present in Laura seeds, as well as lowest PSH and GSH content. Generally, the higher content of phenolics were in sunflower seeds, what could be considered as negative atribut from nutritive point of view. The highest PSH content was observed in soybean seeds of Nena and Olga. Lower PSH and GSH content was noted in sunflower seeds (down to 4 and 7 times, respectively), compared to soybean. The soybean seeds have potentially better antioxidative potential, compared to sunflower, owing to multiple higher PSH and GSH content and lower level of phenolics. Soybean variety Olga is accenuated as high in PSH and GSH, as well as sunflower line l4/ru, which had higher PSH and particulary GSH level, with lower phytate and phenolics content, as possible antinutrients.

Key words: *phytate, phenolics, thiolics, glutathione, soybean, sunflower.*

INTRODUCTION

Antioxidative substances are very important nutritive factor, responsible for seed longevity (Ramarathnam et al., 1986). One of the most important components for phosphorus storage is phytate (Lott et al., 2000), which, from one side represents antinutritive factor, due to its indigestibility for monogastric organisms, and from other side, has positive role as an antioxidant and anticancerogenic agent. Malenčić et al. (2007) un-

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Original scientific paper / *Originalni naučni rad*

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derlined phenolics as bearers of antioxidative activity in soybean seeds, since the seeds with low phenolic's content have poor antioxidative activity, too. Meanwhile, phenolics react with proteins in sunflower seeds, threatening its quality (Gandhi et al., 2008).

One of the most important antioxidative and nutritive factors are protein antioxidants, i.e. free thiolic groups, which participate in stress prevention in seeds, either in green parts of plants (Chernikova et al., 2000; Santos and Rey, 2006). From this point of view, special attention is given to glutathione, as the one of the crucial "free radical" trappers. Free thiolics have important role in soybean and sunflower grain, since they prevent protein components from degradation (De Paula et al., 1996; Awazuhara et al., 2002).

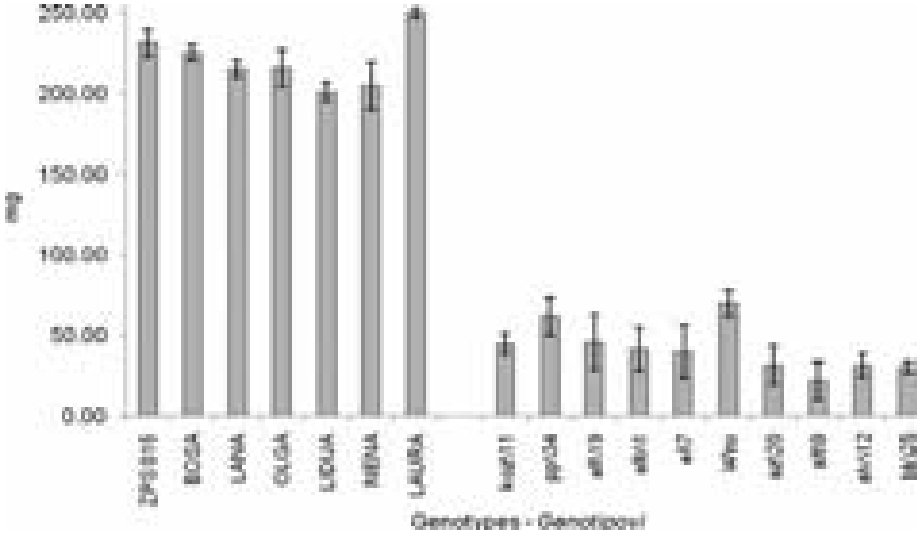
Therefore, the content of phytate, phenolics, free thiolics and glutathione in grain of 7 ZP soybean varieties and 10 sunflower lines were examined in aim to signify their nutritive quality and importance in future breeding program.

MATERIAL AND METHODS

The content of antioxidative substances was analysed in grain of 7 ZP soybean varieties ZPS 015, Lidija, Laura, Lana, Olga, Nena and Bosa) and 10 sunflower lines (koz/11, all/19, bk/25, az/20, alv/12, alt/9, el/7, l4/ru and alb/1). The seeds originated from 2009 and was produced in the experimental field of Maize Research Institute in Zemun Polje. The pericarp was previously removed from sunflower seeds. The soybean and sunflower grain was ground on Tecator Knifetec 1095, then the oil was removed by extraction with petrol-ether (40 – 60 °C) during 12 hours. From grain prepared in such manner, the extraction of antioxidative substances was performed with bidistilled water. The content of particularized antioxidans was analysed: total soluble phenolics, by method of Simić et al. (2004), total soluble thiolics (PSH), by method of deKok et al., (1981), total phytate by modified method of Jočić (1996), while the total gluathione (GSH) was determined after extraction with 5% trichloracetic acid, by method of Sari Gorla et al. (1993). Over against, the content of analysed antioxidants, the was similarity between genotypes was determined b, PATH analysis (Wright, 1923).

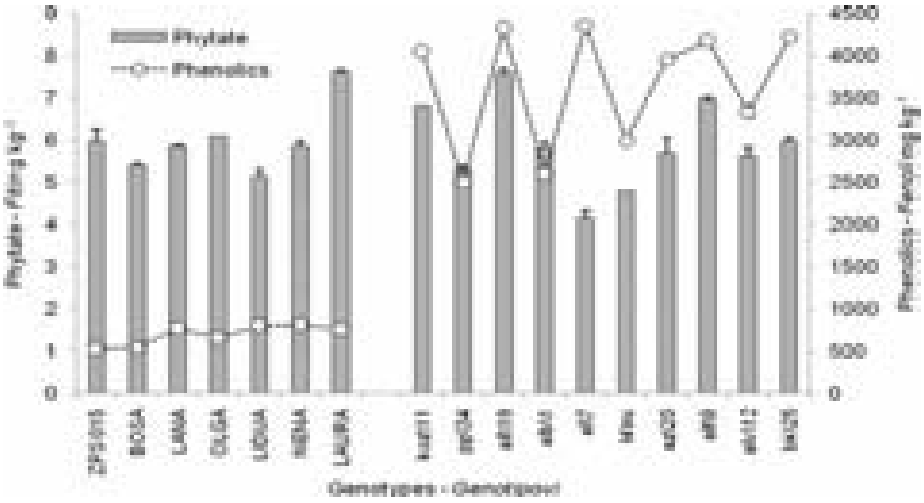
RESULTS AND DISCUSSION

The phytate presents important antioxidant in plants, irrespective to its antinutritive character (Lott et al., 2000). Phytate content differed among soybean varieties in lower degree, to 32% (Graph 2). The highest phytate level was observed in grain of Laura, variety lacking in Kunitz trypsin inhibitor (Perić et al., 2009). On the other hand, a variation of phytate content among sunflower lines was up to 45%. Thereby, it was necessary to emphasize el/7 as line with lowest phytate level. This genotype could be used as valuable source in further breeding programs for low-phytate sunflower hybrids, first of all from the reason of negative aspect of high phytate level in sunflower grain (De Paula et al., 1996; Hídvégi and Lásztity, 2002). Unlike soybean, where positive correlation between seed size and phytate content was observed ($R = 0.80$), the correlation was negative at sunflower and it was insignificant ($R = -0.34$).



