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

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RELATIONSHIPS BETWEEN LEVELS OF NITROGEN COMPOUNDS WITH ANTIOXIDANT PROPERTIES AND SEMEN QUALITY IN BULLS*

EVA TVRDÁ, ZUZANA KŇAŽICKÁ,
PETER MASSÁNYI, NORBERT LUKÁČ1

SUMMARY: Fertility is a complex process, involving multiple interactions between the sperm cells and seminal plasma components. The composition of seminal plasma has great influence on the biological quality of semen, as expressed by sperm motility and viability. The aim of this study was to evaluate relationships between semen characteristics and major nitrogen biochemical components of bovine seminal plasma with antioxidant properties (total proteins, albumin, uric acid, urea). Semen samples from 30 breeding bulls were examined. First, basic measurements were performed – volume (ml), pH and concentration (x 106/mL). Motility analysis was carried out using the CASA system; total protein, albumin, uric acid and urea concentration measurements were based on a colorimetric reaction of the target substance and a spectrophotometric detection at a specific wavelength. The correlation analysis revealed that the spermatozoa motility was positively correlated with total proteins ($r=0.5823$; $p<0.001$), albumin ($r=0.6768$; $p<0.001$) as well as with uric acid ($r=0.5106$; $p<0.001$). Besides, the highest concentration of proteins, albumin and uric acid was found in samples of the best quality. No significant results ($p>0.05$) were found in relation to urea. This study proves the importance of proteins, albumin and uric acid in the semen quality, as well as the crucial role of biochemical analysis of semen in relation to male fertility.

Key words: *bulls, spermatozoa, proteins, albumin, uric acid, urea.*

Original scientific paper / *Originalni naučni rad*

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INTRODUCTION

Artificial insemination has proven to be the most effective tool for genetic improvement of animals of zootechnical importance, especially in the cattle industry. The inspection and handling of semen is considered a key and essential step for assessing fertility and the successful use of semen. Knowledge of fertility in bulls is an objective of great importance for the production of bovine semen, which is achieved by good analysis of the semen, among other assessments. To design an ideal bovine semen analysis to properly assess and predict the fertility of an ejaculate is the goal of many specialists in the reproduction field (Alm-Packalén, 2009).

The seminal plasma mediates the chemical function of the ejaculate. Due to its slight alkalinity (light alkaline buffer) it is also responsible for creating a milieu beneficial for the spermatozoa in a vaginal surrounding that is normally maintained acidic. Other functions of the seminal plasma include activation and augmenting the spermatozoa motility as well as supplying nutrients for the sperm cells (Eghbali et al., 2008). Besides, the seminal plasma possesses a wide antioxidant system to scavenge reactive oxygen species (ROS) and prevent ROS related cellular damage (Sharma and Agarwal, 1996).

This extracellular fluid is a composite mixture of secretions that come from the male accessory reproductive organs (Elzanaty et al., 2007). The composition of seminal plasma has great influence on the biological quality of semen, as expressed by sperm motility and viability. These factors are directly related to the fertilization success. Determining of seminal plasma composition can help to understand the design requirements for preparing the appropriate artificial seminal plasma solutions for short-term storage or cryopreservation (Bozkut el al., 2009).

The protein constituents of seminal plasma long have been considered of importance in the maintenance of proper conditions for spermatozoa. In fact, in the practice of artificial insemination, various protein-containing systems such as milk, gelatin solution, and egg yolk, in addition to various chemical buffer systems, have been used as semen extenders (Stančić et al., 2002; Jonáková and Tichá, 2004).

Non-protein nitrogenous compounds such as ammonia, creatinine, urea and uric acid, the major end products of protein metabolism, are excreted via urine. These compounds are also found in semen, although at much lower concentrations than in urine (Hirsch et al., 1991). They are probably derived from two sources: local production (Ogino et al., 1979) and transudation from the circulation (Setchell, 1974).

Determinations of biochemical constituents with antioxidant ability of seminal plasma are needed for semen evaluation, since physical characteristics of semen alone are not completely satisfactory for semen appraisal in the current practice. Thus, the present study was aimed at studying the relationships between semen characteristics and the major nitrogenous biochemical constituents with antioxidant properties (total proteins, albumin, uric acid, urea) present in bovine seminal plasma.

MATERIAL AND METHODS

Bovine semen samples were obtained from 30 adult breeding bulls (Slovak Biological Services, Nitra, Slovak Republic). The samples had to accomplish the basic quality criteria given for the corresponding breed. The semen was obtained on a regular

collection schedule using an plastic artificial vagina.

After collecting the samples were stored in the laboratory at room temperature (22-25 °C) and basic measurements were performed – volume (ml), pH and concentration (x 106/mL). Motility analysis was carried out using the CASA system – SpermVision (MiniTüb, Tiefenbach, Germany) with Olympus BX 51 phase microscope (Olympus, Japan). Each sample was placed into Makler Counting Chamber (deph 10 μm; Sefi-Medical Instruments, Izrael) and the percentage of motile spermatozoa (motility > 5μm/s) was evaluated.

Subsequently, the samples were centrifuged (15 min, 15 000 rpm, 4°C) to obtain the cell sediment and seminal plasma fraction. The fractions were separated, seminal plasma was transferred into 1.5 ml tubes and kept frozen (-80°C) until analysis.

All of the measurements were based on a colorimetric reaction of the target substance and a subsequent spectrophotometric detection at a specific wavelength.

Albumin concentration was measured using the BioLa Albumin commercial kit (PLIVA-Lachema, Brno, Czech republic). The measurement was based on a reaction between albumin and Bromocresol Green (BCG) at acid pH forming a complex, which was easy to detect photometrically at 578 nm.

Uric acid measurement was characterized by the oxidation of the substance leading to H2O2 and allantoine formation. The formed H2O2 was detected by recating with N-ethyl-N-(2-hydroxy-3-sulphopropyl)-m-toluidine (TOOS) and 4-aminoantipyrine (AOD). The absorbance was measured at 550 nm. BioLa Uric Acid commercial kit (PLIVA-Lachema, Brno, Czech Republic) was used.

The total protein content was measured by the DiaSys Total Protein (DiaSys, Holzheim, Germany) commercial kit based on the Biuret method, where copper ions (copper sulphate) together with proteins formed a violet blue color complex in alkaline solution. The absorbance of the color was directly proportional to the concentration and measured at 540 nm.

Urea detection was based on the urease-GLDH enzymatic UV measurement. Urea with hydrogen peroxide formed ammonium, the reaction was catalyzed by urease. Subsequently, ammonium reacted with 2-oxoglutharate and NADH forming L-glutamate. The conversion was detected at 340 nm. Urea concentration was measured using the Urea DiaSys (DiaSys, Holzheim, Germany) commercial kit.

Albumin and uric acid were measured with the help of Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc, USA). Urea and total protein content were analyzed using the Microlab 300® photomter (Vital Scientific NV, Netherlands).

Statistical analysis of the results was carried out using the statistical program GraphPad Prism 3.02 (Graphpad Software incorporated, San Diego, California, USA). Results are quoted as arithmetic mean±standard error of mean (SEM). Pearson's correlation coefficient (two tailed) test was used to examine correlations between all the parameters of semen. The comparison of semen parameters in the quality groups of samples was carried out by the Tukey's Multiple Comparison Test.

RESULTS

The results of the semen and seminal plasma evaluation in Table 1 show that the total protein content of the seminal plasma was 71.88 ± 0.39g/L, while albumin concentration was 14.66 ± 0.11 g/L. Mean uric acid level was recorded as 109.57 ± 3.08 μmol/L

and urea concentration was detected at 3.91 ± 0.06 mmol/L (Tab. 1).

Table 1. Characteristics of bull semen (mean ± SEM; n = 30)

Ejaculate volume (mL)	6.77 ± 0.03
pH	6.64 ± 0.01
Sperm concentration (x 106 cells/mL)	2,682.50 ± 39.47
Motility (%)	85.73 ± 0.44
Total proteins (g/L)	71.88 ± 0.39
Albumin (g/L)	14.66 ± 0.11
Uric Acid (μmol/L)	109.57 ± 3.08
Urea (mmol/L)	3.91 ± 0.06

The correlation analysis revealed that the spermatozoa motility was positively correlated with total proteins ($r=0.5823$; $p<0.001$), albumin ($r=0.6768$; $p<0.001$) as well as with uric acid ($r=0.5106$; $p<0.001$). No correlation was found between the motility and urea. The concentration was negatively correlated with all of the observed parameters, negative correlations were found with motility ($r=-0.4232$; $p<0.05$) and total proteins ($r=-0.4250$; $p<0.05$). No significant correlations ($p>0.05$) were found when observing pH and volume of the samples. Significant correlations were also observed between albumin, uric acid ant total proteins ($r=0.3660$; $p<0.05$ and $r=0.6254$; $p<0.001$ respectively). Sperm concentration, volume and pH of the samples remained unaffected by the observed biochemical parameters of seminal plasma (Tab. 2).

Table 2. Correlations between the quality parameters of semen and biochemical parameters of seminal plasma

	Concentration	pH	Volume	Motility	Total Proteins	Albumin	Uric Acid	Urea
Concentration	1							
pH	0.1755	1						
Volume	0.06254	0.1522	1					
Motility	-0.4232*	0.05761	0.1181	1				
Total Proteins	-0.4250*	0.09988	-0.02784	0.5823***	1			
Albumin	-0.2163	0.1115	0.06680	0.6768***	0.6254***	1		
Uric Acid	-0.1418	0.05054	0.08649	0.5106**	0.1853	0.3660*	1	
Urea	-0.1451	0.1649	0.3483	-0.07899	-0.06220	-0.03632	-0.09377	1

The correlation analysis was based on the value of the correlation coefficient: ±0.111 - ±0.333: no correlation; ±0.334 - ±
0.666: moderate correlation; ±0.667 - ±0.999: high correlation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

In order to have a better insight of the results and make the range variances narrower, the samples were categorized in three groups of Excellent (Ex, >90% motile; n=16), Good (Go; 80-89% motile; n=8) and Medium quality (Me; <79%; n=6) according to their motility rates (Tab. 3).

Table 3. Average values of observed parameters in the quality groups (mean ± SEM)

	Ex (n=16)	Go (n=8)	Me (n=6)
Volume (mL)	6.44±0.06	7.3 ± 0.10	6.49±0.03
pH	6.69±0.01	6.49±0.03	6.45±0.04
Concentration(x 10^6 cells/mL)	2574.38±69.91	2433.75±56.57	3302.50±311.58
Motiliy (%)	93.92±0.14	85.89±0.30 ^{a**}	63.66±2.10^{b***} ^{c***}
Total Proteins (g/L)	76.54±0.63	70.24±1.03	61.63±2.29^{b*}
Albumin (g/L)	16.22±0.18	13.81±0.33	10.12±0.43^{b***} ^{c**}
Uric Acid (µmol/L)	145.85±5.69	99.55±11.54	26.16±1.53^{b*}
Urea (mmol/L)	3.58±0.11	4.43±0.27	4.08±0.17

[a] Ex vs Go, [b] Ex vs Mo, [c] Go vs Mo. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Mean values for motility were recorded as 93.92±0.14% in Ex, 85.89±0.30% in Go and 63.66±2.10% in Me groups, which were significantly ($p<0.001$) different. The highest concentration of proteins, albumin as well as uric acid were detected in Ex group (76.54±0.63 g/L, 16.22±0.18 g/L and 145.85±5.69 µmol/L respectively) while the lowest concentrations for all three parameters were recorded in the Me group (61.63±2.29 g/L, 10.12±0.43 g/L and 26.16±1.53 µmol/L respectively). Significant differences were observed especially when comparing the content of mentioned parameters between the Ex and Me group. The highest concentration of urea was detected in the Go group (4.43±0.27 mmol/L) and the lowest concentration was in the Ex group (3.58±0.11 mmol/L). No significant differences were detected in relation to this parameter. Also, no significant differences were observed when comparing the concentration, pH and volume of the samples in the quality groups.

DISCUSSION

Evaluation of the sperm quality provides information allowing zootechnicians to determine the optimal time for sperm collection and to devise optimal handling and storage protocols for sperm used in artificial fertilization (Baas et al., 1983). Semen volume, concentration and motility as well as composition of the seminal plasma are common parameters to assess sperm quality in animals (Alavi and Cosson, 2006), with sperm motility being the single most important parameter of semen quality. Therefore, this study was aimed to evaluate the relationship between semen quality parameters and selected nitrogen compounds (total proteins, albumin, uric acid and urea) in bovine seminal plasma which are known to have antioxidant properties.

Seminal plasma proteins participate in events that occur both in the male and the female reproductive organs. Mammalian seminal plasma is a complex mixture of secretions originated from the testis, the epididymis, and the male accessory sex glands (seminal vesicles, ampulla, prostate, bulbourethral glands). Seminal plasma is a very complex fluid containing a wide variety of both organic and inorganic components, among which proteins are an important part of the high-molecular-weight substances (Jonáková and Tichá, 2004).

The proteins secreted into seminal plasma may play an important role during sperm capacitation and fertilization (Rodríguez-Martínez et al, 1998) and may also serve to protect sperm from damage or to maintain their longevity. Seminal plasma

contains many proteins that seem to be important in providing a suitable environment for the survival and function of spermatozoa. Some of these proteins are secreted by the testis (transferrin; Zalata et al., 1996), epididymis (neutral-alpha glucosidase; Yeung et al., 1990), prostate (prostate-specific antigen; Siciliano et al., 2000) and seminal vesicles (Elzanaty et al., 2007). All these proteins are extensively studied in semen in an attempt to determine their physiological role and clinical relevance in the evaluation of male fertility (Chia et al., 2000). Our study confirms the crucial role of proteins on the semen quality. A significantly positive correlation ($r=0.5823$; $p<0.001$) was detected between the protein content and spermatozoa motility. Besides, the highest concentration of proteins was found in the Ex group (76.54±0.63 g/L) and the lowest concentration was detected in the Me group (61.63±2.29 g/L).

Apart from organ-specific proteins, seminal plasma is rich in other proteins, of various origins and functions. One of these proteins is albumin. In this regard, Lindholmer et al. (1974) claimed that most of the albumin in seminal plasma originates from the prostate. Others suggested a testicular and epididymal origin of seminal albumin (Orlando et al., 1988). Albumin is a highly soluble protein present in plasma (Roche et al., 2008), which transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments. In general, albumin represents the major and predominant antioxidant in plasma acting through its multiple-binding sites and free radical-trapping properties (Bourdon and Blache, 2001).

Few studies have addressed the issue of possible correlation between seminal plasma albumin and sperm quality. A weak positive significant correlation between albumin and semen volume, as well as sperm concentration, was reported (Chard et al., 1991). On the other hand, according to Lindholmer et al., 1974, no correlation between albumin and sperm motility was found. Our results show, that albumin has a positive influence on semen quality. Not only it showed to have a positive correlation to motility ($r=0.6768$; $p<0.001$), but it also has been found in larger amounts in the best quality samples (16.22±0.18 g/L).

The main nitrogenous components of urine such as ammonia, urea, uric acid and creatinine also exist in seminal plasma (Hirsch et al., 1991). They are probably derived from local production and transudation from the circulation. Urea enters testicular semen from the bloodstream (Setchell, 1974). Spermatozoa oxidize amino acids from which they produce various metabolites. In bulls, uric acid is probably formed by oxidation of hypoxanthine and xanthine, both of which occur in the seminal vesicles (Leone and Santoianni, 1957). This metabolically inert end product of purine metabolism has been found to act as an important non-enzymatic antioxidant in the blood as well as in other body fluids like seminal plasma (Keel et al., 2002). It can act as an oxidisable co-substrate for any reactive oxidative species and thus can protect the important biomolecules from oxidative damage. It also helps to stabilize the antioxidant activity of ascorbic acid in the seminal plasma (Xu et al., 2004). Reflection of all such protective role of uric acid was also seen the study of Zhang et al. (2009). Highest levels of uric acid were found in normozoospermics followed by asthenoterato, oligoasthenoterato and azoospermics. Moreover, the authors found a positive correlation between seminal uric acid and seminogram parameters like sperm motility and morphology. These findings were similar to that observed by Xu et al. (2004). Regarding our results, uric acid was behaving in a similar way than albumin did. It had a significantly positive cor-

relation with the motility (r=0.5106; p<0.001), the highest uric acid concentration was detected in the Ex group (145.85±5.69 μmol/L) and the far lowest concentration was present in the Me group (26.16±1.53 μmol/L).

Meanwhile, insignificant results were observed when analyzing the content of urea. Urea is considered to be in a relationship with protein metabolism and total protein content, nevertheless we did not find any significant correlations with the quality parameters of semen samples and the highest content of urea was found in the samples of the Go group (4.43±0.27 mmol/L). Equally, Ronquist et al. (1985) reported that urea concentrations in semen were not correlated to any of the sperm motility parameters. According to Crich and Jequier (1978), the observed reduction in spermatozoa motility in their study was more closely related to a reduced osmolality than to pH or urea concentration. Srivastava et al. (1984) examined the levels of urea in males divided as normal, azoospermic, infertile with different sperm numbers and vasectomized. Similarly to other studies, no significant difference in the levels of urea between any of the groups was revealed.

CONCLUSION

Fertilization in mammals is a complex process, involving multiple interactions between the sperm cells and the seminal plasma components of the ejaculate. The distribution of nitrogen compounds with antioxidant properties, such as proteins, albumin, uric acid and urea in semen and seminal plasma should be considered in the interpretation of results obtained in the evaluation of bull fertility. Success in predicting fertility is limited by spermatozoa characteristics, the process of fertilization, and approaches used for *in vitro* evaluation of seminal quality. This study demonstrates the importance of proteins, albumin and uric acid in the bovine semen quality. Additionally we can conclude that analysis of biochemical characteristics, especially of nitrogen substances and antioxidants in semen provides very useful information regarding artificial insemination and male fertility.

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UTICAJ AZOTNIH JEDINJENJA SA ANTIOKSIDATIVNIM SVOJSTVIMA NA KVALITET SPERME BIKA

EVA TVRDÁ, ZUZANA KŇAŽICKÁ, PETER MASSÁNYI, NORBERT LUKÁČ

Izvod

Fertilitet je složen proces, koji uključuje interakciju spermatozoida i komponenata spermalne plazme. Sastav spermalne plazme ima velikog uticaja na biološki kvalitet sperme, meren stepenom progresivne pokretljivosti i preživljavanja spermatozoida. Cilj ovog rada je da se oceni povezanost između osobina sperme i osnovnih biohemijskih azotnih sastojaka semene plazme sa antioksidativnim svojstvima (ukupni proteini, albumin, mokraćna kiselina i urea). Ispitivani su uzorci sperme od 30 priplodnih bikova. Prvo su određeni volumen ejakulata (ml), pH i koncentracija spermatozoida (x 106/mL). Pokretljivost je utvrđena upotrebom CASA sistema. Sadržaj ukupnih proteina, albumina, mokraćne kiseline i mokraće, određen je kolorimetrijskom reakcijom targetne supstance i spektrofotometrijskom detekcijom specifične talasne dužine. Korelaciona analiza pokazuje da je pokretljivost spermatozoida u pozitivnoj korelaciji sa sadržajem ukupnih proteina ($r=0.5823$; $p<0.001$), albumina ($r=0.6768$; $p<0.001$) i mokraćne kiseline ($r=0.5106$; $p<0.001$). Veća koncentracija proteina, albumina i mokraćne kiseline je ustanovljena u ejakulatima naj boljeg kvaliteta. Nije ustanovljena statistički značajna ($p>0.05$) povezanost koncentracije mokraće i stepena pokretljivosti spermatozoida. Dobijeni rezultati pokazuju visok uticaj sadržaja proteina, albumina i mokraćne kiseline na kvalitet sperme, kao i izuzetno važnu ulogu biohemijske analize sperme, u korelaciji sa fertilitetom mužjaka.

Ključne reči: bikovi, spermatozoidi, proteini, albumini, mokraćna kiselina, mokraća.

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POSTLACTATIONAL ESTRUS REACTION AND SOWS FARROWING RATE IN COLD AND WARM SEASONE*

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SUMMARY: The effects of the warm and cold period of the year on the sows postlactational estrus reaction and farrowing rate, were investigated on an intensive pig production farm unit in Vojvodina. Average weaning-to-estrs interval duration was significantly (P>0.01) longer (8.1 vs. 6.0 days) and farrowing rate was significantly (P>0.05) lower (72.9% vs.80.2%) in worm compared with cold season. The present results support the findings of other authors that higher temperature and extended photoperiod, during summer months, are the main factors influencing reduction the reproductive parameters in the breeding sows herds. This phenomenon is known as a "summer sows infertility".

Key words: *estrus, fertility, season, sow.*

INTRODUCTION

Paragenetic factors, as nutrition, parity structure of the herd, housing, AI technology and general healt staus of the breeding animals (sows, boars, gits) has a greater impact on reproductive parameters (Stančić, 2005; Stančić et al., 2010) than genetic factors (breed, race, inbreding). Becouse the genetic heritability of all reproductive traits is low, about 10 to 30% (See, 2002).

In the modern production conditions, the phenomenon known as "summer sows infertility" represents the most significant factor that reduced sows fertility parameters. Namely, for as long as 40 years, considerably lower values of sow fertility parameters have been evident in the warmer part of the year (Almond, 1992). Thus, Aumaitre i sar. (1976) found significantly lower farrowing rates, extended weaning-to-estrus interval, increased number of irregular rebreeding and the reduced number of live-born pigs per

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litter in the warm part of the year (June – September). This phenomenon is related to the negative effect of increased ambient temperature and daily photoperiod duration on sow reproductive functions (Gordon, 1997; Stančić et al., 2011).

The aim of the present study was to investigate the variation of the postlactational estrus reaction and farrowing rate in the sows, within cold and warm season of the year, at the industrial farm unit in AP Vojvodina (Serbia).

MATERIAL AND METHOD

The reproductive performance parameters (weaning-to-estrus interval, farrowing rate and rebreeding rate), for total of 3062 farrowings within one year, was obtained from reproductive evidence of one big farm unit in AP Vojvodina (Serbia).

During lactation, which lasts aproximatively 30 days, sows are kept in individual boxes. The nutrition of sows is adequate, with meals prepared for certain categories of breeding sows. Estrus testing is performed in the presence of boar, once within 24h. Artificial insemination is performed with liquid diluted semenn, with the dozes of 100ml, with aproximatively 5x109 progressive motile spermatozoa. Duble insemination was performed: firstly, a couple of hours after estrus detection, and secondly, 24h later. Rebreeding control was conducted in facilities for inseminated (pregnant) sows, staring around 14 days after insemination.

The values of all the above stated parameters of reproductive performance were analyzed in relation to the parity of farrowing and a season of the year. The year is divided in the warm (May to September) and cold (October to April) season.

The obtained results were processed using "Statistika 7" software program.

RESULTS

The average weaning-to-estrus interval (WEI) duration, for all sows, in both of the seasons, was 6.8 days. In the warm season, WEI was significantly (P>0.01) longer (8.1 days) then in the cold season (6.0 days).

Table 1. Weaning-to-estrus interval duration (days)
Tabela 1. Trajanje intervala zalučenje – estrus (dani)

Parity / *Paritet*	Weaning-to-estrus interval (days) / *Interval zalučenje – estrus (dani)*					
	Period of year / *Period godine*				Total / *Ukupno*	
	Cold / *Hladni*		Warm / *Topli*			
	n	$\bar{x}$	n	$\bar{x}$	N	$\bar{x}$
1.	353	7.8[a]	242	12.5[b]	595	9.7
2. – 5.	1121	5.4[a]	922	7.8[b]	2043	6.4
≥ 6.	238	6.3[a]	186	8.7[b]	424	7.1
Total / *Ukupno*	1712	6.0[a]	1350	8.1[b]	3062	6.8

a, bValues with different superscripts, in the same rows, are statistically significantly different (P>0.01).

a, bVrednosti sa različitim superskriptima, u istom redu, se statistički značajno

razlikuju (P>0,01).

During the whole year, gilts WEI was longer for abou 3 days (average 9.7 days) compared with sows (average 6.8 days). In both gilts and sows, the average WEI was significantly (P>0.01) longer in the warm than in the cold period (Table 1).

Farrowing rate was also significant different (P>0.05), after insemination in the first post-lactation estrus, between the cold (80.2%) and warm (72.9%) period of the year, for all parity sows (Table 2).

Table 2. Farrowing rate after insemination in first estrus after weaning
Tabela 2. Vrednost prašenja posle osemenjavanja u prvom estrusu posle zalučenja

Parity / *Paritet*			Period of year *Period godine*		Total *Ukupno*
			Cold / *Hladni*	Warm / *Topli*	
1.	Inseminated (n) *Osemenjeno (n)*		353	242	595
	Farrowed *Oprašeno*	n	247	141	388
		%	70.0^{A}	58.3^{B}	65.7
2. – 5.	Inseminated (n) *Osemenjeno (n)*		1121	922	2043
	Farrowed *Oprašeno*	n	942	658	1600
		%	84.0^{a}	71.4^{b}	78.8
≥ 6.	Inseminated (n) *Osemenjeno (n)*		238	186	424
	Farrowed *Oprašeno*	n	184	128	312
		%	77.3^{a}	68.8^{b}	73.8
Total *Ukupno*	Inseminated (n) *Osemenjeno (n)*		1712	1350	3062
	Farrowed *Oprašeno*	n	1373	985	2358
		%	80.2^{a}	72.9^{b}	77.0

Values with different superscripts, in the same rows, are statistically significantly different.

Vrednosti sa različitim superskriptima, u istom redu, se statistički značajno razlikuju.

^{A,B} (P>0.01); ^{a,b} (P>0.05).

Gilts achieved a significantly lower (P>0.01) farrowing rate in cold (70%) and warm (58.3%), compared with sows (Table 2).

Table 3. Distribution of regular and iregular returne rate
Tabela 3. Distribucija regularnih i neregularnih povađanja

	Period of year / *Period godine*				Total /*Ukupno*	
	Cold / *Hladni*		Warm / *Topli*			
	n	%	n	%	n	%
Total returne rate *Ukupno povađanje*	339	-	365	-	704	-
Regular returne *Regularno povađanje*[1]	246	72.6^{A}	170	46.6^{AB}	416	59.1
Iregulara returne *Neregularno povađanje*[2]	93	27.4^{B}	195	53.4^{AB}	288	40.9

[1] Within intervals / *U intervalima*: 18 - 24 i 36 - 48 days after AI / dana posle VO.

[2] Within intervals / *U intervalima*: ≤ 17, 25 - 35 i ≥ 49 days after AI / dana posle VO.

[A,B] Values with different superscripts, in the same rows and culumns, are statistically significantly different (P>0,01).

[A,B] *Vrednosti sa različitim superskriptima, unutar istog reda i kolone, se statistički značajno razlikuju (P>0,01).*

It is important to state that the iregular returne rate was significantly (P>0.01) higher in the warm (53.4%) than in the cold (27.4%) season. Regular and iregular returne rate values, in the cold season, was significantly (P>0.01) different (72.6% vs. 27.4%), buth these differences was not significatn in the warm season (46.6% vs. 53.4%) (Table 3).

DISCUSSION

Infertility or reduced fertility in sows can be influenced by genetic and paragenetic factors. The most important paragenetic factors are nutrition, housing, parity structure of breeding herds, climatic conditions (ambient temperature and daily photoperiod duration), treatment with hormonal preparations and general health of breeding animals (Tomes et all., 1982; Stančić, 2005). Significant reduction of sow fertility during the warm period of the year, has been reported by numerous authors. This phenomenon is known as "summer or seasonal infertility syndrome" (Love, 1978; Rozeboom et all., 2000; Stančić et al., 2011). Significant reduction of sows fertility parameters (weaning-to-estrus interval duration, farrowing rates, the rebreeding and abortion rates, as well as the average litter size), within warm season, has been reported since 1970s, by Aumaitre et al. (1976). Summer infertility is considerably more prominent in gilts, compared with sows (Britt et all., 1983). Research results by a number of authors, as stated by Gordon (1997), point out the general conclusion that the parameters of sow fertility, during the warm period, are reduced by 15 to 20% compared with the cold period of the year.

The results of the research in this paper showed that the average weaning-to-estrus interval (WEI) duration was significantly higher (P>0.01) within warm (8.1 days), compared with cold season (6.0 days). Very similar differences in WEI duration, between the cold and warm period of the year, were identified also by certain other authors (Aumaitre et all., 1976; Hurtgen et all., 1980; Peltoniemi et all., 1999; Stančić et all., 2002; Almond and Bilkei, 2005). Thus, for instance, in Eastern European countries Almond and Bilkei (2005) ascertained that this interval lasts 5.9 days on average in the cold and 7.8 days in the warm period of the year. The extended duration of WEI reduces reproductive efficiency of breeding herds both directly and indirectly. Firstly, the reproductive efficiency is reduced directly, as sows with extended WEI achieve lower farrowing rates (%) after insemination in the first post-lactation estrus and have a significantly lower number of pigs per litter (Stančić, 1994; Kemp and Soede, 1996; Stančić, 1997a and 1997b; Borchardt Netto, 1998; Stančić et all., 2000; Stančić, 2002; Timotijević et all., 2003). Secondly, the reproductive efficiency is reduced indirectly, as the extended WEI prolongs the interval between successive farrowing and, consequently, reduces the farrowing index, resulting in the reduced yearly pig production and the increased number of non-productive feeding days (Tomes et all., 1982; Tubbs, 1990; Stančić, 2005). According to the research conducted by Prunier et all. (1996), the extended WEI during the warm period of the year is a consequence of the decreased

capability of hypothalamus to re-establish the normal pulsatile secretion of Gn-RH. This inhibits the release of hypophyseal gonadotropin (FSH and LH), which results in postponement of the first post-lactation ovulation and estrus manifestation.

The farrowing rate after insemination in the first post-lactation estrus was also statistically significant (P>0.01) lower (72.9%) in the warm than in the cold period of the year (80.2%). The results of other authors also indicate lower values of farrowing during the warm period compared with the cold period of the year. Thus, Almond and Bilkei (2005) determined that this value reaches 91% in the cold and 78% in the warm periods of the year. In this research, the iregular returne rate was significantly (P>0.01) higher in the warm (53.4%) than in the cold (27.4%) season. The most frequent reason for farrowing failure, i.e. unsuccessful conception (rebreeding, return to estrus), in summer months is irregular rebreedings (i.e. reestablishment of estrus 25 to 35 days or > 46 days after insemination. Such early disruption of pregnancy is a consequence of embryo mortality (Xue et all., 1994) or the regression of pregnancy corpora lutea (CL) (Wrathall et all., 1986). Namely, recent research indicate that high ambient temperature leads to the increased embryo mortality, and consequently, to disruption of pregnancy (Stančić et all., 2004). Besides, it seems that the increased ambiental temperature inhibits prolactin (LTH) release from hypophysis, which is necessary for enhancement of secretory activity of pregnancy CL after 16th day of gestation, which causes disruption of pregnancy and irregular rebreeding manifestation (Tast et all., 2002; Kirkwood, 2009). Certain authors point out that the stress induced by increased ambient temperature reduces sows immunity to infectious diseases which causes increased mortality and/or fetal mummification (Yeske, 2007; Givens and Marley, 2008). According to the research of certain authors (Christianson, 1992), abortions were most frequently caused by infectious factors, and less frequently by stress induced by increased ambient temperature.

The phenomenon of seasonal infertility in sows is very complex. Precise mechanisms of physiological basis of this phenomenon are not entirely clarified (Kirkwood, 2009). However, the results of all the research consistently indicate that the reduced fertility in sows is a consequence of the interaction of high ambient temperature and extended daily photoperiod in the warm period of the year. These factors act through neuroendocrine mechanisms at the level of cerebral nervous system – hypothalamus – hypophysis – ovary (Tast, 2002).

The results of the present research, clearly indicate that the values of the examined parameters of sow fertility are significantly lower during the warm period compared with the cold period of the year. Additionally, these parameters point to significantly more prominent decrease in gilts than in sows.

CONCLUSION

Obtained results clearly indicate that there are negative effects of the warm period of the year on the examined parameters of sow fertility:

During the warm period the sows weaning-to-estrus interval duration was statistically significantly higher (8.1 days) compared with cold period of the year (6.0 days).

The farrowing rate, after insemination in the first post-lactation estrus, during the warm period is statistically lower (72.9%) compared with the cold period (80.2%).

The iregular returne rate was significantly higher in the warm (53.4%) than in the cold (27.4%) season.

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POSTLAKTACIJSKO ESTRUSNO REAGOVANJE I VREDNOST PRAŠENJA KRMAČA U TOPLOJ I HLADNOJ SEZONI

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Izvod

Ispitivan je uticaj toplog i hladnog perioda godine na postlaktacijsko estrusno reagovanje i vrednost prašenja na jednoj velikoj farmi za proizvodnju svinja u AP Vojvodini (Srbija). Prosečno trajanje intervala zalučenje-estrus je bilo statistički značajno (P>0.01) duže (8 dana) u toplom, u odnosu na hladni period godine (6 dana). Vrednost prašenja, posle osemenjavanja u prvom postlaktacijskom estrusu, bila je statistički značajno (P>0.01) niža (72.9%) od one u hladnom periodu godine (80.2%). Dobijeni rezultati potvrđuju nalaze drugih autora, da povišena ambijentalna temperatura i produžen dnevni fotoperiod, tokom toplih letnjih meseci, značajno redukuju vrednosti parametara reproduktivne performanse krmača. Ovaj fenomen je pozmat kao „letnji infertilitet krmača“.

Ključne reči: estrus, fertilitet, sazona, krmača.

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EDAPHIC CONDITIONS IN MOST COMMON TYPES OF OAK FORESTS AFFECTED BY DRYING*

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SUMMARY: We evaluated the edaphic conditions in three most common types of oak forests affected by different degree of drying. According to Soil Map of Vojvodina Province R 1:50000 meadow black soil was indicated as a dominant systemic soil unit. Content of silt+clay fraction was above 60%, and two major textural classes were loam and clayey loam. The content of hardly available water was the highest in all types of forests with the most prominent drying process (ranging from 21,65 to 24,13%). Chemical soil properties varied only slightly, and the most prominent deviations were related to the content and ratio of carbon and nitrogen. The above mentioned characteristics indicated the need for further monitoring in the common oak stands.