Graph 1. The seed weight of different soybean varieties (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA) and sunflower lines (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25); average value ± SD

Grafik 1. Masa semena različitih sorti soje (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA) i linija suncokreta (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25); prosečna vrednost ± SD

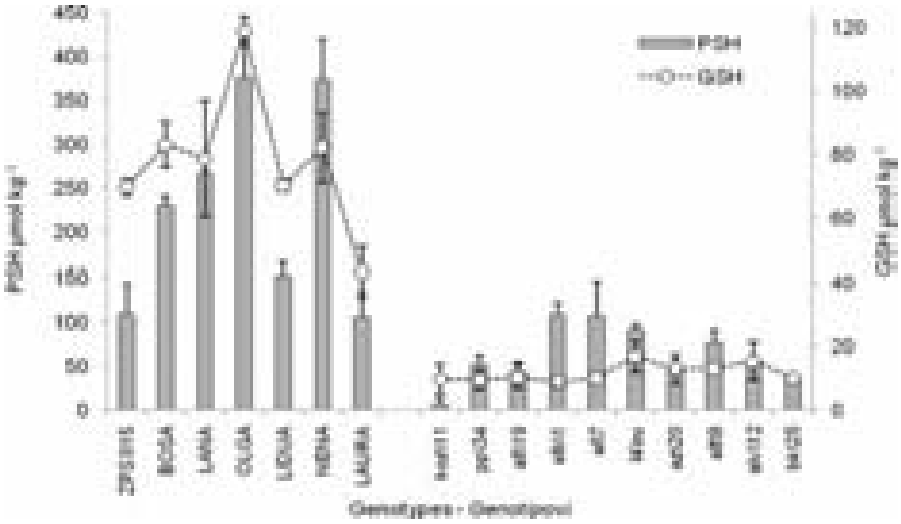


Graph 2. The content of phytate and phenolics in grain of the different soybean varieties (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA) and sunflower lines (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25); ± SD

Grafik 2. Sadržaj fitina i ukupnih fenola u zrnu različitih sorti soje (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA) i linija suncokreta (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25); ± SD

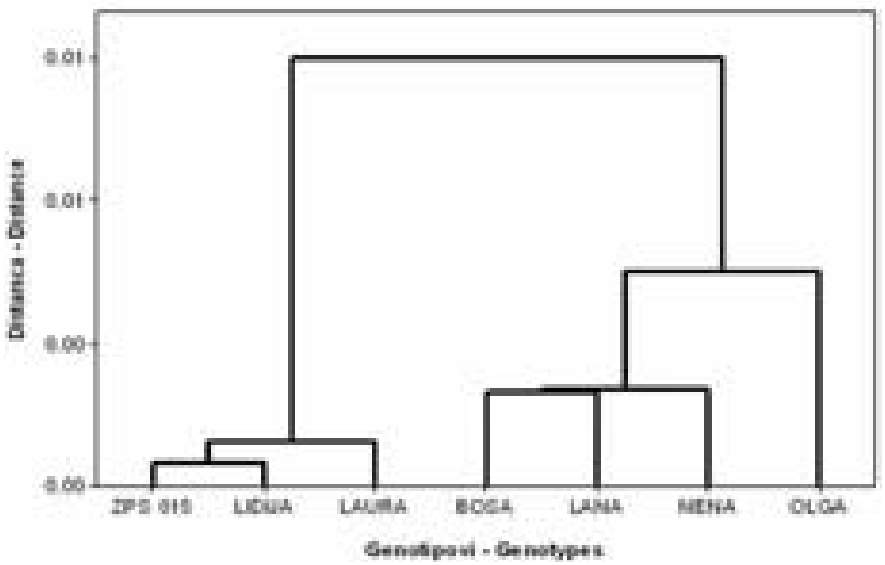
As well, the content of total soluble phenolics varied among soybean varieties to 35%. The group of 4 varieties with higher content of phenolics in grain was seceding:

Lana, Lidija, Nena and Laura, which had 758 – 805 mg kg^{-1} (Graph 2). Considering Laura, as variety with largest seed and highest level of phenolics is aparted from other varieties, the theory of Lee et al. (2008), about negative correlation between grain size (Graph 1) and phenolics content in soybean grain could be confirmed. Beside the high level of phenolycs and phytate in Laura grain (Graph 2), it was important to underline that lowest level of PSH i GSH was present, too (Graph 3) indicating different anti-oxidative profile of this variety, i.e. relatively low antioxidative potential of its protein component in grain. Generally, the higher content of phenolics was present in sunflower grain, while the varying among lines was up to 42%. From the nutritive point of view, higher phenolics level, as it was in grain of koz/11, all/19, el/7 alt/9 and bk/25 is negative characteristics, since it has been responsible for changes in protein quality during the procession of sunflower meal (Gandhi et al., 2008). Ramarathnam et al. (1986) are underlining positive impact of phenolics on rice seed longevity.



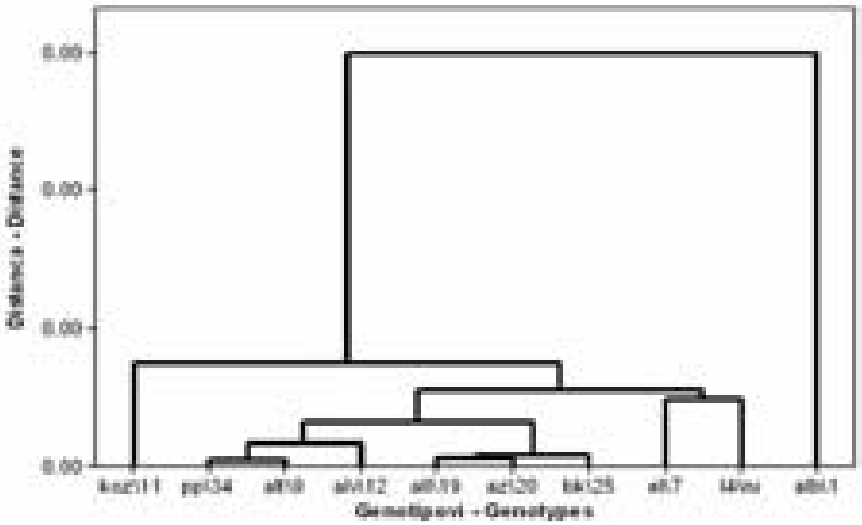
Graph 3. The content of thiolics (PSH) and glutathione (GSH) in grain of the different soybean varieties (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA) and sunflower lines (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25); average value ± SD
Grafik 3. Sadržaj tiolnih grupa (PSH) i glutationa (GSH) u zrnu različitih sorti soje (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA) i linija suncokreta (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25); prosečna vrednost ± SD

The free thiolic groups (-SH) are of importance as anti stress factor (Chernikova et al., 2000; Awazuhara et al., 2002). The highest PSH content was observed in grain of Nena and Olga (Graph 3). Additionally, the highest GSH value was noticed in Olga's grain, which could provide the high anti oxidative potential of this variety, based on thioredoxines (Santos and Rey, 2006). Then again, low PSH i GSH content in Lidija grain could point to low thioredoxine's activity in trapping of free radicals. Other than soybean, 4 times lower PSH and 7 times lower GSH content was noted in sunflower grain. Torres et al. (1996) are emphasizing that GSH is the most important antioxidant in sunflower grain. In regard to that fact alb/1, el/7 i l4/ru were identified as the lines with twice higher PSH level, compared to other lines. It is important to underline line l4/ru, which had the highest GSH level, too.