Key words: *Quercus robur, site conditions, forest types, monitoring.*

INTRODUCTION

English oak (Quercus robur L.) is the dominant tree species of natural forests in the area of flat Srem, and it is linked to a hygrophilic oak-Alno – Quercion roboris forests. The stands are highly productive, and preserved stands in the flat Srem are considered the most valuable forests in Serbia, as in Europe (Thomas et al. 2002). The appearance of a trend toward oak drying in the forest area of Srem imposed the obligation of monitoring the intensisty of drying, both at the time of forest inventory and at implementing the forest managament plans (Medarević et. al 2009), as well as to require mobility of competent scientists and experts to resolve this matter.

Dubravac and Dekanic (2009) mentioned that the middle-aged stands are the most

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prone to stem drying, and that the most intense drying happens in forests communities of English oak in lower positions. Drying of English oak forests causes deterioration of hydrology regimes, microclimate changes, deterioration of soil biological components, which alltogether affects the numerous changes in micro-ecosystem (Pilaš et al., 2007; Dubravac and Dekanić, 2009, Bittner et al., 2010). Ecological preconditions must be created prior to reforestation in order to rejuvenate such surfaces with forest trees of which the stand was previously made (Q. robur and F. angustifolia). In a conceptual framework of the impact of factors in this study (Dubravac and Dekanic, 2009) the analysis of a part of the factors such as soil and water regime will be run, since the regime of flood water wetting, and the level of ground water depends on the fine variations in micro-relief in the alluvial plain of the plain rivers, while the agricultural production is often limited by quantity and temporal distribution of precipitation (Benka et al. 2010).

Great area under English oak forests in the alluvial plain of Sava river is out of the influence of flood waters, which causes the changes in habitat conditions. From that reason determination of edaphic conditions was done in the forests affected by different degrees of drying.

MATERIAL AND METHODS

The study sample plots are situated in defended part of alluvial plain of Sava near Morovic in nine oak forests stands. Depending on the micro-relief conditions, soil profiles were opened for the study of soil morphology and soil samples were taken for the laboratory analysis. Particle size composition (%) was determined by the international B-pipette method with the preparation in sodium pyrophosphate. Soil particle classification in the particle size composition was based on Atterberg's classification. Water retentions at Rv0.1 b and Rv0.33b (% mass) were determined in Pressure plate extractor and water retention Rv6.25b (% mass) was determined by Richards pressure membrane and differential mercury regulator. Available water capacity (Kvk) (% mass) was calculated as the difference between Rv0.33b and Rv6.25b by the formula Kvk = Rv0.33b – Rv6.25b,. Chemical characteristics were determined by the following methods: humus (%) by Turin's method, modification by Symakov; CaCO3 (%) volumetric method, by Scheibler unit calcimeter; pH in H2O electrometric method with combined electrode on Radiometer pH meter; C and N on CHN analyzer; total contents of Ca, K and Mg on AAS extracted with HNO3.

The trial was conducted in three types of forests that make up about 65% representation of all forest types in Srem region. In this stands are observed weak, moderate and strong drying process.

71 (IV2) – Forest of pedunculate oak and ash (Fraxino-Querceto roboris typicum) on drier varieties of hydromorphic black soil (humosemigley), Jović et al. (1994)

73 (IV4) – Forest of pedunculate oak and ash with common maple and tartar maple and rich shrub layer (Fraxineto Quercetum roboris aceretosum) on the driest varieties of hydromorphic soils and meadow black soils with the signs of lessivage (humosemigley to eugley with signs of lessivage), Jović et al. (1994)

111 (VI3) – Forest of pedunculate oak, hornbeam and ash in floodplain (Carpino-Fraxino-Quercetum roboris inundatum) on alluvial brown soil, Jović et al. (1994)

RESULTS

The most frequent type of soil is meadow black soil in loess i.e. loess-alluvium (table 1). Maximum carbonate content did not exceed 11,7% (Table 2).

Total content of silt+clay ranged from 52,8 to 78,5 % (table 3), and textural class ranged from loam to clayey-loam depending on the content of this fraction (Table 2). Loamy textural class was determined in the most cases in the humus-accumulative horizon, while prevailing fraction in deeper profile layers was clay to clayey-loam.

Table 1. Soil types
Tabela 1. Tipovi zemljišta

Soil type Tipovi zemlji- šta	IV2	IV4	VI3
Weak / Slab	Meadow black soil on loess-alluvium Livadska crnica na lesoalu -vijumu	Meadow black soil on loess / Livadska crnica na lesu	Meadow black soil on loess Livadska crnica na lesu
Mode- rate / Srednji	Riparian black soil on loess-alluvium Ritska crnica na lesoalu-vijumu	Meadow black soil on loess-alluvium Livadska crnica na lesoalu-vijumu	Meadow black soil on loess-alluvium Livadska crnica na lesoalu-vijumu
Strong / Jak	Meadow black soil on loess-alluvium Livadska crnica na lesoalu -vijumu	Meadow black soil with fossil horizon Pogrebe-na ritska crnica	Meadow black soil on loess-alluvium Livadska crnica na lesoalu-vijumu

Table 2. Textural class
Tabela 2. Teksturna klasa

Text- ural class Tekst- urna klasa	IV2	IV4	VI3
Weak / Slab	Loam / Ilovača	Loam / Ilovača	Loam / Ilovača
	Clayey loam / Glino-vita ilovača	Loam / Ilovača	Loam / Ilovača
Mode rate / Srednji	Loam / Ilovača	Loam / Ilovača	Silty loam / Praš-kasta ilovača
	Clayey loam / Glino-vita ilovača	Clayey loam / Glino-vita ilovača	Clayey loam / Glino-vita ilovača
Strong / Jak	Silty loam / Praša-sta. ilovača	Loam / Ilovača	Silty loam / Praška-sta ilovača
	Silty loam / Praška-sta ilovača	Clayey loam / Glino-vita ilovača	Clayey loam / Glino-vita ilovača

Content of easily available water in the profile ranged from 75,87 to 86,59% (table 5). The largest quantity of easily available water was recorded for low drying intensity, while the highest content of hardly available water was related to soils with the highest degree of drying (Table 6). The content determined in these soils ranged from 21,65 do 24,13%.

Table 3. Contents of silt+clay %
Tabela 3. Sadržaj praha+gline

Contents of silt+clay Sadržaj praha+gline	IV2	IV4	VI3
Weak / Slab	63,2	54,8	60,9
	64,8	52,8	54,7
Moderate / Srednji	68,1	54,0	78,9
	66,4	75,9	70,5
Strong / Jak	74,4	67,4	71,6
	73,3	78,5	70,0

Table 4. Contents of C/N ratio
Tabela 4. Odnos C/N

C/N ratio Odnos C/N	IV2	IV4	VI3
Weak / Slab	10,6	13,6	17,9
Moderate Srednji	13,5	10,72	22,46
Strong / Jak	8,32	9,76	12,43
	0,83	0,00	0,00

Table 5. The ammount of water R0.1b-R6,25b
Tabela 5. Količina vode R0.1b-R6,25b

Ammount of water m^3/ ha; % *Količina vode m^3/ha; %*	IV_2	IV_4	VI_3
Weak / *Slab*	2772,55	3356,28	2604,42
	84,00	86,59	82,42
Moderate / *Srednji*	2613,68	2585,52	3172,40
	80,21	76,30	80,96
Strong / *Jak*	2237,72	2373,60	2952,59
	75,87	76,02	78,35

Table 6. The ammount of water R6.25b-R15b
Tabela 6. Količina vode R6.25b-R15b

Ammount of water m^3/ ha; % *Količina vode m^3/ha; %*	IV_2	IV_4	VI_3
Weak / *Slab*	528,06	519,64	555,36
	16,00	13,41	17,58
Moderate / *Srednji*	644,80	803,00	746,26
	19,79	23,70	19,04
Strong / *Jak*	711,56	748,80	815,93
	24,13	23,98	21,65

Quantity of carbon was the smallest in forests with low drying intensity and it ranged from 2,26 to 3,93% (table 7). The similar trend was observed for nitrogen content in the soil (Table 8).

Table 7. Contents of carbon
Tabela 7. Količina ugljenika

C (%)	IV_2	IV_4	VI_3
Weak / *Slab*	3,93	2,59	2,26
Moderate/ *Srednji*	3,08	3,85	1,76
Strong / *Jak*	5,45	3,52	2,62

Table 8. Contents of nitrogen
Tabela 8. Sadržaj azota

N (%)	IV_2	IV_4	VI_3
Weak / *Slab*	0,371	0,196	0,126
Moderate/ *Srednji*	0,228	0,357	0,075
Strong / *Jak*	0,655	0,361	0,257

* in A horizon

Ratio between carbon and nitrogen revealed (Table 4) pronounced humification in the forests affected by a high degree of drying. In stands with high drying intensity this ratio ranged from 8,32 to 12,43.

The widest Ca:Mg ratio in the humus-accumulative horizon was determined for low degree of drying in forest types IV4 and VI3 (table 9). In other types of forest and for high degree of drying the ration of Ca:Mg was uniform.

Table 9. Ca/Mg ratio
Tabela 9. Odnos Ca/Mg

Ca/Mg ratio *Odnos Ca/Mg*	IV_2	IV_4	VI_3
Weak / *Slab*	1,34	12,49	12,44
	6,03	4,04	2,52
Moderate *Srednji*	1,62	1,22	0,96
	0,79	1,01	0,87
Strong / *Jak*	1,44	1,80	1,88
	1,08	1,67	1,37

Table 10. K/Mg ratio
Tabela 10. Odnos K/Mg

K/Mg ratio *Odnos K/Mg*	IV_2	IV_4	VI_3
Weak / *Slab*	1,51	1,34	1,18
	0,89	0,24	0,25
Moderate *Srednji*	1,93	1,74	1,56
	1,12	1,53	1,34
Strong *Jak*	2,06	2,50	2,03
	1,46	1,70	1,19

It can be seen form table 10 that the widest ratio of K:Mg was the greatest for high degree of drying in all studied forest types.

DISCUSSION

The basic physical soil characteristics in the studied objects were in accordance with previous investigations in the area of Srem (Ivanišević et al., 2001). More detailed studies were dedicated to water-airy characteristics of soil. It was determined that the greatest degree of drying was recorded in the soils with the greatest quantity of hardly available water. The mentioned phenomena can be associated with the lack of additional wetting by surface waters, and insufficient wetting by ground waters. Even greater deficit of water in the soil, and thus the more pronounced drought can be expected since decreased amount of rainfall can be expected in the future (Galić et al., 2009). The consequence could be even more pronounced drying, and thus deforestation. Deforestation leads to further deterioration of water regime, and then to micro climatic changes, deterioration of biological soil characteristics, which all affects the numerous changes in micro ecosystem.

The changes in micro eco system are reflected by the content of carbon and nitrogen, as well as by the ration between carbon and nitrogen. The mentioned factors had the greatest values expressed in degrees with pronounced drying, which can be linked to the opening of the stand, and thus to faster mineralization of organic matter.

CONCLUSIONS

In this study the analysis of physical, water-airy and chemical soil characteristics in some types of English oak forest affected by different degree of drying was carried out. There were no greater changes in relation to physical soil characteristics.

The content of hardly available water was the greatest in forest with excessive drying, and the lack of additional wetting by flood waters did not provide enough water. Further drying of trees directly or indirectly linked to climatic changes can be expected in the future.

Changes to the micro ecosystem were also indicated by the content and ratio of carbon and nitrogen, and further monitoring could provide understanding of the process and the ability to mitigate the effects of forest drying.

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EDAFSKI USLOVI U TIPOVIMA ŠUMA HRASTA LUŽNJAKA ZAHVAĆENIH SUŠENJEM

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Izvod

U radu su proučeni edafski uslovi u tri najzastupljenija tipa šume hrasta lužnjaka zahvaćenih različitim stepenom sušenja. Dominantna sistematska jedinica zemljišta je bila livadska crnica. Istraživana zemljišta su sa sadržajem frakcije praha+gline preko 60% i teksturnom klasom zemljišta od ilovače do glinovite ilovače. Sadržaj teškopristupačne vode je bio najveći u svim tipovima šuma sa najizraženijim procesom sušenja (od 21,65 do 24,13%). Hemijske osobine zemljišta su sa manjom varijabilnošću, a najizražnija odstupanja su vezana za sadržaj i odnos ugljenika i azota. Navedene karakteristike ukazuju na potrebu daljeg monitoringa u sastojinama hrasta lužnjaka.

Ključne reči: *Quercus robur*, stanišni tipovi,tipovi šuma, monitoring.

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ECONOMIC GAIN OBTAINED BY CHOICE OF SPECIFICALLY-ADAPTED SUNFLOWER HYBRIDS FOR CROPPING

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SUMMARY: The aim of the study was to determine specifically adapted sunflower hybrids based on 2007 testing network grain yield results, and also to show economic gain obtained by minimizing crossover interaction (COI) and utililising specific adaptation. Twenty commercial sunflower hybrids and 16 locations were included in testing. SREG (sites regression) analyses were done for the set of all tested locations, set of locations with above-average mean yield and set of locations with below-average mean yield, to obtain GGL biplot with "which-won-where" patterns. When giving recommendations for growing sunflower hybrids, their adaptability examined in past years via GGL biplot methodology must be taken into account. Based on the results of GGL biplot comparative analyses, the specifically adapted hybrids identified were: Duško for Rimski Šančevi, Kikinda, Bačko Gradište, Bačka Topola set of above-average mean yield („better") locations, and for Neuzina, Neštin, Vršac set of below-average mean yield („poorer") locations; Bačvanin for Kula Vitovnica, Đurđin, Zaječar, Zrenjanin set of „poorer" locations; Branko for Kula location. Significant economic gain was obtained when cropping these three specifically adapted hybrids, in comparison to cropping those not adapted to particular locations.

Key words: *sunflower hybrids, GGE biplot analysis, grain yield, economic gain.*

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INTRODUCTION

Sunflower represents the most important crop for edible oil production in Serbia with an average grain yield of 1.902-2.419 t ha-1, with areas harvested ranging from 154.793-187.822 ha and with total production of 294.502-454.282 tonnes for the 2006-2009 period (Food and Agriculture Organization of the United Nations, 2009).

For general cultivation of any crop plant, testing by multi-environment trial (MET) is very important to ensure that the selected hybrids/cultivars have acceptable performance in variable environments within the target region (Kang et al., 2005). Genotype × environment interaction (GEI), commonly known as the differential response of genotypes to diverse environmental conditions, indicates the importance of environments and their variable factors on genotype adaptability and stability, and is a favorable phenomenon only if it is correlated with above-average yield (Yan and Hunt 2003). As a component of phenotype variability it reduces correlation between phenotypic and genotypic values, causes heritability and genetic progress from selection to decrease (Kang, 2002) and hinders identification of superior genotypes in METs (Shaffi and Price, 1998). MET is organized as a part of breeding program efforts to (i) investigate performance and stability of genotypes of interest; (ii) identify groups of locations with low crossover GEI (COI) for single and multiple years; (iii) check mega-environment differentiation for the target tested region; (iv) examine representativeness and discriminating ability of the test locations; and (v) determine specifically and generally adapted cultivars.

Effective interpretation and utilization of the MET data in making selection decisions, however, remain a major challenge to researchers. Yan et al. (2000) referred to biplots based on singular value decomposition (SVD) of environment-centered or within-environment standardized GE data as "GGE biplot" and this term emphasizes that both genotype (G) and genotype × environment (GE) effects are relevant to genotype evaluation and must be considered simultaneously (Yan and Tinker, 2006). GGE biplot offers opportunity to portray the relationship between the genotypes and environments for each attribute graphically. GGE biplot represents the most appropriate multivariate technique for this purpose, because of all used multivariate models used like Additive main effect and multiplicative interaction effects (AMMI) (Gauch and Zobel, 1996), Shifted multiplicative model (SHMM)(Seyedsadr and Cornelius 1992), Completely multiplicative model (COMM) (Cornelius et al., 1992), it is the only to possess feature of „which-won-where" biplot view revealing specific adaptation.

Determination of specifically adapted genotypes for different crop cultivars is a valuable aim for national breeding programs, in which yield gain from GEI utilisation within the state, can help increase competitiveness of national seed companies in comparison to international ones. It is considered as a rule that cultivars that have wide or general adaptation show yield stability over a great range of environmental conditions and different environments, but lower mean performance, in comparison to specifically or narrowly adapted cultivars that possess genetic potential for high yield potential and accomplish high yield in favorable evironments and lower yields in less favorable environments (Annicchiarico, 2002). Sunflower prices creating may affect a number of factors some of which are general and some specific. General factors may be costs level, competition, and even the relationship of supply and demand (Woodworth, 1977). Particular factors may be durability in demand or fluctuations in supply under the influ-

ence of natural factors, because natural factors affect both the quantity and the quality of the goods offered.

The objective of this study was to determine specifically adapted sunflower hybrids based on multilocation trial in 2007, and also to show economic gain obtained by minimizing COI and utililising specific adaptation.

MATERIAL AND METHODS

Genetic material used in this research was represented by 20 commercial sunflower hybrids from the collection of Oil Crops Department of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Sunflower hybrids were tested in multi-location trial over 16 locations distributed in the sunflower growing region in Serbia. Variety trials were organized by Oil Crops Department of the Institute of Field and Vegetable Crops, in 2007 in Serbia. Names, codes, mean yield values for hybrids and locations used in 2007 sunflower testing network are presented in Table 1.

Table 1. Mean grain yield (t/ha) of sunflower hybrids across locations for 2007 testing network
Tabela 1. Prosečan prinos zrna (t/ha) hibrida suncokreta u višelokacijskom ogledu za 2007 godinu

Hybrid / Hibrid	Code/ Kod	*Regions* / Regioni															
		RS	KU	BG	BT	DU	SO	AS	KI	ZR	NZ	VS	NS	KV	KG	NE	ZA
Ns-h-45	1	3,63	2,88	3,48	3,78	2,54	2,71	2,24	3,49	2,71	2,85	2,09	3,65	2,14	2,34	1,37	2,24
Vranac	2	3,78	3,20	3,68	3,86	2,61	2,72	2,33	3,38	2,41	2,31	2,27	3,38	2,36	2,22	1,24	2,02
Rimi	3	3,76	2,51	3,34	3,76	2,21	3,27	2,28	3,39	2,47	2,38	2,17	2,97	2,36	1,75	1,10	2,00
Bačvanin	4	3,73	2,79	3,57	4,01	3,01	3,71	2,55	3,46	2,51	2,39	2,54	3,50	2,71	2,13	1,05	1,97
Ns-h-111	5	4,10	2,82	3,84	3,94	2,42	3,12	2,28	3,10	2,73	2,47	2,51	3,99	2,56	1,76	1,26	2,25
Velja	6	4,18	3,06	3,92	4,02	2,67	3,29	2,54	3,74	2,39	2,99	2,84	3,80	2,31	2,05	1,16	1,90
Krajišnik	7	3,66	2,54	3,49	3,71	2,52	3,24	2,13	3,10	2,66	2,20	2,50	3,84	2,59	1,88	0,97	1,87
Perun	8	3,72	2,93	3,65	3,58	2,67	2,55	2,04	3,12	2,29	2,92	2,67	3,55	2,28	1,89	1,26	2,14
Pobednik	9	3,75	2,62	3,51	3,94	2,58	3,40	2,49	3,15	2,24	2,49	2,56	3,99	2,30	2,06	0,99	2,03
Baća	10	3,97	3,13	3,24	4,35	2,84	3,48	2,19	3,54	2,38	2,35	2,29	4,09	2,40	1,88	0,71	1,57
Sremac	11	4,24	2,85	3,83	3,99	2,52	3,29	2,46	3,89	2,27	2,79	2,19	3,79	2,61	2,05	1,15	2,13
Somborac	12	3,83	2,87	3,91	3,91	1,75	3,17	2,51	3,22	2,23	2,66	2,42	3,90	2,12	2,11	1,04	1,94
Šumadinac	13	4,11	3,30	3,86	4,41	2,51	3,07	2,31	3,36	2,60	2,78	2,64	3,94	2,41	2,12	1,38	2,06
Kazanova	14	4,31	2,92	3,67	4,22	2,54	3,22	2,50	3,78	2,67	2,84	2,45	3,39	2,28	2,23	1,22	1,95
Olivko	15	3,17	3,12	3,63	3,54	2,24	2,71	2,04	2,56	2,28	2,29	2,23	3,01	1,94	2,41	1,00	1,71
Plamen	16	3,84	2,94	3,59	3,71	1,88	3,12	2,33	3,53	2,22	2,62	2,51	3,60	2,18	2,49	0,70	1,32
Duško	17	4,02	3,14	3,53	4,04	2,61	3,28	2,44	4,54	2,38	3,54	2,54	4,00	2,34	2,23	1,39	1,93
Branko	18	4,06	3,15	3,54	3,56	2,38	3,07	2,21	3,02	2,37	2,72	2,21	4,32	2,08	2,37	1,42	1,74
Novosađanin	19	3,67	3,45	3,45	3,81	2,70	2,73	2,13	3,26	2,44	2,76	2,26	4,20	2,44	2,04	1,37	1,72
Oliva	20	4,07	3,02	3,85	4,07	2,79	3,11	2,65	3,55	2,17	2,31	2,24	3,21	2,45	1,96	1,08	2,27

RS-Rimski Šančevi, KU-Kula, BG-Bačko Gradište, BT-Bačka Topola, DU-Đurđin, SO-Sombor, AS-Aleksa Šantić, KI-Kikinda, ZR-Zrenjanin, NZ-Neuzina, VS-Vršac, NS-Neštin, KV-Kula Vitovnica, KG-Kragujevac, NE-Negotin, ZA-Zaječar.

Experimental data were mean grain yields (t ha-1) of tested hybrids over tested locations. The experimental design was a randomized complete block design with four replications and plot size was 28 m2. Two middle rows of four were used for analysis and border plants were excluded. Elementary plot size was 13.3 m2 (0,7 x 0,25 x 76). Parcels on which sunflower had not been cultivated for four last consecutive years, soybean and oil seed rape for three years and maize as preceding crop were not treated with herbicides based on Atrazine, was choosen for experiment implementation. Winter ploughing was done on 30 cm soil depth. Fertilisation included application of mineral fertilisers with 50 kg ha-1 of nitrogen, 80-90 kg ha-1 of phosphorus oxide (P2O5) and 60 kg ha-1 of potassium oxide (K2O). The whole quantity of phosphorus and potassium, and half of nitrogen was added during primary tillage in autumn and other half of nitrogen quantity was added during secondary tillage. Sowing was done mechanically during April. Grain yield evaluation was performed by measurment of grain mass for each elementary plot, and grain yield (t ha-1) with 11% moisture ha-1 was calculated.

In order to evaluate hybrids specific adaptation both genotype (G) and genotype by location interaction (GL) effects must be considered simultaneously, and we used the sites regression (SREG) model (Crossa and Cornelius 1997) to obtain GGL biplot with "which-won-where" pattern. SREG analyses were done for the set of all tested locations, locations with above-average mean yield and locations with below-average mean yield, within R computing environment (R Development Core Team 2010). For plotting the biplot, Excel (macro), according to Lipkovich and Smith (2002), was used.

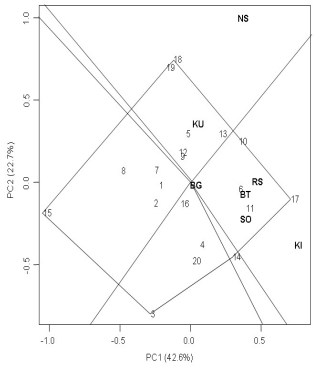
RESULTS AND DISCUSSION

Plant breeders and agronomists have found GGL biplot analysis to be useful for simultaneous evaluation of genotype performance and stability, mega-environment investigation and adaptability studies (Yan et al., 2001; Lee et al., 2003; Butron et al., 2004; Samonte et al., 2005; Malvar et al., 2005; Voltas et al., 2005; Kang et al., 2005; Dardanelli et al., 2006; Fan et al., 2007; Mohammadi et al., 2010; Goyal et al., 2011).

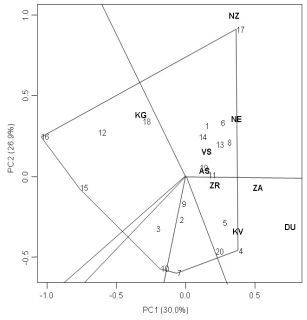
One of the most attractive features of a SREG biplot is its ability to show the "which-won-where" pattern of the dataset (Yan and Tinker, 2006). Many researchers find this use of a biplot intriguing, as it graphically addresses important concepts such as crossover GE, mega-environment differentiation, specific adaptation, etc (Yan and Hunt, 2003). A polygon is drawn on hybrids that are furthest from the biplot origin so that all other hybrids are contained within the polygon. Hybrids located on the vertices of the polygon performed either the best or the poorest in one or more locations. The perpendicular lines drawn to each side of the polygon or it extension, starting from the biplot origin represent equality lines which divide the biplot into sectors, and the winning hybrid for each sector is the one located on the respective vertex (Yan and Tinker, 2006).

By selecting a set of locations with above-average mean yield ("better" locations) and set of locations with below-average mean yield ("poorer" locations) from the whole set of tested locations, and by comparing all three "which-won-where" patterns (Graph. 1; Graph. 2) for all three sets of locations, we determined specifically adapted hybrids (graph for the set of all locations is not shown). Our procedure can be explained by the fact that varietal differences becomes more obvious when extracting a set of "better" or set of "poorer" locations from analysis because genetic backgrounds of varieties

and their adaptability to various environmental conditions differ according to Lin's et al. (1986) definition of an adaptability as the consistence of genotype performance in space. Based on the results of biplot analysis for the 2007 testing network specifically adapted hybrids determined were: G17 (Duško) for RS, KI, BG, BT („better" locations), for NZ, NE, VS („poorer" locations); G4 (Bačvanin) for KV, DU, ZA, ZR („poorer" locations); G18 (Branko) for KU.



Graph. 1. GGL biplot („which-won-where" view) for hybrids grain yield across set of locations with above-average mean yield for 2007 sunflower testing network.
Grafikon 1. GGL biplot („which-won-where" prikaz) prinosa zrna hibrida za set lokaliteta sa iznad-prosečnim prinosom višelokacijskog ogleda suncokreta za 2007 godinu.



Graph. 2. GGL biplot („which-won-where" view) for hybrids grain yield across set of locations with below-average mean yield for 2007 sunflower testing network.
Grafikon 2. GGL biplot („which-won-where" prikaz) prinosa zrna hibrida za set lokaliteta sa ispod-prosečnim prinosom višelokacijskog ogleda suncokreta za 2007 godinu.

The average annual purchase price of sunflower in 2007. amounted to 25,64 din/kg or 25.640 din/t. (Statistical Office of the Republic of Serbia, 2007). Prices of agricultural products take into account both purchase and selling price. Purchase prices are the prices at which companies and organizations authorized purchase of agricultural

products from private households and of course they do not include incentive premiums. On the other hand, selling prices are those prices at which agricultural enterprises and organizations in the domestic market sell products from their own production. Average purchase and selling prices are calculated based on the report of the entire purchase and sales from the owner's production (Koester and Zarić, 2009).

By correct use of specifically adapted sunflower hybrids for cropping in specific locations in Serbia significant economic gain can be obtained. The context of economic gain referred to in our paper meant the difference (increase) in total revenue obtained with the hybrid identified as compared with other not adapted hybrids. By cropping specifically adapted hybrid Duško in the Kikinda location significant economic gain of 1.692.240 dinars and of 1.346.100 per 50 hectares is obtained in comparison respectively to Olivko and Somborac (Table 2). Olivko and Somborac were poorly adapted hybrids to this location, as was shown by GGL biplot. Also economic gain obtained by cropping Bačvanin, specifically adapted hybrid to Đurđin location was 1.615.320 dinars and 641.000 dinars per 50 hectares in comparison to cropping not adapted hybrids Olivko and Vranac, according to GGL biplot, respectively (Table 2).

Table 2. Economic gain expressed in dinars achieved with correct and incorrect choice of sunflower hybrids for cropping based on biplot adaptability analyses
Tabela 2. Ekonomska dobit izražena u dinarima ostvarena pravilnim i nepravilnim izborom hibrida suncokreta za gajenje na osnovu biplot analiza adaptabilnosti

Location-Hybrid */Lokalitet-Hibrid*	Economic gain expressed in dinars for / *Ekonomska dobit izražena u dinarima ostvarena na*				
	1 ha	5 ha	10 ha	20 ha	50 ha
RS-Duško	103.072,8	515.364	1.030.728	2.061.456	5.153.640
RS-Olivko	98.201,2	491.006	982.012	1.964.024	4.910.060
RS-Somborac	93.073,2	465.366	930.732	1.861.464	4.653.660
KI-Duško	116.405,6	582.028	1.164.056	2.328.112	5.820.280
KI-Somborac	89.483,6	447.418	894.836	1.789.672	4.474.180
KI-Olivko	82.560,8	412.804	825.608	1.615.216	4.128.040
BG-Duško	90.509,2	452.546	905.092	1.810.184	4.525.460
BG-Somborac	89.227,2	446.136	892.272	1.784.544	4.461.360
BG-Novosađanin	85.637,6	428.188	856.376	1.712.752	4.281.880
BT-Duško	103.585,6	517.928	1.035.856	2.071.712	5.179.280
BT-Novosađanin	96.406,4	482.032	964.064	1.928.128	4.820.320
BT-Branko	95.124,4	475.622	951.244	1.902.488	4.756.220
NZ-Duško	90.765,6	453.828	907.656	1.815.312	4.538.280
NZ-Olivko	68.202,4	341.012	682.024	1.364.048	3.410.120
NZ-Oliva	58.715,6	293.578	587.156	1.174.312	2.935.780
NE-Duško	35.639,6	178.198	356.396	712.792	1.781.980
NE-Olivko	26.665.6	133.328	266.656	533.312	1.333.280
NE-Baća	18.204,4	91.022	182.044	364.088	910.220
VS-Duško	65.125,6	325.628	651.256	1.302.512	3.256.280
VS-Pobednik	57.946,4	289.732	579.464	1.158.928	2.897.320
VS-Somborac	53.587,6	267.938	535.876	1.071.752	2.679.380
KV-Bačvanin	69.484,4	347.422	694.844	1.389.688	3.474.220
KV-Plamen	58.459,2	292.296	584.592	1.169.184	2.922.960
KV-Olivko	54.356,8	271.784	543.568	1.087.136	2.717.840
DU-Bačvanin	77.176,4	385.882	771.764	1.543.528	3.858.820

DU-Vranac	64.356,4	321.782	643.564	1.287.128	3.217.820
DU-Olivko	44.870,0	224.350	448.700	897.400	2.243.500
ZA-Bačvanin	50.510,8	252.554	505.108	1.010.213	2.525.540
ZA-Duško	47.946,8	239.734	479.468	958.936	2.397.340
ZA-Branko	33.844,8	169.224	338.448	676.896	1.692.240
ZR-Bačvanin	64.356,4	321.782	643.564	1.287.128	3.217.820
ZR-Olivko	57.177,2	285.886	571.772	1.143.544	2.858.860
ZR-Perun	55.638,8	278.194	556.388	1.112.776	2.781.940
KU-Branko	80.766,0	403.830	807.660	1.615.320	4.038.300
KU-Bačvanin	74.868,8	374.344	748.688	1.497.376	3.743.440
KU-Krajišnik	72.304,8	361.524	723.048	1.446.096	3.615.240
ZR-Bačvanin	64.356,4	321.782	643.564	1.287.128	3.217.820
ZR-Olivko	57.177,2	285.886	571.772	1.143.544	2.858.860
ZR-Perun	55.638,8	278.194	556.388	1.112.776	2.781.940
KU-Branko	80.766,0	403.830	807.660	1.615.320	4.038.300
KU-Bačvanin	74.868,8	374.344	748.688	1.497.376	3.743.440
KU-Krajišnik	72.304,8	361.524	723.048	1.446.096	3.615.240

RS-Rimski Šančevi, BG-Bačko Gradište, BT-Bačka Topola, DU-Đurđin, KI-Kikinda, ZR-Zrenjanin, NZ-Neuzina, VS-Vršac, KV-Kula Vitovnica, NE-Negotin, ZA-Zaječar.

Total economic gain calculated for Duško, Bačvanin and Branko, specifically adapted hybrids determined via GGL biplot comparative analyses, were in the range of 1.282-33.845 dinars per hectare and of 64.100-1.692.240 dinars per 50 hectares, in comparison to not adapted hybrids.

CONCLUSION

When giving recommendations for growing sunflower hybrids, their adaptability examined in past years via GGL biplot methodology must be taken into account. Based on the results of GGL biplot comparative analyses for 2007 sunflower testing network, specifically adapted hybrids determined were: Duško for RS, KI, BG, BT („better" locations), and for NZ, NE, VS („poorer" locations); Bačvanin for KV, DU, ZA, ZR („poorer" locations); Branko for KU. Significant economic gain was obtained when cropping these three specifically adapted hybrids, in comparison to cropping those not adapted to particular locations.