Graph 4. Cluster analysis dendrogram for content of analysed antioxidants in soybean grain (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA)

Grafik 4. Dendrogram klaster analize za sadržaj analiziranih antioksidanata u zrnu soje (ZPS015, BISA, LANA, OLGA, LIDIJA, NENA, LAURA)



Graph 5. Cluster analysis dendrogram for content of analysed antioxidants in sunflower grain (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25)

Grafikon 5. Dendrogram klaster analize za sadržaj analiziranih antioksidanata u zrnu suncokreta (koz/11, pp/34, all/19, alb/19, el/7, l4/ru, az/20, alt/9, alv/12, bk/25)

Considering the total content of analysed antioxidants, a cluster analysis classified 7 soybean genotypes into two groups: first subcluster composed of cultivars ZPS015, Lidija and Laura and second group comprised of cultivars Bosa, Lana, Nena and Olga

(Graph 4), which were characterised by high level of protein antioxidants (Graph 3). Sunflower lines (Graph 5) were clustered into several smaller groups, while two lines were outliers: koz/11 with the lowest level of protein antioxidants (Graph 3) and relative high content of phenolics (Graph 2), and alb/1, with the highest content of phenolics and PSH.

CONCLUSION

Based on obtained results, it could be concluded that soybean grain has potentially better antioxidative potential, in relation to sunflower, owing to multiple higher content of PSH and GSH, as well as lower level of phenolics. Special accent is given to soybean variety Olga, which has high PSH and GSH content and sunflower line l4/ru, which had higher GSH and PSH content, too, with lower level of phytate and phenolics, as potential antinutrients.

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ANTIOKSIDANTI U SEMENU SOJE I SUNCOKRETA

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VESNA PERIĆ, MIRJANA SREBRIĆ

Izvod

Cilj ogleda je bio da se ispitaju razlike u sadržaju antioksidanata: fitina, fenola, slobodnih tiola i glutationa u semenu 7 ZP sorti soje i 10 linija suncokreta i time ukaže na njihov nutritivni kvalitet i značaj za dalji proces selekcije. Kod soje nije bilo znatnijih variranja u pogledu sadržaja fitina, dok se kod suncokreta izdvaja el/7, kao linija sa najnižim učešćem fitina, koja bi mogla poslužiti u postupku oplemenjvanja niskofitinskih hibrida suncokreta. Kod Laure je pored relativno visokog sadržaj fenola i fitina bio prisutan i najniži sadržaj PSH i GSH. Kod suncokreta je generalno bio prisutan znatno veći sadržaj fenola u zrnu, što je sa nutritivne tačke gledišta negativno. Kod ispitivanih sorti soje, najveći sadržaj PSH je bio kod Nene i Olge. U semenu suncokreta je prosečno bilo 4 i 7 puta manje PSH i GSH. Seme soje poseduje potencijalno bolji antioksidativni potencijal u odnosu na suncokret, zahvaljujući višestruko većem sadržaju PSH i GSH i nižem udelu fenola. Posebno se ističe sorta soje Olga sa visokom sadržajem PSH i GSH, kao i linija suncokreta l4/ru, koja je imala veći sadržaj PSH, a posebno GSH i niži udeo fitina i fenola, kao potencijalnih antinutritiva.

Ključne reči: fitin, fenoli, tioli, glutation, soja, suncokret.

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ORGANIC CEREAL PRODUCTION - OPPORTUNITY FOR AGRICULTURE IN SERBIA*

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SUMMARY: Areas under certified organic production in the world are constantly increasing. The most present plant species in organic production in the world are cereals and forage crops, and from permanent crops - olives, fruits and grape vines. Trend of constant increasing in human population imposes a constant need to increase the production of small grain, while the specific nutrition requirements suggest use of alternative grain in addition to conventional. These usually involve species that are produced in relatively small areas, whose production is in most cases labor intensive, but from the unit area provides greater profit compared to the production of conventional crops. Organic production in Serbia is recent date compared with EU countries, and it is based mainly on the production of vegetable and fruit. Since the cereals are most represented in organic production in the world, our goal is to present the basic recommendations and the possibilities of their growing in these systems in our country. Special accent was placed on the specificity of next alternative plant species: durum wheat, spelt, millets, grain sorghum and buckwheat.

Key words: *organic production, small grains, alternative crops*

INTRODUCTION

According to the *International Federation of Organic Agriculture Movements (IFOAM)*, *«Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects».* Organic produc-

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Review scientific paper / *Pregledni naučni rad*

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tion is based on basic agroecological principles, by including household management of resources with respect for all elements of environmental protection. In recent decades, there is intensively developed awareness that the quality of food and raw materials is necessary to monitor, improve and regularly control, more than ever *(Malešević et al., 2008).* Besides that, accent is put on the control of use of various agrochemicals, whose improper application negatively affects agro- and natural ecosystems, and directly or indirectly influences people's health (*Malešević et al., 2009).* According to *Bavec and Bavec (2007),* in human- and environment-friendly crop production and food processing systems, use of chemicals (fertilizers, pesticides, pharmaceuticals, growth regulators, and unhealthy additives), gene modified organisms (GMOs), and non-resistant cultivars to diseases are maximally reduced.

Thus, the notion of organic agriculture usually refers to the way of growing plants without using mineral fertilizers and pesticides *(Kovačević and Oljača, 2005).* Long term maintenance of soil fertility is of crucial importance, and it is obtained mostly by applying integral and preventive measures. Organic production is based on the principles of agroecology *(Oljača, 2001; Molnar et al., 2005; Milošev and Šeremešić, 2007),* and it is integral part of sustainable agricultural development which uses scientific knowledge regarding the natural laws and establishment of environment protection principles, for production of healthy and safe food (*Lotter, 2003; Lazić and Malešević, 2006).*

Due to a number of advantages in comparison to other systems of growing plants, the areas under certified organic production are increasing every year. Organic production methods have been applied in over 150 countries in the world, on over 35 million ha, from which 8,2 (about 23%) million ha are in Europe. The most common plants in organic arable land (total 4,6 million ha) are cereals (~2 mill. ha, or 44%; the countries with the largest areas in 2008 are Italy, USA and Germany) and field fodder crops (32%), and among perennial plants - olives, fruit and grape vines *(Source: FiBL & IFOAM Survey 2010; most data collected are from 2008).*

As cereals are the most common species in organic production in the world, our goal is to point out basic principles of their cultivation, with a special review of alternative grain species which are becoming more and more popular in organic production. According to *Bavec and Bavec (2007)*, the introduction of alternative crops into rotation will contribute to more natural production systems. Alternative crops (old, ancient, neglected, disregarded, or new) can also help to reduce natural over-sensitivity by sowing more resistant genotypes to plant diseases, increasing the population of natural predators, changing weed population, and helping us to produce healthy food without synthetic pesticides.