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EKONOMSKA DOBIT OSTVARENA KORIŠĆENJEM SPECIFIČNO ADAPTIRANIH HIBRIDA SUNCOKRETA ZA SETVU

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Izvod

Cilj istraživanja je obuhvatao utvrđivanje specifično adaptiranih hibrida suncokreta na osnovu rezultata prinosa zrna hibrida u višelokacijskom ogledu organizovanom 2007 godine, kao i izračunavanje ekonomske dobiti ostvarene minimiziranjem interakcije sa izmenom ranga (COI) i iskorišćavanjem specifične adaptacije. U testiranje je bilo uključeno 20 komercijalnih hibrida suncokreta i 16 lokaliteta. SREG (sites regression) analiza je urađena za set svih testiranih lokaliteta, set iznad-prosečno prinosnih lokaliteta i za set ispod-prosečno prinosnih lokaliteta, da bi se dobio GGL biplot sa "which-won-where" obrascima. Radi davanja preporuke za gajenje hibrida suncokreta, njihova adaptabilnost utvrđena za prethodne godine GGL biplot metodologijom, mora biti uzeta u obzir. Na osnovu GGL biplot komparativne analize utvrđeni su specifučno adaptirani hibridi: Duško za Rimske Šančeve, Kikindu, Bačko Gradište, Bačku Topolu, set iznad-prosečno prinosnih ("boljih") lokaliteta, i za Neuzinu, Neštin, Vršac, set ispod-prosečno prinosnih ("lošijih") lokaliteta; Bačvanin za Kula Vitovnicu, Đurđin, Zaječar, Zrenjanin, set ispod-prosečno prinosnih ("lošijih") lokaliteta; Branko za Kulu. Značajna ekonomska dobit je ostvarena gajenjem navedena tri specifično adaptirana hibrida suncokreta u poređenju sa gajenjem neadaptiranih za određene lokalitete.

Ključne reči: suncokret, hibridi, GGE biplot analiza, prinos zrna, ekonomska dobit.

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EFFECT OF DESICCATION DATE ON OIL CONTENT IN SUNFLOWER SEED*

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SUMMARY: The trial was conducted at Rimski Šančevi experimental field in 2009 in order to assess the effect of chemical desiccation on oil content in seed of three sunflower inbred lines (L1, L2 and L3). Reglone forte (2 l/ha) was used for desiccation. Desiccant application was performed at 7-day intervals from the end of flowering to harvest maturity. Seed moisture content was measured prior to each treatment. Seed oil content was determined after harvest. The three lines showed highly significant differences in seed oil content. The highest oil content was found in L3, the lowest in L2. The highest oil content was obtained with the treatment done 21 days after flowering (DAF), when seed moisture was 45.6%. There was no subsequent increase in oil content. Regression analysis showed that the three lines achieved maximum oil content at different seed moisture levels at the time of desiccation (19% to 39%).

Key words: *desiccation, oil content, sunflower, seed moisture.*

INTRODUCTION

Desiccation as an agrotechnical measure was introduced in agricultural practice several decades ago. The basic idea is to accelerate plant drying in order to make the crop ready for early harvest. The measure is advantageous in cool climates, where spring crops often mature in late fall, when weather conditions make harvest difficult. Chemical desiccation largely solves the problems occuring in mechanized sunflower harvest (Miklič et al., 2001). Desiccation is recommendable for sunflower production, especially seed production, because early harvest reduces seed loss, facilitates the har-

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vest, makes additional seed drying unnecessary and prevents adverse effect of frost on seed germination (Đukić et al., 2006). Early harvest reduces the intensity of occurence of parasites of the head (Maširević and Glušac, 1999). Desiccation is particularly important for double cropped sunflowers (Liović et al., 2010).

Desiccation timing is associated with physiological maturity of plants. Physiological maturity is defined as the moment when assimilate supply to seed is completed and when contact between seed and parent plant is disrupted. In the sunflower, this moment is counted by the number of days after flowering. This method is unreliable because the sunflower head flowers in concentric circles and it is difficult to determine when precisely each flower ends flowering. Besides, beginning of physiological maturity varies widely depending on the genotype and weather conditions. Visual methods for determining the beginning of physiological maturity are based on color change of the reverse side of the head, bottom leaves drying or bracts drying (Kaya et al., 2004) and they are subjective. The best method is obviously the one related to the moisture content in seed (Miklič et al., 2006).

The sunflower (*Helianthus annuus*) is an important crop due to its high oil content in seed. Sunflower oil is an exceptional edible oil which is widely used in human nutrition. It is used directly for cooking and as an ingredient in a variety of food products (Jevtić et al., 1986). Oil synthesis in sunflower seed begins soon after pollination and it gradually intensifies (Vratarić et al., 2004). It is important to determine how the date of desiccation affects oil content, i.e., what is the optimal date for its performance.

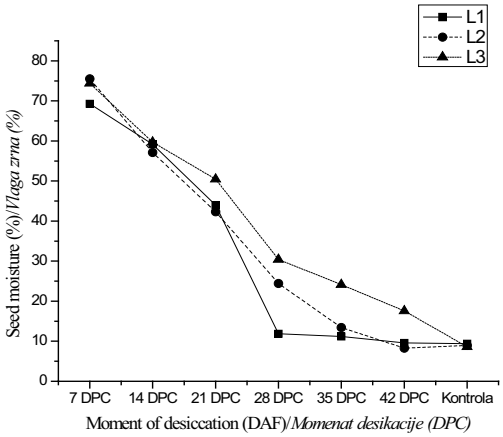
MATERIAL AND METHODS

The trial was conducted at Rimski Šančevi experimental field in 2009. It included three new cytoplasmic male sterile lines of sunflower developed at Institute of Field and Vegetable Crops (L1, L2 and L3). Desiccation was performed with Reglone forte (2 l/ha) applied at 7-day intervals from the end of flowering (DAF) to harvest maturity. Treatments were performed with a knapsack sprayer. Seed samples from several sunflower heads were collected directly before each treatment in order to test the moisture content in seed at the time of treatment. Seed moisture was determined in the laboratory by the conventional gravimetric method. When harvest time was reached, 12 heads were taken from each treatment for making average samples for seed oil content analysis. The analysis was performed by the conventional Soxhlet method in three replications.

The obtained results were statistically processed. The analysis of variance of two factorial experiment (split-plot design) and the regression analysis were used. Significance of regression was tested with the F-test.

RESULTS AND DISCUSSION

The highest average moisture content in seed at the time of desiccation was found in the line L3 (37.9%), the lowest in the line L1 (30.64%). The graph shows that the rate of seed moisture reduction was the fastest in the line L1 and the slowest in the line L3 (Graph 1).



Graph. 1. Moisture content in seed at the time of desiccation
Graf. 1. Sadržaj vlage u semenu u momentu desikacije

The highest average oil content was found in the line L3 (42.97%), the lowest in the line L2 (38.15%). The differences in the average oil content among the three lines were highly significant (Table 1).

Table. 1. Seed oil content (%)
Tabela. 1. Sadržaj ulja u semena(%)

Line-L *Linija-L*	Desiccation time -R / *Rok tretiranja -R*							Average *Prosek* (L)
	7 DAF	14 DAF	21 DAF	28 DAF	35 DAF	42 DAF	Control *Kontrola*	
L 1	35.66	43.73	41.56	40.77	37.24	37.73	37.03	39.10
L 2	29.56	35.87	41.40	39.68	40.29	40.34	39.89	38.15
L 3	32.52	39.04	43.45	45.57	46.38	46.99	46.83	42.97
Average *Prosek* (R)	32.58	39.55	42.13	42.00	41.30	41.69	41.25	-

LSD	L	R	L*R	R*L
5%	0.11	0.55	0.89	0.96
1%	0.18	0.74	1.20	1.29

The treatment 21 DAF, when seed moisture was 45.6%, brought the highest average oil content (42.13%). The lowest oil content (32.58%) was found in the treatment 7 DAF. The average oil content in the treatment 21 DAF was highly significant in relation to those achieved in the treatments 7 DAF, 14 DAF, 35 DAF and the control. No significant difference in average oil content was registered among the treatments 21 DAF, 28 DAF and 42 DAF. Miklič (2001) found the highest oil content in the control, while after the treatment 21 DAF, at seed moisture of 44.34%, there was no significant increase in oil content, which is consistent with the results of our experiment. On the other hand, Radić (2006) found the highest oil content much later, 42 DAF, Rana et al. (1990) report-

ed of finding the highest oil content 45 DAF, while Baydar and Erbas (2005) found the highest oil contents 30 and 35 DAF, after which dates the content decreased slightly.

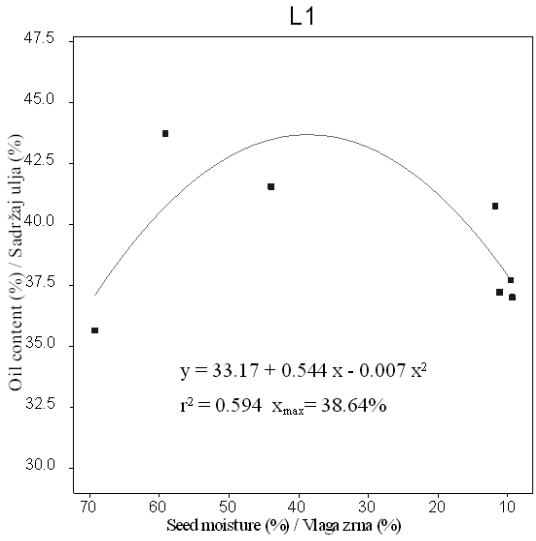
In the case of the line L1, the highest oil content was obtained with the treatment 14 DAF (43.73%), the lowest with the treatment 7 DAF (35.66%). The treatment 14 DAF had a highly significant oil content in relation to all other treatments and the control. The highly significant decrease in oil content may be attributed to the increase in seed weight upon the completion of oil accumulation, which lowered the proportion of oil in the total seed weight.

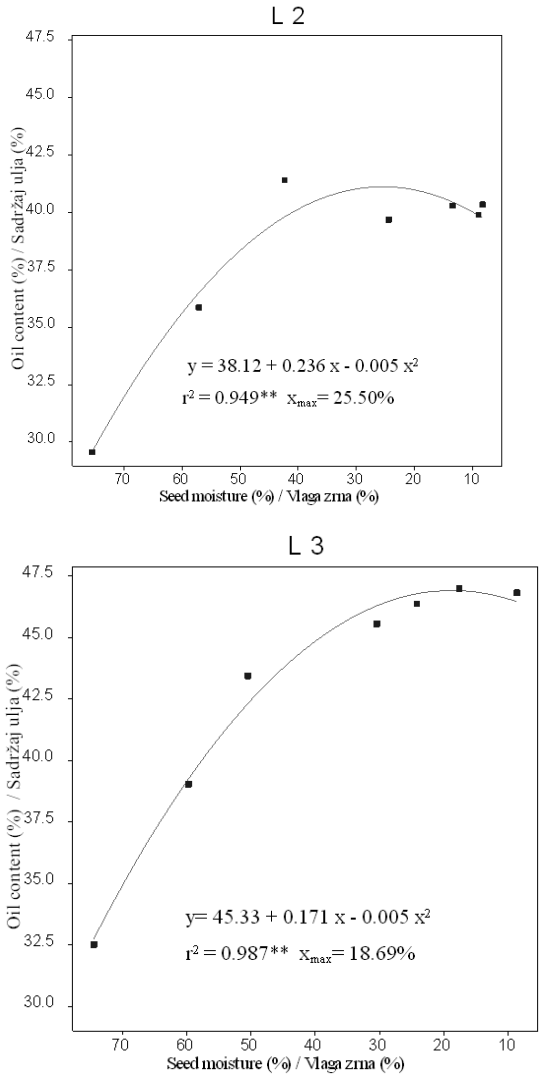
In the case of the line L2, the highest oil content was obtained with the treatment 21 DAF (41.4%), the lowest with the treatment 7 DAF (29.56%). The treatment 21 DAF had a highly significant oil content in relation to the treatments 7 DAF, 14 DAF, 28 DAF and the control, and significant in relation to the treatments 35 DAF and 42 DAF.

In the case of the line L3, the highest oil content was obtained with the treatment 42 DAF (46.99%), the lowest with the treatment 7 DAF (32.52%). The treatment 42 DAF had a highly significant oil content in relation to the treatments 7 DAF, 14 DAF and 21 DAF, and significant in relation to the treatment 28 DAF. The treatments 35 DAF, 42 DAF and control showed no significant difference in oil content.

In the case of treatments 7 DAF and 14 DAF, the highest oil content was registered in the line L1 (35.66% and 43.7%), and it was highly significant in relation to other two lines.

In the case of all other treatments and the control, the line L3 had the highest oil content (43.45%, 45.57%, 46.38%, 46.99% and 46.83%). Oil content in the line L3 was highly significant in relation to other two lines.





Graph 2. Effect of seed moisture at the time of desiccation on oil content

Graf. 2. Uticaj vlage zrna u momentu desikacije na sadržaj ulja

The regression analysis showed that the oil content in the three lines was differently affected by seed moisture level at the time of desiccation (Graph 2). The greatest impact was observed in the line L3, medium in the line L2 and lowest in the line L1, 0.987 **, 0.949** and 0.594, respectively. Those impacts in the lines L2 and L3 were highly significant, while the effect of moisture content on oil content in the line L1 could not be explained by the quadratic regression. The regression curve exhibited maximum oil contents in L1, L2 and L3 at moisture contents of 38.64%, 25.5% and 18.69%. Similarly, Miklič et al. (2001) and Radić (2006) found a significant effect of seed moisture at the time of desiccation on oil content in sunflower seed.

CONCLUSION

The following conclusions were drawn on the effect of desiccation timing on oil content in sunflower seed.

The differences in average oil content between the three lines were highly significant. The highest oil content was found in the line L3, the lowest in L2.

The treatment 21 DAF, at seed moisture of 45.6%, brought the highest oil content. There was no further increase in oil content with the later treatments.

The three lines achieved maximum oil contents at different seed moisture levels at the time of desiccation, from 19% to 39%.

The results of this study seem to indicate that oil accumulation in sunflower seed ends well before harvest maturity.

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UTICAJ VREMENA DESIKACIJE NA SADRŽAJ ULJA U SEMENU SUNCOKRETA

PETAR ČANAK, VELIMIR RADIĆ, MILAN JOCKOVIĆ, MIHAJLO ĆIRIĆ, NADA LEČIĆ, JELENA MRĐA, VLADIMIR MIKLIČ

Izvod

Ogled je postavljen 2009. godine na Rimskim Šančevima sa ciljem da se ispita uticaj hemijske desikacije na sadržaj ulja u semenu kod tri roditeljske linije suncokreta. Desikacija je vršena preparatom *Reglone forte* (2 l/ha) svakih 7 dana od završetka cvetanja i oplodnje do žetvene zrelosti. Pre svakog tretmana utvrđena je vlažnost semena. Sadržaj ulja u semenu utvrđen je po dostizanju žetvene zrelosti. Između sve tri linije utvrđena je visoko značajna razlika u sadržaju ulja u semenu. Najviši sadržaj ulja utvrđen je kod linije L3, a najniži kod linije L2. Kod tretmana izvršenog 21 dan posle cvetanja (DPC), pri vlazi semena od 45,6 %, utvrđen je najviši sadržaj ulja, posle čega nije utvrđen rast sadržaja ulja. Regresionom analizom, kod sve tri linije, maksimum sadržaja ulja utvrđen je pri različitoj vlažnosti semena u momentu desikacije, od 19 % do 39 %.

Ključne reči: desikacija, sadržaj ulja, suncokret, vlaga semena.

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TRADITIONAL AND NONCONVENTIONAL RENEWABLE ENERGY SOURCES

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SUMMARY: The modern development of energy sources should be based on highly effective non-polluting power technologies. The non-traditional energetic uses renewable energy sources (geothermal heat of the Earth, the Sun, wind, inflow etc.) and are ecologically friendly. High economic efficiency and environmental awareness became main reasons of using widely non-traditional power technologies in the remote regions of the World. The constant reduction of the mineral fuels on the Earth and the perspective of their price increase and also the rise o of the environmental pollution caused by harmful wastes are the reasons for the active search and development of the alternative energy sources. At the time being the world community has recognized that in the recent years and possibly, centuries, the discovery and development of the totally new source of energy will not succeed. That's why the main hopes and nearest plans on the development of the world energetic complex are directed to the renewable sources of energy, including geothermal one, the energy of the sun, wind , bio-mass.

Keywords: *Energetic, power supply, renewable energy sources, electric and heat energy.*

INTRODUCTION

The search for new forms of energy has always been one of the main tasks of the humanity. There was always lack of energy. To obtain steady harvests, produce goods, learn to manage space technologies we are all in need of different types of energy: electrical, heat, optical and others. The continuing progress of the world community is connected with the growth of energy consumption in it's different final forms. During 80 years of the last century the world's power consumption increased more than 110 times and reached the consumption which equals to 10 billions of tons of equivalent

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Original scientific paper / *Originalni naučni rad*
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fuel (Amerhanov et al., 2002; Kirushatov, 1991). The problems with the energy supply which have recently become obvious, can be explained by the growth of the primary energy consumption per capita, depletion of resources convenient for using traditional types of mineral fuels (oil, natural gas), the growth of the expenses for extracting them, worsening ecological conditions because of their burning in various heat-energetic installations.

The growth of power consumption on average makes up 4,5 % annually. It means that the power consumption doubles every 15 years. Up to middle of the 1970th the speedy growth of the world energetic depended on the cheapness of oil and other mineral fuel production. The important role was given to the detection of efficient oilfields in the Persian Gulf's region. For example, one oil well can produce up to 500-1000 tons of oil (Harchenko, 1991)

The analyses of the modern situation in energetic makes us draw a conclusion that the problem of the reliable and economical energy supply cannot be successfully solved without a considerable change of the energetic structure, without introducing new types of energy sources. Considering the exhaustion of mineral resources trend and the price growth of energy carriers the issues of energy saving are the recent problems. The important role in improving the efficiency of using of traditional energy resources and mineral fuel saving is given to nontraditional renewable energies. The global transfer to the renewable sources of energy doesn't take place only because the industry, machinery and equipment in the past were intended to use a cheap mineral fuel. Existing technologies and equipment using mineral fuels at a large extent are rather developed and they were economically beneficial for us. At the same time, the problems of exhaustion of the mineral fuels and also the problems of the environment caused by its burning, issues of transporting fuels into the zones with decentralized energy supply show the necessity of searching for the alternative sources of energy and fuels. The main advantages of renewable energy sources (RES) are inexhaustibility and ecological compatibility. Energy technologies based on RES are related to "soft" technologies which practically don't harm the environmental and don't change the energetic balance of the Earth. RES plays a considerable role in solving of 3 global problems which the humanity faces: energetic, ecology, food producing issues. The important advantage of the renewable sources of energy is that they are able to provide the humanity with the energy for many centuries to come. Many types of the RES at present are competitive at the energy market, especially the geothermal energy. Paces of RES development in developed countries are so high, that they will have replaced 70% of traditional sources up to 2050 year, according to Bansal et al. (1994) and Erikson (1990).

The issues of the RES development and using are one of the main problems in foreign policy of different countries. Countries with developed RES technologies will get obvious and considerable ecological and economical advantages. Many countries at present are profoundly concerned with the perspectives of their own energy supply.

From short review mentioned above it is clear that engineering solutions of energy problem can be concluded as the following:

1) Considering the small density of the solar radiation, which doesn't make it possible to heat the carrier to the consumer' temperature (90°C), it is necessary to develop concentrators of solar radiation which will be able to work in negative temperature. These plants can be used for heating of buildings.
2) Developing of low-powered (2…4 kW), simple, reliable constructions of wind tur-

bines and organization of their production.

3) Developing of devices using the solar radiation for different technological processes, for example- water feed from water sources.

MATERIALS AND METHODS

Working on the engineering solution of the energy supply problem in Stavropol State Agrarian University a pilot plant of conical solar concentrator was developed and made. The distinctive peculiarity of the conical solar concentrator (figure 1) is in the fact that the sunlight rays which get into cone's aperture are reflected to heat absorber and fully transformed to heat. The advantages of conical solar concentrator will be realized only with the special construction of heat absorber and water feeder which will make it possible to reduce heat leakages as a result of the convective heat transfer, thermal radiation and thermal conductivity.

These abilities are provided by the heat absorber (figure 2) presenting by itself absorber- converter 2 which is connected to the truncated cone 1. The absorber-converter includes annular closed cavities 3 located concentrically towards axe of the truncated cone. Cavities are connected with each other pipelines 4 and extreme annular cavity 3 is provided with the input 5 and output 6 fittings.

From other side, the absorber-converter 2 is covered by low-profile cavity 7 connected by joining pipelines 8 and 9 to 5 and 6 fittings. The inlet of the absorber-converter 2 from the truncated cone side 1 is closed by the transparent doubled glass 10. The outer side of the glass is covered by the heat insulator 11. Constructively, the absorber-converter is made with the possibility of filling cavities 3 and 7 by the heat carrier 12.

Figure 1. Conical solar concentrator

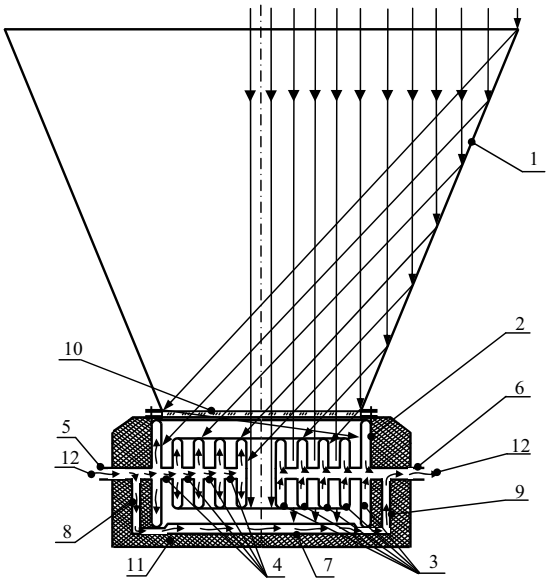

Figure 2. Principal scheme of conical solar concentrator
1 – cone reflector, 2 – absorber-converter, 3 – annular closed cavities, 4 – pipelines, 5 – inlet fitting, 6 – outlet fitting, 7 – low-profile cavity, 8, 9 – joining pipelines, 10 – transparent cover, 11 – heat insulator, 12 – heat carrier.

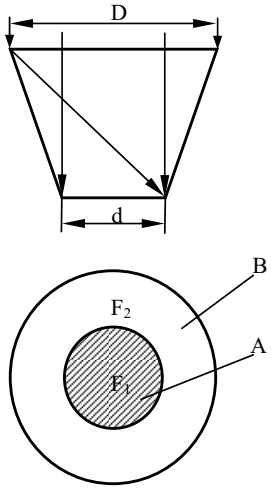

Figure 3. Solar radiation distribution on solar concentrator

Solar flux $\hat{O}_{conc}$, falling to heat absorber of conical solar concentrator, will be formed from direct solar flux which falls to active surface A and reflected by cone's surface B (figure 3) solar flux, i.e

$$\hat{O}_{conc} = \hat{O}_A + \hat{O}_B, \quad (1)$$

where $\hat{O}_A$ - power of solar radiation on surface A, kW

$\hat{O}_B$ - power of solar radiation on surface B, kW

The power of forward flow of solar radiation calculated in the following way:

$$\hat{O}_A = \mathring{A}_D \cdot \frac{\pi \cdot d^2}{4}, \quad (2)$$

where $\mathring{A}_D$ - density of solar radiation, kW / m^2;

d – diameter of small bottom of the cone, m;

$\frac{\pi \cdot d^2}{4} = F_1$ - square of small bottom of the cone, m^2;

The power of reflected flow of solar radiation calculated in the following way:

$$\hat{O}_B = \mathring{A}_E \cdot \frac{\pi(D^2 - d^2)}{4}, \quad (3)$$

where $\mathring{A}_E = \mathring{A}_D R^n$ – density of solar radiation without concentration, with a glance to loss in the time of n- multiple solar beam's reflection, kW / m^2.

R - reflectivity factor; n - quantity of reflections; d – diameter of large bottom of the cone, m; So, the power of solar concentrator will be:

$$\hat{O}_{conc} = \frac{\mathring{A}_E \cdot \pi}{4}\left[(D^2 - d^2)R^n + d^2\right], \quad (4)$$

The density of the solar radiation will be:

$$E_E = \frac{4 \cdot \Phi_{conc}}{\pi \cdot [(D^2 - d^2)R^n + d^2]} \quad (5)$$

The solar flux $\hat{O}_{conc}$ changing of solar radiation density $\mathring{A}_E$ of the cone concentrator subject to reflectivity factor R and quantity of reflections n presented in a table 1 ($D = 1m$).

Table 1. The solar flux $\hat{O}_{conc}$ changing of solar radiation density $\mathring{A}_E$ of the cone concentrator subject to reflectivity factor R and quantity of reflections n

Quantity of reflections n	$\frac{d}{D}$	F_1, m^2	F_2, m^2	R=0,8		R=0,9		R=0,97	
				Φ_{conc}, kW	E_E kW/m^2	Φ_{conc}, kW	E_E, kW/m^2	Φ_{conc} kW	E_E, kW/m^2
0	1	0,78	0,78	0,51	0,65	0,51	0,65	0,51	0,65
1	0,42	0,14	0,78	0,42	3,03	0,46	3,32	0,49	3,54
2	0,32	0,08	0,78	0,34	4,23	0,42	5,22	0,48	5,84
3	0,24	0,05	0,78	0,27	5,97	0,38	8,41	0,46	10,17
4	0,17	0,02	0,78	0,21	9,20	0,34	14,55	0,45	19,40
5	0,13	0,013	0,78	0,17	12,80	0,30	22,60	0,43	32,40
6	0,12	0,011	0,78	0,13	11,50	0,27	23,58	0,42	36,68

Having regard to the state of changing of the solar radiation density $\mathring{A}_E$ for the cone solar concentrator with reflective surface (reflectivity factor R=0,8), quantity of reflections $n \leq 3$. For the reflectivity factor R=0,9 $n \leq 4$ and for reflectivity factor R=0,97 $n \leq 5$. Having regard to loss of energy subject to quantity of reflections n of sunbeam it's reasonable to take R=0,8 and n =1, for R=0,9 n=2 and for R=0,97 n=3.

RESULTS AND DISCUSSION

The renewable sources of energy, particularly the use of the electric- magnet solar energy, are gaining the increasing popularity in the world. One of the directions of using of the solar radiation is the use of the cone solar concentrator. The mathematic model of the cone solar concentrator was worked out; it includes energetic, optical geometrical parameters. The said model allows calculating the parameters of the concentrator depending on the applied mass expenditure and the final temperature of the carrier. Using the cone solar concentrator helps to increase the density of the stream of the solar radiation with the number of the reflection of the sun ray from 1 to 4 and the index of reflection from 0,8 to 0,97 up to 16 times. The practical number of reflections is n=3, in which the density of the solar radiation increases up to 8 times, while the loss of the ray energy is 18 %.

In the solar concentrator testing time the water in the accumulator box (volume 65 liters) had been heated up to 95°C for 40 minutes. In winter time with the outside temperature of - 8°C, the temperature of water in the capacity of the accumulator (volume 85 liters) was heated up from 15°C to 80°C. for 120 minutes. We think that the solar concentrator has good perspectives for using it in a system of winter heating of building. For the purpose of improving of developed equipment in Stavropol State Agrarian University the training ground where the concentrator is placed was created which is open for viewers.

CONCLUSION

The solar radiation utilization factor in the cone solar concentrator amounted 75% when for low-profile collector it is only 40%.

Using the cone solar concentrator helps to increase the density of the stream of the solar radiation with the number of the reflection of the sun ray from 1 to 4 and the index of reflection from 0,8 to 0,97 up to 16 times. The practical number of reflections is n=3, in which the density of the solar radiation increases up to 8 times, while the loss of the ray energy is 18 %.

In winter time with the outside temperature of - 8°C, the temperature of water in the capacity of the accumulator (volume 85 liters) was heated up from 15°C to 80°C. for 120 minutes.

The developed cone solar concentrator can be used for heating and hot water supply of individual houses.

The issues of developing of low-power devices which work from renewable sources should be long term scientific program and independent branch of energetic.

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TRADICIONALNI I NEKONVENCIONALNI IZVORI OBNOVLJIVE ENERGIJE

VYACHESLAV NEFEDOV, ANDREY ZEMTSEV

Izvod

Savremeni razvoj izvora energije treba da se zasniva na visoko efikasanoj tehnologiji, koja ne zagađuju okolinu. Netradicionalni izvori energije (geotermalna toplotni izvori, Sunce, vetar, snaga vode, i td.) su ekološki, jer ne zagađuju okolinu. Visoka ekonomska efikasnosti i ekološka svest postaju glavni razlozi za široko korišćenje netradicionalnih energetskih tehnologija u udaljenim regionima sveta. Konstantno smanjivanje mineralnih goriva na Zemlji i perspektive rasta njihove cena, kao i sve veći

porast rizika od zagađenja životne sredine, usled štetnog energetskog otpada, su razlozi za aktivno iznalaženje i razvoj alternativnih izvora energije. U ovom trenutku svetska zajednica je prepoznala da u narednih nekoliko godina, a možda, i tokom ovog veka, neće uspeti otkriće i razvoj potpuno novog izvora energije. Zato su glavne nade i najviše planova, o razvoju svetskog energetskog kompleksa, usmereni na obnovljive izvore energije, uključujući geotermalne izvore, energiju sunca, vetra i bio-mase.

Ključne reči: energetika, snabdevanje energijom, obnovljivi izvori, električna i toplotna energija.

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INFESTATION WITH SMALL (*DICROCOELIUM DENDRITICUM*) AND LARGE FLUKE *(FASCIOLA HEPATICA)* IN TWO DEER HUNTING GROUNDS IN THE NORTH-WEST OF AP VOJVODINA (SERBIA)*

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SUMMARY: Fluke (fascioloides) is a parasitic liver disease that infests deer in the flatland forest and wetland plains hunting areas, rarely in the mountains. The causes of this disease are two types of liver fluke which belong to a group of flatworms: the large fluke (Fasciola hepatica seu Distomum hepaticum) and a small fluke (Dicrocoelium dendriticum). The aim of this study was to determine: (a) the impact of disease progression on the deer population, (b) optimal methods of drug treatment, (c) the number of infested deer in population after continuous drug treatment over several years and (d) the impact of this deer disease on the quality of tourism offer in the area of the Special Nature Reserve "Gornje Podunavlje". The hunting experts estimate, based on established health condition of the hunted deer, that fluke infestation has steadily increased from initial 20% up to 90%. Fluke has, undoubtedly, contributed to the overall health status deterioration of deer population, with very high mortality rate. The situation has significantly changed in 2006, when the deer were treated with an anti-parasitic preparation (Albendazole), mixed in concentrated food and salt. This treatment proved to be a very successful therapy for Fluke disease.

Key words: *large fluke, small fluke, American fluke, hunting ground, deer.*

Original scientific paper / *Orignalni naučni rad*

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INTRODUCTION

Fluke (*fascioliasis*) is a parasite which influences liver disease, which occurs in deer in flatland forest and wetland plain hunting areas, rarely in the mountains. The causes of this disease are two types of liver fluke which belong to a group of flatworms: the large fluke (*Fasciola hepatica seu Distomum hepaticum*) and a small (or lancet) fluke (*Dicrocoelium dendriticum*).

In situation of a liver fluke invasion in a small number, especially in case of good shape adult deer, clinical signs of disease were not observed. In the cases of a strong invasion on adult deer, the disease has a chronic course. Clinical signs of illness cannot be noticed at the time of lush vegetation, but the signs become visible in the months when there is a shortage of food. In these cases, the infested animals were thin, manifested by general weakness (due to anemia), changing hair more slowly, followed by the lower development and also signs of deformation of antlers. Later, with improved nutrition and better pasture, the condition improved. In case of a strong invasion at young deer, the disease has an acute course and often leads to death (Florijančić et al., 2011). Preventive measures for this disease are difficult to implement in practice (field reclamation, drainage, bar reclamation, dispensing chemicals for the destruction of pastures snails, etc.). Therefore, a method of prevention and disease control in areas contaminated with fluke is shooting weak animals and animals suspected to be diseased. By this method, the animals that are a potential source of fluke infestations are eliminated from the hunting area, and deer individuals of good body shape and good health are kept. Antihelmintic preparations are usually performed for fluke therapy in deer populations, by individual or group animal treatment (Foreyt and Todd, 1976; Qureshi et al., 1994; Janicki et al., 2005).

The aim of this study was to determine: (a) the impact of disease progression in the deer population, (b) results of group method treatment with Albendazole preparation, during several years and (c) the impact of this deer disease on the quality of tourism offer in the area of the Special Nature Reserve "Gornje Podunavlje" (North-West AP Vojvodina, Serbia).

MATERIAL AND METHOD

The Special Nature Reserve (SNR) "Gornje Podunavlje" includes two hunting grounds: "Apatinski rit", Apatin and "Kozara", Backi Monostor, located in the North-West area of AP Vojvodina (Serbia). Hunting areas are situated in the municipalities of Apatin and Sombor (Tomic et al., 2004; Stojanovic, 2005; Stojanovic, 2002). The hunting ground "Apatinski rit" occupies the area of 6579ha in a typical wetland environment by the river Danube and is completely enclosed. The main big game species are deer and wild boar, and a roe deer as an accompanying species. This hunting area is a lowland type, with the altitude from 82 to 89 m (Ristic, 2006; Ristic, 2006).

The hunting ground "Kozara" occupies the area of 11,764 ha and is completely enclosed. The main types of big game in this hunting ground are deer and a wild boar, accompanied by a roe deer (Novakovic, 1999).

Determination of fluke presence and its causes in the mentioned hunting areas took place from 1996- 2009. In order to determine the presence of the liver fluke, examination of the liver and bile ducts was carried out on all hunted deer (Cervus elaphus

L.). Detection of liver fluke eggs in feces is a good diagnostic method, but is not reliable in terms of rating the degree of therapeutic effect of applied drugs (Foreyt and Todd, 1976).

Fluke therapy began in 1997, followed by comparative analysis of the number of infected animals, cured animals and animals without infestations. The therapy was performed by antihelmintic preparations, based on Albendazole. The treatment was performed with doses of 5 mg Albendazole per 1kg of body weight/daily, by mixing adequate amount of preparation in deer concentrated feed and salt.