The basic principles of organic production in field crops management

Successful farming, as well as *organic production of field crops*, is based on *soils* rich in organic matter, with a good structure, water-air properties and ample in living microorganisms, wich represents a basis for production of healthy plants. This type of soil is good for providing plants with water and elements of mineral nutrition which occurs due to the activity of soil microorganisms, and can be assured by the proper manipulation of harvest residues *(Malešević et al., 2009b; Latković et al., 2009).* Fixation of atmospheric nitrogen can be successfully done on such soil as well as normal circulation of matter, including organic matter from farm and fertilizers permitted in this kind of production *(Malešević et al., 2009a).* Organic farming is founded on the idea that soils with sufficient organic matter content, good structure, ample and variegated in living

microorganisms can provide a base for healthy crops. *Đurić et al. (2008)* refers that application of manure as a result had an increase of microbial C-biomass by 100% in soil profile from 0-60 cm. During the organic maize production, input of manure induced the increase of number of investigated group of microorganisms in soil.

Crop rotation has the crucial role in achieving goals of organic production *(Milošev and Šeremešić, 2004)* because it systematically harmonizes and optimizes all agrotechincal measures. The impact of *previous crops (precrops)* is manifested through consumption of water and nutrients, quantity of harvest residues, time of leaving the field etc. Pre-crops also dictate the method and intensity of soil cultivation, as well as dynamics of fertilizing. Thus, crop rotations have a great importance and potential in the development of integral systems of plant production, especially in the systems of organic farming.

In order to preserve soil fertility potential, it is important to carefully choose which annual and perennial plant species will be grown, therefore, crop rotation is most important agrotechical measure in organic production *(Lazić and Malešević, 2004; Šeremešić and Milošev, 2006)*. Organic production has greater capacity for improvement of soil quality in comparison to the conventional production systems *(Karlen et al., 1997)* because it uses different crop rotations, organic fertilizers and smaller intensity of soil cultivation *(Liebig and Doran, 1999).* In the organic production system, *forage and leguminous crops* should be more present in the structure of crop rotation. Crop rotations should include growing of *stubble and cover crops, intercrops, companion- and catch crops,* as well as other various modifications. By sowing inter- and cover crops, organic production can be remarkably improved. The main goal of growing intercrops is not the yield, but primarily protection of agroecosystems, decreasing or avoiding mineral fertilizers, pesticides and increase in biodiversity *(Ćupina et al., 2004)*.

Release of *nutrients* into easily accessible forms to plants is directly affected by the activity of microorganisms, and their activity is conditioned with temperature and presence of water and air. Nutrient management in organic systems is based on fertility-building leys that fix atmospheric nitrogen, combined with the recycling of nutrients via bulky organic materials such as farmyard manure and crop residues, and with only limited inputs of permitted fertilizers *(Gosling and Shepherd, 2005). Soil fertility control,* which includes measuring of content of easily available nutrients in the soil, must be included in this segment of organic production *(Malešević, 2008b)*. Amount of nutrients in the soil and their availability depend to a great extent on fertilizing system before conversion to the organic production as well as during the conversion period *(Čuvardić et al., 2006).*

Organic fertilizers are irreplaceable when it comes to soil revitalization, i.e. to the improvement of its physical, chemical and biological characteristics. They are important in all concepts of sustainable and organic agriculture as those concepts almost completely exclude application of mineral fertilizers *(Čuvardić, 2006)*. Also, a significant amount of fresh organic matter is introduced by plowing down *green manure*, and *leguminous crops* which can enrich soil by nitrogen. Green manuring improve soil physical and water characteristics, and above all, enhance structure and increase biological activity, as significant factor of soil fertility. Tasks of organic management system are to maximize the contributions of on-farm resources such as animal manures, composts, and green manures to soil fertility. However, purchased off-farm nutrients - including mineral fertilizers, fortified composts, and plant and animal meals - may be necessary

to ensure adequate nutrient availability during transition to an organic program *(ATTRA, 2003).*

Special attention in organic crop production should be devoted to *nitrogen*, as major yield bearer *(Malešević, 1987).* Animal manures and legumes are two major sources of nitrogen in organic systems. Legume cover crops, plowed down to provide green-manure nitrogen, also contribute to soil tilth and increase of organic matter content. During decomposition, legumes can provide 50 to 170 kg N ha^{-1}. Small grains can also receive supplemental nitrogen from crop rotation patterns that include perennial legumes like alfalfa and clover *(Sullivan, 2003).* Plant demands for nitrogen can be compensated besides symbiotic, with the unsymbiotic nitrogen fixing bacterias. Possibilities for use of useful microorganisms in organic agriculture are great, especially as bio-fertilizers and biofertilizators *(Đorđević, 2005).* Nowadays, for such intentions there are numerous microbiological preparations in Serbia *("Nitragin", "Azotofiksin", "Azotobakterin").* Leguminous plants that fix and accumulate nitrogen in soil should be grown in rotation with plant species that consumes greater amounts of this nutrient.

Native *phosphorus and potassium* fertility may be enhanced using animal manures and conserved through good management of cover crops and crop residues. Rock phosphate can serve as an alternative or supplementary phosphorus source when necessary *(Sullivan, 2003).*

The right selection of adaptable cultivars and hybrids of agricultural plants for specific ecological conditions, as well as quality and health conditions of seed material, represents one of the most important pre-conditions for successful crop production. Moreover, the choice of the variety and hybrids must be founded on precise evaluation of consumptive habits in the specific market as well as on the expected financial results. Modern breeding technologies offer great choice of varieties and hybrids of field crops species suitable for organic growing. According to *Berenji (2009),* creation of varieties with an outstanding resistance towards the most important diseases and pests, and adapted to the conditions of low-input, rational investments as well as on abiotic stresses - represent a special challenge in breeding.

Crop density should be adjusted to the characteristics of variety (height, tillering, branching, yield structure etc.) and to the chosen methods of plant protection against weeds and diseases. Crops with lower density have a better developed root system and they are more immune to stresses. Moreover, in such lower density crops, the possibility of mechanical control of weeds is much simpler. Crop density should be also formed on the basis of available water quantities.

In a dynamic and highly sensitive agroecosystems in which an organic production is carried out without the use of synthetic preparates, for suppression of diseases, pests and weeds it is necessary to develop and promote natural mechanisms as self-regulatory function of their populations. In the ecological croping systems, the occurrence of different weed species was significantly higher and similarly in this system the weight of weed dry matter was much higher than in the integrated one *(Týr and Lacko-Bartošová, 2007).* Weed control in organic agriculture should be based on long-term strategy that includes prevention of their growth, exhaustion and destruction.

Preventive measures in suppressing weeds are very important factor in efficient protection in all systems of plant production. They include all measures whose goal is protection of field from weeding, which refer to all those measures that stop occurrences of weed seeds and their vegetative organs in the field *(Kovačević i Momirović,*

2008). From all direct agrotechnical measures used in weed control; the most important are all methods and systems of field cultivation, fertilization, sowing, care measures, and especially plant production systems where crop rotation is playing a important role. Also, growing resistant varieties and hybrids, utilisation of alelopathy, passive barriers and monitoring of weather forecast, have significant place in efficient plant protection in organic production.

Harvest and crop reaping are defined and determined by plant growing systems. The moment of harvest is also determined by dynamics of plant maturation, forming of the quality of products, methods of drying and storing.

Organic small grains production

Growing of small grains organically means using sustainable methods that exclude the use of standard artificial fertilizers and other synthetic matter like pesticides, preservatives, and growth regulators. The group of small grains suitable for organic production includes wheat, barley, oats, rye and triticale (*Triticosecale*). From *Triticum* genus, because of their high nutritive values, more and more interesting are *Durum (hard) wheat*, *Spelt wheat*, *Einkorn*, *Emmer, Kamut (Khorasan wheat)* and many others. By knowing requirements of these species to environmental conditions is extremely important when choosing the appropriate growing technology. The role of people here primarily refers to mitigate the negative effects of climate extremes on plants, especially by the right choice and on-time applying of agrotechnical measures.

Ecology and biological characteristics of small grains. Small grains have winter and spring forms. Each species has great number of varieties, and some of them have sub-species and ecotypes. If huge number of varieties is added, it is clearly why small grains have the widest growing area in the world. Small grains belong to the species of moderate climate. Biological temperature minimum for germination is 4-5 °C, and for the formation of generative organs and maturation about 10-12 °C. Their production optimum for germination is 6-12 °C, for forming vegetative organs 12-16 °C, for generative ones 16-20 °C and for maturation 16-22 °C. Higher temperatures (over 30 °C) significantly decrease vegetation period of small grains, by decreasing yield. Barley endures them best, and rye and oats the least. The relation towards low temperatures is extremely important for winter forms of small grains. In our conditions rye can endure even -25 to -30 °C without snow, triticale, spelt and common wheat -15 to -20 °C, winter barley and durum wheat up to -12, winter oats -10 to -12 °C, while spring barley and oats with minor damages can endure -6 to -8 °C. Relation towards low temperatures depends on the variety and growing stage.

All small grains require a continuous supply of water throughout the vegetation period. The optimal soil moisture for this species is around 60-80% of full field water capacity. Transpiration coefficients of small grains are ranged from 300-400 at barley, to 500-600 in oat and wheat. Critical periods regarding to water plants supply are periods from sowing to emergence; stem elongation to intensive growth and grain filling. Under the continental climate conditions, reserves of winter precipitation in soil are very important. Without them, only with vegetative precipitation, high yields cannot be achieved.

Good yields of small grains can be achieved in the fertile, humous *soils*, mostly by neutral reaction, pH 6-7. Rye can endure acid reaction up to pH 5,5, triticale 5,5-6, and some wheat varieties can endure pH values about 8. It is very important that soils are rich in organic matter and that they have great microbiological activity. These soil

characteristics are crucial for dynamics of adopting nutritive matter in small grains.

Details of **small-grain production practices** - such as planting dates, seeding rates, varieties, and harvesting methods - vary widely among regions, but are largely the same for conventional and organic systems. Compared with conventional agriculture, where agrotechnical measures (cultivation, fertilization, plant protection etc.) can be individually planned and optimized, organic small grain production technology is more complex because of its expected cumulative effects *(Olesen, 1999, Kovačević et al., 1999).*

Variety selection. Variety or hybrid is the basis of high yield and quality of small grains. When selecting cultivars, great attention must be paid to their yield, yield stability and grain quality (especially from the aspect of nutritive value and possibility of 'healthy food' production), then their resistance to lodging, diseases and abiotic stresses *(Bedo and Malešević, 2001).* Also, it is important to know duration of vegetation period for specific variety, in order to avoid critical periods in which high temperatures may appear. Appropriate genotypes for organic production can be found among very rich collections of small grain varieties in Serbia. Modern breeding offers great opportunities for this selection, and in the existing 'gene banks' eminent older varieties convenient for systems of organic crop production can be found. Many of serious seed companies in the world started with production of organic seed of some grown species. In Serbia, possibilities for this kind of production were getting by adoption of Organic agriculture law *(Milovanović et al., 2009).*

Table 1: Needs of small grains for nutritive elements in kg t^{-1} and for adequate straw quantity (average values by various authors)
Tab. 1: Potrebe strnih žita za hranljivim elementima u kg t^{-1} zrna i odgovarajuću količinu slame (prosečne vrednosti date od strane više autora)

Small grain species – *Vrsta žita*	kg t^{-1} grains + straw* - *kg t^{-1} zrna + slama**				
	N	P_2O_5	K_2O	CaO	Mg
Wheat - *Pšenica*					
Winter - *ozima*	30	12	22	6	4
Spring - *jara*	32	12	24	4	3
Barley - *Ječam*					
Winter - *ozimi*	24	10	25	10	3
Spring - *jari*	20	10	21	10	3
Spring oats – *Jari ovas*	25	13	30	6	4
Winter rye – *Ozima raž*	25	11	25	8	3
Winter triticale – *Ozimi tritikale*	25	12	18	6	3
Durum wheat – *Durum pšenica*					
Winter - *ozima*	30	12	24	7	4
Spring - *jara*	34	15	24	7	3
**Given values vary ± 15-20% according to authors, growth conditions and yield height*					

Small grains nutrients requirements. The adoption of elements of mineral nutrition in cereal begins after forming the 2 leaf of seedlings, when young plants transfer to autotrophic nutrition. The fertilization of small grains is based on their needs for NPK nutrients and dynamics of their adoption. It is important to know critical periods during vegetation, when the nutrients requirement is the greatest and when their deficiency

causes the highest yield decrease. Needs of small grains for nitrogen, sulphur and potassium, in kg t^{-1} of grains and adequate quantity of straw are shown in the Tables 1 and 2 *(Malešević et al., 2008a)* and these plants requirements should be compensated by using organic and mineral fertilizers allowed in organic production.

According to presented data, the highest uptake/removal of nitrogen with grains has durum wheat and common spring wheat (Table 2). In the straw of oats and durum wheat, the nitrogen content is the highest, so the removal of this element is significant. At the same time, these cereals have the unfavorable grain to straw ratio. Harvest index is also very variable and it depends from environmental conditions. In favorable years harvest index is much better than in the unfavorable ones. Lodging of crops extremely lowers grain percentage in the total yield of small grains.