In order to determine the presence of various developed young (cocoon) and grown up (adult) forms of liver fluke, pathomorphological examination of the studied deer liver was conducted. Finding thickened bile ducts, presence of scar tissue, cysts or other pathomorphological changes in the liver, yet not finding any parasites, was considered as the healing of fluke. Pathomorphological changes (edema, ascites, etc.) on mucous membranes and abdominal handkerchief (omentum) were recorded, too. These pathomorphological findings are not presented in this paper.

RESULTS AND DISCUSSION

The large American liver fluke (*Fascioloides magna*) from the group of large liver flukes is present in the hunting ground within the area of SNR "Gornje Podunavlje". Pathomorfological liver examination of the hunted deer shows that deer populations from the region of SNR "Gornje Podunavlje" intensively suffer from parasitosis caused by the infection of the large liver fluke. Fluke occupancy was first recorded in serious deer numbers during 1996/97 hunting season. Treatment started immediately. From 1997 to 2006 various antiparasitics (Vermitan, Clozan) were used, mixed with concentrated nutrients but with very little or no results.

According to hunting experts' estimation, based on established health condition of hunted deer, fluke occupancy has steadily increased over time, from initial 20% to 90%. Fluke had, undoubtedly, a significant impact on the deterioration of the overall health status of deer population, with very high mortality. The situation significantly changed in 2006 because antiparasitics treatment of the entire population started with implementation of antihelmintic preparation Albendazole, which was mixed in the concentrated feed and salt. This method, undoubtedly, gave very good results after deer treatment in the hunting grounds "Kozara" and "Apatinski rit" (Table 1 and 2)

Table 1. Results of deer treatment with Albendazole in the hunting ground "Kozara"
Tabela 1. Rezultati tretmana jelena sa Albendazolom u lovištu "Kozara"

Year *Godina*	Shot *Ulovljeno* (n)	Infested *Infestirano* (%)	Cured *Izlečeno* (%)	Without fluke *Bez metilja* (%)
2004/05	505	-	-	-
2005/06	513	-	-	-
2006/07	345	75	5	20
2007/08	203	35	30	35
2008/09	244	30	30	40
2009/10	314	15	25	60

Table 2. Results of deer treatment with Albendazole in hunting ground "Apatinski rit"
Tabela 2. Rezultati tretmana jelena sa Albendazolom u lovištu "Apatinski rit"

Year *Godina*	Shot *Ulovljeno* (n)	Infested *Infestirano* (%)	Cured *Izlečeno* (%)	Without fluke *Bez metilja* (%)
2004/05	137	36	28	36
2005/06	148	99	1	0
2006/07	115	-	-	-
2007/08	23	17	83	0
2008/09	90	0	13	87

After the usage of Albendazole, the number of infested individuals decreased from 99% to 0% in the hunting ground "Apatinski rit", and from 75% to 15% in the hunting ground "Kozara". The effectiveness of the fluke therapy in white-tailed deer, with preparations based on Albendazole, was scientifically researched in the USA by Quershi et al. (1990). These authors found that Albendazole treatment effectively inhibits the development of liver fluke eggs in the digestive tract and reduces their number in feces, also causing significant mortality of adult form of parasites in the deer body. Treatment success was around 85% of the total number of treated animals.

Treatment of animals infested by various types of liver fluke is being successfully carried out by individual or group animal antihelmintic treatment (Janicki et al., 2005). Some products that are used in the treatment of liver fluke are shown in Table 3.

Table 3. A review of some antihelmintic preparations used in fluke treatment
Tabela 3. Pregled nekih preparata antihelmintika, koji se koriste u tretmanu metilja

Active ingredient *Aktivna supstanca*	Trade name *Trgovački naziv*	Dose / *Doza* (mg/kg-1)	Remark / *Napomena*
Bis-hydroxy-3,5-dichlorfenyl-sulfoxid	BHS	50	No impact on the juvenile stages *Nema efekta na razvojne forme*
Albendazole	Valbazen, Vermitan	8.5-16.5	Has an impact only on the adult stages (82-84%) *Ima efekta samo na adultnu formu*
Diamphenethid	Coriban	140	Has an impact only on the juvenile stages *Ima uticaja samo na razvojne forme*
Rafoxanid	Ranide	10-15	Dubious results / *Neizvesni rezultati*
Triklabendazol	Fasinex	10	Has an impact only on the juvenile stages *Ima uticaja samo na razvojne forme*
		50-60	Has an impact only on the juvenile stages *Ima uticaja samo na razvojne forme*

Source / *Izvor:* Florijančić et al. (2011).

Individual treatment is the most effective method of fascioliasis treatment (therapy). Anti-trematode preparations are applied in the rumen (intrarumenally) by using probe to a previously calmed animal (application of sedative). Thereafter, the treated animals should be kept in quarantine for 30 days until liver fluke eggs are fully extracted through feces (Florijančić et al., 2011). However, this method is quite difficult to perform in practical terms, especially when performed on adult deer. Namely, capture, sedation and placement of the probe intrarumenally are very risky procedures for the animal as well as for people who perform this procedure.

Group therapy of the entire population of deer is performed by mixing anti-trematode drugs in concentrated nutrients. However, success of this method greatly depends on whether the deer consume sufficient amounts of offered concentrated feed (Qureshi et al., 1994). Namely, in the absence of food in an open hunting ground, deer visiting feeding places with concentrated feed more frequently so they consume the required dose of medicine, and vice versa (Florijančić et al., 2011).

The success of an individual, and particularly group treatment, with antiparasitics application in nutrients depends on whether the deer will take this food, because taste and smell can be changed due to these medicaments. Therefore, chemical substances are added in, which, more or less successfully, neutralize a distinct taste and fragrance of medicaments. In any case, the effectiveness of group therapy treatment is, however, significantly lower than that achieved by the method of individual therapy. One of the measures to prevent the fluke infestation is extinction of water (pond) snails, which are transient host of liver fluke in its development cycle. For this purpose wetlands are treated with various molucid preparations. However, this method of prevention is not recommended because of the potential negative effects on the environment. Moreover, avoiding feeding grounds with proven presence or reasonable suspicion of the liver fluke presence can also be used as a measure of the prevention. Also, hay or other nutrients from such places should not be used to feed the wild and domestic animals. To prevent and fight liver fluke, it is necessary to have continuous veterinary control of all hunted deer (Corn and Nettles, 2001; Konjevic et al., 2002).

In European hunting grounds, infestation of deer by a large American liver fluke (*Fascioloides magna*) is more and more frequently recorded. Thus, the infestation of deer by a large American liver fluke was found in Croatia (Baranja) in 2000. It is assumed that the parasites enter into this area through the natural migration of deer from neighboring Hungary (Marinculic, et al., 2002). The occurrence and treatment of this disease, in the same region of Croatia, was more studied later by Janicki et al. (2005). They have achieved good results of treatment, using antihelmintic treating of individual animals as well as group treatment in open hunting grounds. The presence of this type of liver fluke is noticed also in deer in hunting areas around the river Danube in Austria. Antihelmintic treatment was successful, but failed to complete eradication of this disease (Usprung et al., 2006). Pathogenesis of large American liver fluke, and the pathological changes caused in the liver, lungs, diaphragm and organs of the digestive tract, are described in detail in Presidente et al. (1980).

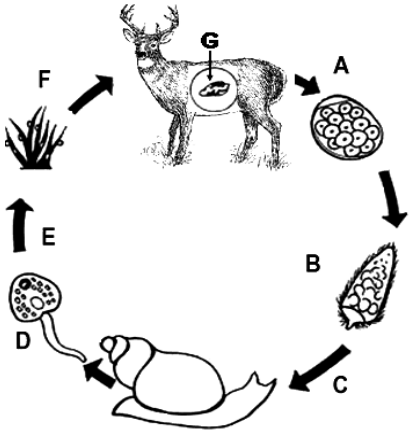
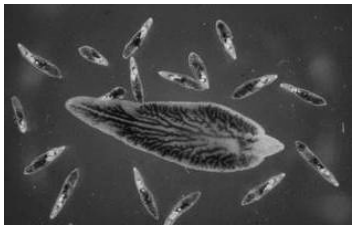


Photo 1. Life cycle of a large fluke *(Fasciola hepatica)*
Slika 1. Životni ciklus velikog metilja (Fasciola hepatica)

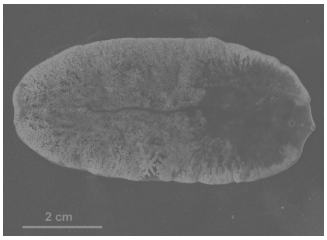
A - Eggs passed in faeces / *Jaja se izbacuju fecesom*; B – Miracidium released / *Oslobađa se Miracidium*; C – Miracidium invades snail (intermediate host) / *Miracidium invadira puža (prelazni domaćin)*; D – Cercariae leave snail / *Cerkarija napušta puža*; E – Cercariae swim until encyst on vegetation, forming metacercariae / *Cerkarija pliva, dok se ne prihvati za vodeno bilje, gde formiracistu, matacerkariju*; F – Metacercariae ingested by Deers, or other wild or domestic animals / *Metecerkariju pojede jelen ili neka druga divlja ili domaća životinja*; G-Adult fluke in liver / *Adultni oblik metilja u jetri*.

A

B



B1



C

Photo 2. A - Fasciola hepatica; B - Dicrocoelium dendriticum; B1 – Large and small fluke (size comparation);
C – Fascioloides magna (American large liver fluke)

Slika 2. A - Fasciola hepatica; B - Dicrocoelium dendriticum;B1 – Veliki i mali metilj (kompa-racija veličine);

C - Fascioloides magna (Američki veliki jetrin metilj)

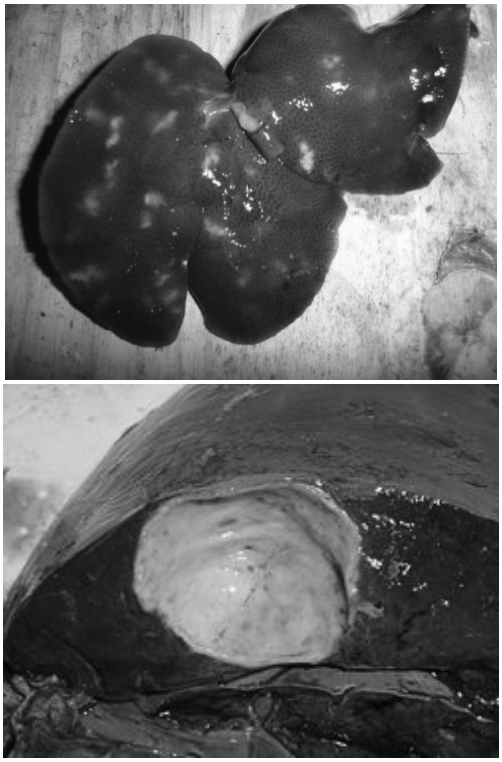


Photo 3. The fibrous capsule in the liver parenchyma of red deer

Slika 3. Fibrozna kapsula u parenhimu jetre crvenog jelena

CONCLUSION

The last few years, it was noticed that in the hunting grounds of the Special Nature Reserve “Gornje Podunavlje”, “Apatinski rit”, Apatin and Kozara “Backi Monostor”

there was a steady increase in deer infested with large liver fluke (*Fasciola hepatica still Distomum hepaticum*) and small liver fluke (*Dicrocoelium dendriticum*).

This has resulted in significant damage in natural and economic terms, due to reduced quality of deer offer in hunting tourism.

By applying the continuous group therapy treatment in deer population in these hunting grounds, by applying antiparasitics Albendazole concentrated in nutrients and salt, very high rate of heeling after treatment has been achieved.

In order to fight this disease, therapeutic and preventive measures are required in all affected areas, especially in deer hunting grounds. In this regard, it is necessary to continuously monitor epizootiological situation in hunting grounds, veterinary examination of the liver and other internal organs of each hunted deer.

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INFESTACIJA JELENA MALIM (*DICROCOELIUM DENDRITICUM*) I VELIKIM METILJEM *(FASCIOLA HEPATICA)* U LOVIŠTIMA SEVERO-ZAPADNE AP VOJVODINE (SRBIJA)

ZORAN A. RISTIĆ, JELENA APIĆ, DRAGAN BOŽIĆ, MARKO CINCOVIĆ

Izvod

Metilji (*fascioloides*) su paraziti, koji prouzrokuju bolest jetre jelena, u lovištima niskih šuma i močvarnog zemljišta, a ređe u planinskim lovištima. Obolenje prouzrokuju dva tipa metilja, koji spadaju u plosnate crve: veliki metilj (*Fasciola hepatica* seu *Distomum hepaticum*) i mali metilj *(Dicrocoelium dendriticum*). Cilj ovog rada je da se ustanovi: (a) uticaj ovog obolenja na populaciju jelena, (b) optimalne metode tretmana lekovima, (c) broj infestiranih jelena posle continuiranog tremana lekovima, tokom nekoliko godina i (d) uticaj ovog obolenja na kvalitet turističke ponude u specijalnom rezervatu prirode »Gornje podunavlje«. Na osnovu ustanovljenog zdravstvenog stanja ulovljenih jelena, eksperti su ustanovili da se infestacija stalno povećava, sa početnih 20« do 90%. Metiljavost, nedvosmisleno, utiče na pogoršanje opšteg zdravstvenog stanja, sa visokom stopom smrtnosti. Situacija se značajno poboljšala od 2006. godine, kod jelena koji su bili lečeni dodavanjem antiparazitskog preparata (Albendazole) u hranu i so. Ovaj tretman se pokazao vrlo uspešnim.

Ključne reči: veliki metilj, mali metilj, američki metilj, lovište, jelen.

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THE EFFECTS OF COMPLETE MIXTURES FROM VARIOUS DOMESTIC PRODUCERS IN NUTRITION OF GROWING PIGS

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SUMMARY: The effects of nutrition using complete mixtures from five producers on production performance, digestibility of nutrients and cost of 1 kilogram of gain in nutrition of growing pigs were investigated. Obtained results showed that: the best pig production is realized using control mixture A, slightly lower results with diet B, followed by mixtures C, D and E. Degree of utilization of dry, organic matter and protein was best in diets A and B, and slightly lower in remaining C, D and E diets. The cheapest gain was realized by using mixtures A and C, followed by mixture B, and the least favourable cost of gain was recorded in pigs fed diets D and E, without any established differences between them. In general, obtained results showed that by using the mixtures from various manufacturers certain differences in production performance were established, also in the degree of utilization of nutrients and cost of kilogram of gain in growing pigs.

***Key words**: quality of mixtures, various producers, pigs, growth.*

INTRODUCTION

For extensive, efficient and economical production, as well as preservation of pig health, the requirements for about 40 different nutrients, where in addition to energy, also requirements for 10 amino acids, 15 vitamins, 15 mineral substances have to be fulfilled, and also use of additives is desirable. One part of the nutrient requirements animals meet by consuming natural food, but some have to be added in form of synthetic substances.

Previous researches of the quality of vitamin-mineral pre-mixtures showed the

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Original scientific paper / *Originalan naučni rad*

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presence of differences in production of sows and piglets (Živković et al., 2010) and fattening pigs (Živković et al., 2010).

Objective of this paper was to study comparatively quality properties of complete mixtures from various manufacturers in nutrition of growing pigs.

MATERIAL AND METHODS

The study which included total of 108 growing pigs of Swedish landrace breed, and initial body mass of approx. 30 kg, was carried out on Experimental pig farm of the Institute for Animal husbandry, Belgrade-Zemun.

Based on standard criteria, origin, sex and initial mass, piglets were distributed in groups taking into consideration the uniformity of their age and body masses, and also to avoid placing siblings in same groups. Every group contained 24 animals, with even sex ratio.

Animals were fed flour mixtures and had access to water ad libitum. First, control group, was fed farm mixture produced in the feed mill of the Institute for Animal Husbandry. The remaining four groups of animal(the exceptio for E group)s were fed mixtures produced by four different animal feed producers in Serbia.

In addition to production performance, in the trial also the utilization of nutrients in pig nutrition was studied. Digestibility of nutrients was analyzed using the direct method with Cr_2O_3 markers, when pigs reached body mass of around 50 kg.

In evaluation of obtained results the following parameters were considered: average daily gain, use of food per feeding day and per animal and use of food per 1 kg of gain, utilization of dry, organic matter, ash, crude proteins, ether extracts, fibre and NFE (nitrogen free extracts) in the diet. At the end of fattening, based on the cost of mixtures and realized feed conversion ratio, the cost of 1 kilogram of gain of pigs in trial was calculated.

Obtained pig growth results, coefficients and digestibility of nutrients were statisticaly processed by variance analysis and average values, by using t-test.

RESULTS AND DISCUSSION

In the trial, the effect of nutrition using the mixtures from five animal food manufacturers on production performance, digestibility of nutrients and the cost of kilogram of gain in growing pigs was studied.

a) performance

Obtained results (tab. 1) showed that the first, control group of pigs (producer A), in the initial fattening period, realized the best daily gain, with good daily food intake and the lowest use of food per 1 kg of gain. Use of other mixtures lead to lower gain, in average by 8.8% in case of food producer B, by 12.6% in producer C, by 24.5% in producer D ($P<0.01$). The lowest gain was recorded in group of pigs fed mixture from E producer, which was by 25.4% lower compared to the control mixture.