Table 2: Removal of nitrogen with grain and straw yield by various small grains (kg t^{-1})
Tabela 2: Iznošenje azota prinosom zrna i slame kod različitih vrsta strnih žita (kg t^{-1})

Small grain species *Vrsta žita*	Grain *Zrno*	Strow *Slama*	Totally *Ukupno*	Harvest index - *Žetveni index* (%)	
				Grain - *Zrno*	Strow - *Slama*
Wheat - *Pšenica*					
Winter - *ozima*	21	7	28	49	51
Spring - *jara*	25	7	32	50	50
Barley - *Ječam*					
Winter - *ozimi*	20	6	26	48	52
Spring - *jari*	18	8	26	46	54
Oat - *Ovas*					
Winter - *ozimi*	21	9	30	40	60
Spring - *jari*	22	12	34	38	62
Winter rye –*ozima raž*	22	6	28	42	58
Durim wheat - *Durum pš.*					
Winter - *ozima*	24	9	33	45	55
Spring - *jara*	26	11	37	42	58
Winter triticale – *oz. tritikale*	23	8	31	49	51

Dynamics of small grain nutrients uptake is in close correlation with the process of forming organic matter, i.e. growth and development of small grains. After the winter vegetation break, when temperatures become stable at about 5 °C, nutrients uptake continues with much greater intensity in comparison with autumn period. Tillering of winter crops lasts until the end of March and the beginning of April. Presence of sufficient quantities of easy-available forms of nutritive elements in the zone of root system is of crucial importance.

From the stage of stem elongation until the stages of flowering-pollination-beginning of kernel and milk development, cereals adopt remaining quantities of NPK nutrients, about 75%. From the beginning of kernel development until the complete maturity (45-50 days), most of small grains do not uptake new quantities of NPK, but translocate them from the older organs towards ear and grain, respectively. In about 45 days (in favorable years ~50), big quantities of dry matter are formed in grain. Due to high and intensive accumulation of organic matter in the period from pollination until

complete maturity, of crucial importance for plants is that they are supplied with sufficient amounts of water and nutritive elements in soil solution, to have undamaged leaves and absence of weeds. Among abiotic factors, temperature is very important (daily about 25-26 °C, and nightly 11-12 °C) as well as direct sun radiation, without significant cloudiness. High temperature shortens the stages of kernel development and ripening.

Sowing. One of the most delicate technological measures in growing small grains is determining the optimal vegetative area for specific species and varieties of cereals. It depends from sowing time, tillering potential and weather conditions *(Malešević et al., 1994).* In organic production, time and sowing quality are especially important. Lower sowing rates should be used, and sowing time should be adjusted to specific agroecological conditions. Information in the Table 3 are valid for plain areas in Serbia and its surrounding.

Table 3: Time and rate of seeding of small grains in plain areas of Serbia
Tabela 3: Vreme i gustina setve strnih žita u ravničarskim krajevima Srbije

Small grain species *Vrsta žita*	Optimal seeding time *Optimalno vreme setve*	Average seeding rate *Orijentaciona gustina setve*	
		Grains/m^2	kg ha^{-1} of seed
Winter wheat – *oz. pšenica*	1. X - 25. X	450-500-600	180-200-260
Spring wheat – *jara pšenica*	1. II - 5. III	500-550-650	200-220-260
Winter barley – *oz. ječam*	20. IX - 5. X	250-300-400	140-160-180
Spring barley – *jari ječam*	5. II - 10. III	300-350-500	150-170-200
Winter oats – *oz. ovas*	20. IX - 10. X	350-400-450	100-120-160
Spring oats – *jari ovas*	1. II - 10. III	350-450-500	120-130-150
Winter triticale – *oz. tritikale*	20. IX - 10. X	400-450-500	170-180-230
Winter durum wheat – *oz. durum*	15. X - 1. XI	350-400-500	200-240-260
Spring durum wheat – *jari durum*	1. II - 5. III	400-450-550	200-250-270
Winter rye – *oz. raž*	15. IX - 5. X	350-400-500	160-180-220
Spelt wheat - *spelta*	25. IX - 10. X	350-500	160-240

Small grains demand special cultivation measures during the whole vegetative period, whose purpose is better development and protection of yield potential and quality. One of the most important measures is rolling after sowing, which enables quick and equal growing, and helps better root development. Rolling is also used at the end of winter, if the surface layer of the soil is soft. Harrowing is used on poorly structured soils, whose purpose is better aeration and provoke of tillering. Comb harrow can be used for the weeds suppression. These measures are especially significant for barley, oats, durum wheat and spelt. Bunt *(Tilletia sp.)* and Fusarium *(Fusarium sp.)* are the most important diseases as they are transferred by seed or occur as a consequence of frequent returning of grain at the same field. Late sowing and slow growth encourage the diseases development. Preventive measures listed in previous chapters are possible and efficient in suppressing diseases and pests.

The highest yield and the best quality of small grains are at the end of wax maturity, when grain moisture is between 20 and 26%. At this stage harvest losses are minimal, regardless of the fact if it is done in two or in one phase.

Alternative cereals suitable for growing in organic production

Many authors include *archaic wheat forms* in alternative crops. Archaic forms were grown in ancient times and have almost completely disappeared from modern production. These are Spelt (*Triticum spelta* L.), Einkorn (German name for one-grained wheat, also called small spelt - *Triticum monococcum* L.), Emmer (two-grained hulled wheat - *Triticum dicoccum* [Schrank] Schübl.) - as representatives of hulled wheats (they have tough glumes (husks) that tightly enclose the grains), and Kamut - Khorasan wheat *(T. turanicum* L.*; Triticum turgidum* L. *ssp. turanicum (Jakubz))* - archaic free-threshing wheat species. They all have significant advantages in relation to standard wheat forms, not only in nutritional aspect but in the productive and agro-economic aspects as well.

Alternative cereals can include and some species from standard production which are used a lot in the world, however, in Serbia they are not grown much or they are not grown at all. These cereals are important for nutritive and diet food completion with the status of *functional products (Demin and Žeželj, 2009)*. Rye *(Secale cereale),* Triticale *(Triticosecale),* Grain sorghum *(Sorghum bicolor* L. Moench*),* various Millets and similar species belong to this group. Special group of alternative crops comprises of grain species that are not botanically included in cereals, but are lately used in different ways as raw materials in baking industry or production of special organic products. Buckwheat *(Fagopyrum esculentum* Moench*)* and Grain amaranth *(Amaranthus sp.)* can be included in this group.

Spelt (*Triticum spelta* L.) – (Serb. *spelta, krupnik, pir, pira*; Engl. *spelt*, *German wheat, large spelt*, Germ. *Dinkel*)

Production of spelt in the world is constantly increasing, so its products (baking products and pastas) can be found in Serbian markets and healthy food stores. It has minor needs for fertilization, and thus it is very popular in the world in organic production systems. Spelt belongs to the group of hulled wheat types. In its spikelets there are usually two grains, which must be extracted by peeling. Share of the grain in the spikelet of spelt is about 75%, while the rest are glumes (husks). Mass of 1000 grains is about 25-30g.

Chemical content and diet features of spelt: spelt grain has much more proteins (15-18%) compared to common wheat, which is closer to oats from the point of nutritive values. Furthermore, spelt has higher content of vitamins and some microelements (selenium) so its products have and antioxidant effects. Of particular importance is its higher fiber content, which makes it suitable for weight-loss diet or in various other diets for people exposed to stress and intellectual effort. Spelt has various uses: it is cooked, which is the simplest way to use it, and it is usually combined with vegetables. Spelt flakes are a common food for breakfast or as a component of musli. Very good food and dietetic properties has a spelt seedling, which can be used in a raw state or in the form of flakes, which is much more convenient for storage and preparation. Spelt bread with no additives is visually bad (not growing), but the nutritionally is very useful because it is much better digested than common wheat bread. White bread of spelt longer stays soft and flexible compared to common wheat bread, and bread from integral flour (of whole grain) stays fresh longer (up to one week), and has a pleasant, sweet taste. From spelt flour can make the dough without adding eggs, so this bread can eat and people sensitive to albumin. Also, people allergic to glutenin from other cereal can use spelt bread - it does not cause allergic reactions.

Spelt has a modest demand on the climatic and soil factors and agrotechnics, is

tolerant to pests and diseases, and is very suitable for organic production. Its cultivation does not differ significantly from conventional wheat breeding.

Kamut *(Triticum turanicum* L.*; Tritucum turgidum ssp. turanicum*) - also called *Khorasan wheat*, and some taxonomists suggest the names *T. turgidum ssp. polonicum* and Egyptian durum wheat *(T. turgidum ssp. durum*), because of this type of wheat originate from Egypt to the Tigris-Euphrates valley. In many countries in the world this wheat species gained its place in the production and use as one of the most perspective cereal. Kamut has a large, high-quality grain with high gluten level in protein content. The grains are used for all products like common wheat, due to the similar kernel characteristics to durum wheats. Kamut is a free-threshing type of wheat, with big grains, glassy grain structure, with specific, often branched ear. Absolute grain mass is about 60 g. Grain usually contains about 30% more protein than common wheat, and has better mineral and amino acid balance. Also, it is characterized by a much better nutritional value and dietary properties. Products of kamut can consume people allergic to gluten, too.

In the Mediterranean regions it is grown as winter form. Although it is low yielded (1-2 t ha^{-1}), the kamut grain's ability to produce high quality without artificial fertilizers and pesticides makes it an excellent crop for organic farming. Technology of kamut growing does not differ significantly from common wheat growing.

Rye (*Secale cereale* L.): The increasing demand for rye bread, as the specialty of bakery products, has made producers to return again to the production of rye as alternative crop to wheat. The reasons for that are its specific taste and aroma of its products. In addition to the specific tastes, rye bread is attributed to certain medicinal value - it has been found reduced of diseases and mortality of cancer in the countries with stronger participation of rye bread in the diet. An important agronomic characteristic of rye are its thickness and rapid growth, and is a great precrop in crop rotation, as effectively suppress weeds.

Triticale (*x Triticosecale* Wittm. & Camus; syn. *x T. rimpaui* Wittm., x *Triticale* Müntzing) is an artificial cereal species created from a cross between wheat and rye. Favorable nutritional features and very high yields have made triticale one of the most perspective cereals, not only in human diet but in the feed production as well.

One of the many agronomic advantages of triticale is its high grain yielding, which with the application of appropriate agricultural technology, in our country can reach up the level of 7 to 8,5 t ha^{-1} *(Panković and Malešević, 2006).* Triticale is much more resistant to diseases in relation to wheat, and in this aspect is not behind the rye. It was also found that well-tolerated adverse agro-climatic conditions, especially to low temperatures. Triticale is appropriate for production in areas that are less favorable for wheat production; it is a highly promising cereal in hilly regions where wheat and barley production presents a certain risk *(Bavec, 2000).* It can also successfully replace extensive rye cultivars.

Grain sorghum (*Sorghum bicolor* (L.) Moench): Grain of modern, low-tanine sorghum hybrids today is almost the same in quality as the corn grain. It is edible, and in many countries around the world used for human consumption. Sown areas of grain sorghum in our country continue to rise, and domestic F1 hybrids Alba and Gold proved to be very well adapted, high yielding and favorable grain quality *(Berenji et al., 2008).* Quality tests have shown that the grain of grain sorghum can successfully replace corn in the diet of different categories of domestic animals.

The plant is native to tropical regions, adapted to temperate climate conditions. Due to the C4-type of photosynthesis sorghum is characterized by high potential of biomass production. Sorghum is a thermophilic species. It can endure tropical heats during the middle of vegetation better than corn. In conditions of limited soil and air humidity sorghum is capable of bigger biomass production than other crops. The most important factor of its drought tolerance is its powerful developed root system. Very effectively adopts the nutrients from the soil, and uses them more efficiently than corn.

As a precrop, sorghum dries soils a lot and intensively adopts nutrients from soil, especially nitrogen. Thanks to the effective adoption and the modest demands of plant towards nutrients, sorghum is satisfied with more rational fertilization.

Optimal time for regular seeding is in the second decade of April. Grain sorghum is sown on 50-70 cm x 7-8 cm in a row; and the planting depth should be at 3-4 cm. Rolling dry soil after planting encourages germination. Slow initial growth and development takes about a month after sowing. Care of crops consists of inter-row cultivation and hoeing, if necessary.

Millets - the *Panicum* genus includes more than 400 species, but only two are important in production: proso millet (*Panicum miliaceum* L.), and foxtail millet (*Panicum italicum* L., syn.: *Setaria italica ssp. maxima* Alef.)

Proso millet (common millet, broom-corn millet, Russian millet, Indian millet), was already being used as food in the early Stone Age. Its primary genetic centre is thought to be Middle and East Asia. Today, proso millet is produced in the U.S., South America, Australia, Japan, and some European countries (including Greece, Hungary). In Slovenia, during the years l986 to 1990, an average of 240 hectares were sown and the average grain yield was 1850 kg ha^{-1} *(Bavec, 2000a).*

It is the plant of warmer, south areas, which grows well in drier regions, too. Its distribution nearly matches with the zone of corn growing, but due to the short vegetation its growing area is wider than the one of corn. It has a relatively high yield potential. Average grain yield of this plant in our conditions is 2-3 t ha^{-1}. In a stubble-crop production, the yield is about 30% lower. *'Biserka'* (white) and '*Rumenka*' (red grain color) are the first two domestic types of proso millet. Both are characterized by short vegetation (which makes them convenient for stubble-crop production), also they have short stem (tolerance factor on lodging), and they are stand out by high yielding *(Berenji et al., 2008).*

For the development of common millet best temperature condition are between 18 and 24 ºC. Seeding is done when soil reaches the temperature 12-15 ºC. Low temperatures adversely affect growth at all stages of millet, especially during flowering and ripening. Millet has smaller demands towards humidity in comparison to other cereals. Hairiness of leaves and deep root system enables it to withstand stronger drought.

Millet can be cultivated in a monoculture, but in the system of organic crop farming it should be avoided. The best precrops for it are grain and forage legumes and fertilized intertilled crops. Pre-seeding preparation demands special attention because of its quite small seeds. Because of unequal seed maturation, two-phase harvest gives the best results; when the seeds from the middle part of the tassel are at the beginning of wax maturity.

Foxtail, foxtail millet *(Panicum italicum* L., *Setaria italica (L.) Beauv.) (Serb.* italijansko proso, bar proso)*:* Peeled foxtail grains gives highly rated, tasteful and nutritious groats, which can be cooked easily and quickly. In animal nutrition is used grain

and aboveground mass, in the green state or as hay. Whole or milled seed is excellent food for poultry, too.

The length of vegetation period of the earlier foxtail varieties is 100-110 days and at the later ones 120-130 days, so in various organic farming systems it's suitable for stubble-crop growing. According to biological characteristics it is similar to common millet. It has slow initial growth and development. Until tillering it grows slowly, so because of that it requires soil cleaned of weeds. Foxtail is tolerant to drought and high temperatures, and has smaller demands towards soil than the common millet.

Buckwheat *(Fagopyrum esculentum* Moench*)* (Serb. *heljda, ajda, eljda, jeljda;* Germ. *buchweizen, heidenkorn):* used to grow primarily in Asia (Pendzab, Tibet and Poamur regions); today, however, wild plants can be found in China (Himalaya Mountains), Siberia, and the Far East. Buckwheat is grown for its fruits (achene), which when peeled are used in human nutrition. Kernel is characterized with high nutritive value; it is easily digested and very tasteful. Grain of buckwheat consists of kernel and outer hull (shell) that is 25-35% of grain mass. The useful part is the kernel (65-75% grain mass) that comprises of endosperm and germ. It is very rich in proteins, fat and vitamins, which makes grain very nutritious. By milling grains very respected buckwheat flour is gained, whose most important ingredients are proteins (11-15%), carbohydrates (starch; about 70%), fat (2-3%), raw fiber (10-20%) and mineral matter (1.5-2.5%). Due to the lack of gluten, buckwheat is especially interesting as diet food for the ill and older persons, and in the diet of children. Special interest for buckwheat was shown by nutritionists and dieticians as it does not contain gluten and has increased content of lysine and soluble proteins. Buckwheat is one of the main late bees grazing and it is the plant that has high honey potential (it can give up to 200 kg/ha of honey, dark colored). Finally, with numerous usages of buckwheat, in recent times especially emphasize and its medicinal properties *(Jiang et al., 1995).*

Agronomic importance of buckwheat lies in the fact that it grows fast and suppresses weed plants. It adopts phosphorus from hardly available compounds. Thus, it is an excellent precrop (especially as green manure) to other crops. Its main agricultural value is its short vegetation, so it can give yield in such geographical places and altitude where other plants would fail. Very short vegetative period enables buckwheat to be grown as stubble crop which enables two harvests in the year. Traditional way of growing buckwheat in Slovenia is stubble-crop production, but grown as a full-season crop it yields more *(Bavec et al., 2002).*

Cold and wet climate is the most suitable for buckwheat, which is typical for hills and mountains, but it has equal yield in plains, too. Minimal temperature for germination of buckwheat is 4 °C. It is very sensitive to frosts and it is damaged at -2 °C.

Buckwheat has no special demands when it comes to precrops, although it reacts well on growing after annual legumes, root plants and winter small grains. Monoculture is not recommended in intensive production. Buckwheat responds very good on fertilizers from the previous crop and it uses them efficiently, and reacts especially well on the manure. Sowing of buckwheat starts when the soil warms up at about 15 °C till depth of 10 cm. It can be sowed as main crop or stubble-crop, in narrow or in wide rows. Assortment of buckwheat varieties was earlier based on old Russian varieties, while today in our country there are registered varieties of buckwheat as well as some local varieties. After seeding in dry soil if necessary rolling is conducted. If weed and crust occur before germination, harrowing with light harrows is recommended. After germination of

buckwheat, if planted in wide rows, cultivation between rows should be done. Depending of the of weeds population density and soil compaction, 1-2 cultivations are recommended till 10 cm depth, together with manual weeding in the rows. Crops in dense rows are weeded manually. Specific measure of buckwheat care, that can increase yield up to 500 kg/ha is pollination with the help of bees by bringing hives close to the field. Due to the long period of flowering and forming fruits, buckwheat matures unequally, so it is necessary to carefully determine harvest time. It is the best to do the harvest when about 2/3 of fruit are matured.

Grain yields are variable. In unsuitable soil or in conditions without pollination by insects, the yield may be as low as 500 kg ha^{-1}. The approximate yield for buckwheat is between 800 and 1000 kg grain ha^{-1}, but in favorable conditions, it can reach 2200 kg grain yield ha^{-1} *(Bavec, 2000b). Bavec et al., (2002)* suggest that the best-yielding buckwheat genotypes should be determined and introduced separately for stubble-crop or full-season production systems. Buckwheat grown as a full-season crop has a higher leaf area index, more clusters, better developed seeds, and 42% higher yield than the stubble-crop buckwheat *(Bavec et al., 2006).*

CONCLUSION

Cereals are inevitable plant species in organic production. Numerous species, subspecies, forms, types and varieties make their growing possible in the whole world and in almost all agricultural areas. In Serbia, there are valuable genetic collections of cereals which enable breeding of new genotypes intended for organic production, but they also give opportunity to revitalize old, authentic varieties.

Number of organic producers and areas under certified organic production in Serbia, especially in Vojvodina, are constantly increasing due to the growing market demands for healthy and safe food. Because of the rising demands and impossibility of production due to significant soil and air pollution, as well as violated relations in nature i.e. unexistence of basic agroecological preconditions for organic production, in developed countries there is a great lack of organic products in the market. That is why less developed countries, in which agro-ecosystem is undisturbed, have chance to increase their export through organic products.

Regarding the potentials we have, development of organic agriculture could give one new quality in life of local communities and our country as a whole. Development of organic agriculture should contribute to the optimal use of natural resources, increase of local production and overall improvement of status of inhabitants in rural regions.

The future of organic agricultural production in Serbia is still uncertain. There are conditions for its further development. Also, methods and technologies of organic production are known and available in many books, and even more on the many internet pages. However, it is necessary to interest potential producers by continual education and greater state encouragements and in that way provide grater penetration into world of ’organic’ trends.

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ORGANSKA PROIZVODNJA ŽITARICA - PRILIKA ZA POLJOPRIVREDU SRBIJE

MIROSLAV MALEŠEVIĆ, JANOŠ BERENJI, FRANC BAVEC, GORAN JAĆIMOVIĆ, DRAGANA LATKOVIĆ, VLADIMIR AĆIN

Izvod

Površine pod sertifikovanom organskom proizvodnjom u Svetu se konstantno povećavaju. Od njivskih kultura, u organskoj proizvodnji najzastupljenije su žitarice i krmno bilje, a od višegodišnjih zasada masline, voće i vinova loza. Stalni porast ljudske populacije nameće konstantnu potrebu za povećanjem proizvodnje žita, dok istovremeno specifični zahtevi tržišta u ishrani nameću potrebu da se pored konvencionalnih koriste i alternativna žita. Organska proizvodnja u Srbiji novijeg je datuma u odnosu na zemlje EU, a intenzivirana je uglavnom u proizvodnji povrća. Obzirom da su žita najzastupljenije vrste u organskoj proizvodnji u Svetu, cilj nam je da iznesemo osnovne postavke i mogućnosti njihovog gajenja u ovim sistemima i u našoj zemlji. Poseban akcenat dat je na specifičnostima «alternativnih» vrsta; kao što su durum pšenica, spelta, kamut, tritikale, razne vrste prosa, sirak za zrno i heljda.

Ključne reči: organska proizvodnja, žita, alternativna žita

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