Table 1. Performance of growing pigs in the experiment
Tabela 1. Proizvodni pokazatelji kod svinja u porastu u eksperimentu

	Growing Pigs, 30-55 kg/*Svinje u porastu, 30 – 55 kg*				
Group/*Grupa*	1 control/ *kontrola*	2	3	4	5
Feed producer/Proizvođač hrane	A	B	C	D	E
Av. daily gain/*Pros dnevni prirast*, %	100.0^{A*}	- 8.8^{b}	- 12.6	- 24.5^{Ab}	- 25.4
Daily Feed Intake/*Dnevna konzum*., %	100,0	+ 11.0	+ 9.8	0	- 7.5
Feed conversion/*Konverzija hrane*, %	100.0	+ 23.1	+ 26.3	+ 20.2	+ 21.5

* The small letter over the average value designate the statistical difference on the level P<0.05, and the big one on the
level P<0.01/*Ista mala slova iznad redova označavaju statistički značajnu razliku na nivou P<0.05, a velika slova na nivou P<0.01.*

Animals fed mixtures produced by B and C producers consumed by 11.0% and 9.8%, respectively, more food than the control group and the group of pigs fed mixture D, for which no differences were established. Lower food intake by average 7.5% compared to the control group was established in the group of pigs fed mixture of the producer E.

The best feed conversion ratio was realized by animals fed the control mixture, whereas the remaining four pig groups consumed from 20.2 to 26.3 (respectively 20.2% -D, 21.5% - E, 23.1% - B and 26.3% - C producer).

b) digestibility of nutrients

Table 2. Digestibility of nutrients in the experiment, %
Tabela 2. Stepen iskorišćvanja hranljivih materija u eksperimentu, %

	Growing Pigs, 30-55 kg/*Svinje u porastu, 30 – 55 kg*				
Group/*Grupa*	1 control/*kontrola*	2	3	4	5
Feed producer/Proizvođač hrane	A	B	C	D	E
Dry matter/*Suva materija*	75.9	77.4	75.2	74.4	72.5
Organic matter/*Organska materija*	80.8	81.2	78.6	77.9	77.0
Ash/*Pepeo*	27.0^{ABC*}	38.6^{A}	24.8^{BDE}	34.6^{DF}	16.7^{CEF}
Crude protein/*Sirovi protein*	72.3^{a}	72.1	70.3^{b}	70.6^{C}	62.7^{abC}
Ether extract/*Sirove masti*	64.5^{ab}	66.1^{c}	71.3	72.1^{ad}	77.7^{bcd}
Fiber/*Celuloza*	43.1^{A}	47.3^{B}	29.4^{C}	45.1^{ABCd}	35.3^{d}
N. F. E./*B. E. M.*	85.8^{a}	87.9^{aBC}	86.3^{BD}	84.2^{CD}	85.4

*The small letter over the average value designate the statistical difference on the level P<0.05, and the big one on the level P<0.01/*Ista mala slova iznad redova označavaju statistički značajnu razliku na nivou P<0.05, a velika slova na P<0.01.*

Degrees of utilization of dry and organic matter, as well as proteins, were according to following order: animal food producers A and B followed by C, D and E (tab. 2). In regard to ether extracts, the utilization was according to following order: E, D, C, followed by A and B without established differences. In regard to ash, the best use was recorded in group of pigs fed diet D, followed by pigs fed diets B, A, C and E, and in fibre, the order was following: B, D, A, E and C. Nitrogen free extracts were best used

by pigs fed diet B, followed by C, and A and E without significant differences, and the lowest degree of utilization of these nutrients was determined in mixture D.

c) economical indicators

The calculation of cost of mixtures showed (tab. 3) that the mixtures C, B and D were cheaper and the mixture E more expensive than the control diet. Taking into consideration the food intake per 1 kg of realized body gain, it was established that the the cost of 1 kg of gain was the most favourable when the diet A was used (control), and slightly more expensive gain (by 1.1%) when the diet C was used, whereas the remaining mixtures, B – by 17.1%, E – by 23.2% and D – by 23.8% caused increase of cost of gain compared to the cost of gain in animals fed the control mixture.

Table 3. The price of 1 kg gain of growing pigs in the experiment
Tabela 3. Cena 1 kg prirasta kod scinja u porastu u eksperimentu

	Growing Pigs, 30-55 kg/*Svinje u porastu, 30 – 55 kg*				
Group/*Grupa*	1 control/*kontrola*	2	3	4	5
Feed producer/Proizvođač hrane	A	B	C	D	E
The price of mixture/*Cena smeše,* %	100.0	- 4.9	- 20.0	- 3.0	+ 1.4
Feed conversion/*Konverzija hrane, %*	100.0	+ 23.1	+ 26.3	+ 20.2	+ 21.5
The price of 1 kg of gain/ *Cena 1 kg prirasta,* %	100.0	+ 17.1	+ 1.1	+ 23.8	+ 23.2

In regard to the quality of studied mixtures in fattening pigs, in present research, even though all nutrients are important, the attention of researchers is mainly focused on energy, proteins, vitamins and micro elements, as well as additives. It was established long time ago (Braude et al., 1960) that different proteion levels in the mixtures can have impact on economical results in fattening of pigs. Other studies (Miller and Keith, 2011) do not suggest gradual increase of crude protein content by 17% to 27% in mixtures. It is desirable to combine the positive properties of feeds, for instance soybean meal and fodder peas (Chrenková et al., 2011) or use of sorghum (Hal.e and Lyman, 1961).

It is not necessary to increase protein content in the mixtures (Sokha et al., 2008) but it is necessary to increase dietary amino acid levels in concept with increase in energy content (Chiba et al., 1991; Mitchell et al., 2011). Provided that energy requirements are met, better production in the optimum environment temperatures can be expected (Noblet et al., 1985), and in the mixtures the content of lysine can slightly be reduced (Jin et al., 2010) with maximum share of this amino acid in synthetic form of up to 0.3% in the mixtures (Hale and Lyman, 1061). Interaction between energy level and sex of fatteners was observed for voluntary feed intake, daily gain and final body weight (Fagunes et al., 2009). Role and importance of the quality of vitamin- mineral pre-mixtures (Zlatić, 1983; Živković and Kovčin, 1983) depend on the genetic potential, housing and environment conditions, health, etc. (Uremović et al., 1985; Kovčin , 1992; Kasalica et al., 1995; Pomar et al., 2009). The optimum nutrient density of the feed chosen will depend upon the efficiency of feed utilization, the cost of the feed and the income derived from the end product (Ferguson et al., 1999).

In general, results obtained in this study showed that use of mixtures manufactured by five different producers resulted in differences in the performance, degree of utilization of nutrients and the cost of kilogram of gain of growing pigs.

CONCUSLION

The effects of nutrition of growing pigs with the mixtures produced by five different producers on production performance, digestibility of nutrients and cost of kilogram of gain were studied.

Obtained results showed that:

- The best production is realized in pigs fed control mixture A, slightly lower production with mixture B, followed by mixtures from producers C, D and E.
- Degree of utilization of dry organic matter and proteins was the best in mixtures A and B, and slightly lower in remaining mixtures C, D and E.
- The cheapest gain was realized in pigs fed mixtures A and C, followed by mixture B, and the least favourable cost of gain in pigs was recorded in group of pigs fed mixtures D and E, without any established differences between the two groups.

In general, results obtained in this study showed that use of mixtures manufactured by different producers resulted in differences in production performance, degree of utilization of nutrients and cost of kilogram of gain of growing pigs.

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EFEKTI POTPUNIH SMEŠA RAZLIČITIH DOMAĆIH PROIZVOĐAČA U ISHRANI SVINJA U PORASTU

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Izvod

Ispitivani su efekti ishrane smešama od pet proizvođača na proizvodne rezultate, svarljivost hranljivih materija i cenu kilograma prirasta u ishrani svinja u porastu. Dobijeni rezultati su pokazali da se: najbolja proizvodnja kod svinja postiže ishranom kontrolnom smešom A, nešto slabiji obrokom B, pa smešama C, D i E proizvođača. Stepen iskorišćavanja suve, organske materije i proteina je bio bolji kod obroka A i B a nešto slabiji kod preostalih C, D i E obroka. Najjefiniji prirast je ostvaren kod A i C smeša , zatim sledi kod smeše B a najnepovoljije cenu prirasta svinje su bile na obrokcima D i E između kojih nije bilo razlika. U celini dobijeni rezultati u ovom radu su pokazali da se, korišćenjem smeša različitih proizvođača u Srbiji ostvaruju razlike u proizovodnim rezultatima, stepenu iskorišćvanja hranljivih materija i ceni kilograma prirasta svinja u porastu.

Ključne reči: kvalitet smeša, razni proizvođači, svinje, porast.

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THE STABILIZATION AND ASSOCIATION AGREEMENT: THE IMPACT ON THE IMPORT AND EXPORT O F FISH AND FISH PRODUCTS*

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SUMMARY: The research of the economics of fisheries in Europe has long been a topical issue, while in Serbia a small number of papers are dedicated to this subject. In this paper the import and export of fish and fish products are being analyzed for the period from 2006 to 2010 in Serbia, as well as the effect of the Agreement on Stabilization and Association between the EU and Serbia on the import and export of fish and fish products. Standard statistical methods are used for the analysis such as: calculation of average values and calculation of indices.

Key words: *The Stabilisation and Association Agreement, fish, import, export.*

INTRODUCTION

The Stabilisation and Association Agreement (SAA) is an international treaty, signed on the 29th April 2008 between the Republic of Serbia and the European Union (EU). Two most important obligations for the Republic of Serbia will be the establishment of free trade and harmonization of the legislation of the Republic of Serbia with the EU. The agreement creates free trade between Serbia and the EU for a transitional period of six years. The deadline for the liberalization of trade is determined in accordance with the capacity of Serbian industry and agriculture to adapt to free trade, but

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also with Serbia's desire for faster completion of reforms and accession to the European Union. Serbia's obligation consists of the gradual abolition of tariffs on imported goods originating in the EU for a transitional period. On the other hand, with this agreement European Union confirms free access to goods from Serbia to the EU market. The pace of liberalization and the degree of protection depends on the degree of sensitivity of products to the industry of Serbia. Three groups of industrial products were defined based on the sensitivity, whose liberalization will be achieved after a period of two, five or six years. For products which are not on the lists, duty will be abolished at the moment the agreement enters into force. The SAA also foresees the obligation of the Republic of Serbia to harmonize domestic legislation according to the agreed timetable with the regulations that fall under the EU acquis. The priority areas that have direct impact on creating free trade zone between the EU and Serbia were determined in relation to the scope of EU legislation: protection of competition and control allocation of state subsidies, intellectual property rights, public procurement, standardization and consumer protection.

The Serbian Parliament ratified on 9th September 2008 the Stabilization and Association Agreement (SAA) and Interim Agreement. The SAA and the Interim Agreement were signed on 29th April 2008 in Brussels. Since the 1st of February 2009, Serbia has been unilaterally implementing the Interim Trade Agreement.

With the ratification of the Stabilization and Association Agreement (SAA) Serbia became an associate member of the European Union (not an EU member state!). So far, the SAA has been ratified by 17 EU member states and the European Parliament and it is expected that further 10 EU member states will ratify this Agreement. Although the content of this agreement is mixed, it is essentially a trade agreement because the greatest effects are made by creating free trade zone, that is being created through gradual elimination of all tariffs on industrial goods and almost all tariffs on agricultural products, and gradual removal of non-tariff barriers to trade. In this sense, it is important that the agreement is indefinite.

MATERIALS 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, where the data about the import and export of fish and fish products are taken from. In this paper the standard statistical methods, such as the calculation of average values and calculation of indices are being used.

This paper will explore the impact of the Stabilization and Association Agreement (SAA) on the import and export of fish and fish products.

RESULTS AND DISCUSSION

Free trade involves the trade without custom duties and quantitative restrictions except in cases authorized by this agreement. The agreement provides that all restrictions on exports to the EU are immediately abolished (with some exceptions), while imports are to be liberalized within 5 years. Within this period tariffs of industrial products will be reduced in stages, so that in the sixth year they are zero, while some agricul-

tural products will remain with a certain tariff protection, but significantly smaller.

All industrial products are divided into four groups: insensitive, sensitive, very sensitive and the most sensitive products, and each group has its own dynamics of liberalization. The grouping was done according to the current level of tariff protection, the economic, fiscal and social effects of liberalization, the importance of the sector, etc. On the other hand, each tariff line of agricultural products has its own dynamics and the „bottom" liberalization is applied.

ARTICLE 33

Protection of geographical indications for agricultural, fish and food products, with the exception of wine and spirits

- Serbia shall ensure the protection of appellations of origin community registered in the Community by Council Regulation (EC) No. 510/2006 from 20th March 2006 on the protection of geographical indications and designation of origin for agricultural products and food in accordance with the provisions of this article. Geographical indications from Serbia can be registered in the Community under the terms of the above-mentioned regulation.

DUMPING AND SUBSIDIES

- Nothing in this Agreement shall prevent any party to take defensive trade measures in accordance with paragraph 2 of this article and article 41.
- If one of the parties establishes that the trade with the other side implements dumping and/or subsidies on the basis of which it is possible to introduce compensatory measures, that party may take appropriate measures against this practice in accordance with the WTO Agreement on Implementation of Article VI of GATT 1994.

It is characteristic that fishery accounts for less than 10 % of the GDP of agricultural and fishery activities (Marković and Jovanović, 2010). There are large possibilities for our country to develop its fisheries. Domestic production of fish is inadequate, despite the favourable bio-ecological characteristics of our region (Miščević and Ćirković, 2008). During the period of ten years (2001-2010) the fish production recorded the increase of 11.51% (Marković et al, 2011).

The total value of imports of fresh fish and fish products in recent years is characterized by enormous growth. The total imports in 2006 were 41 million US dollars while in 2010 it was increased and was 50 million US dollars (table 2). Almost half of the imported fish in Serbia was hake which are usually imported from Argentina and Norway (Miščević, 2004). However, the situation has drastically changed in 2008 when our country began to intensively import fish from Vietnam. In 2010 the import of fish from Vietnam twice surpassed imports from Argentina. There are no drastic changes in imports from EU countries in relation to total imports and it is in the range of 18-20%. It means that the unilateral application of the SAA has not brought the expected changes.

The import of the following products into Serbia originating in the Community is subject to the following concessions (table 1).

Table 1: Concession on Serbian fish products originating in the EU
Tabela 1: Koncesije Srbije na riblje proizvode poreklom iz EU

Tariff code / *Tarifna oznaka*	Nomination / *Naimenovanje*	Tariff rate / *Carinska stopa* (% of MFN)					
		2008	2009	2010	2011	2012	2013th and years to come
0301	Fish, alive:						
	Other live fish:						
0301 91	Trout (*Salmo trutta, Oncorhynchus mykiss,*						
0301 91 90	Other	90	75	60	40	20	0
0301 92 00	Eel (*Anguilla spp.*)	90	75	60	40	20	0
0301 93 00	Carp	90	85	80	75	65	60
0301 99	Other:						
	Freshwater fish :						
0301 99 19	Other	90	75	60	40	20	0
0302	Fish, fresh or chilled, excluding fish fillets and other fish meat of tariff code 0304:						
	Salmonidae, excluding livers and roes:						
0302 11	Trout (*Salmo trutta, Oncorhynchus mykiss,*	90	75	60	40	20	0
0302 69	Other:						
	Freshwater fish:						
0302 69 11	Carp	90	75	60	40	20	0
0302 69 19	Other	90	75	60	40	20	0
0302 70 00	Livers and roes	90	75	60	40	20	0
0303	Fish, frozen, excluding fish fillets and other fish meat of fariff code 0304:						
0303 21	Trout (*Salmo trutta, Oncorhynchus mykiss,*	90	75	60	40	20	0
0305	Fish, dried, salted or in brine;	90	75	60	40	20	0
1604	Prepared or preserved fish, caviar and caviar substitutes	90	75	60	40	20	0

Source: "Official Gazette of the Republic of Serbia", No. 90/2010

Table 2: Import of fish and fish products
abela 2: Uvoz ribe i proizvoda od ribe

(000 US dollars)

Year *Godina*	Import from EU *Uvoz iz EU*	Index *Indeks*	Import total U*voz ukupno*	Index *Indeks*	Share of imports from EU in total imports *Zastupljenost uvoza iz Eu u odnosu na ukupan uzvoz* (%)
2006	7486	100	41210	100	18
2007	10715	143	51024	124	21
2008	11133	149	57290	139	19
2009	11172	149	57755	140	19
2010	9278	124	50518	123	18

Source: Statistical Office of Serbia

Fish export emphasizes the production of fish and the necessity of creating conditions to increase the prior minor export. In relation to this it is especially important with

certain systemic measures of economic policies to create the conditions for activating the export of fish and fish products. Export is an expensive affair and it is necessary that the state supports it (Miščević et al, 2009). Fish import is increasing from year to year and it is necessary to increase production in order to mitigate the extremely high import of fish. It is also necessary to conduct market research as a necessary precondition for making timely and rational marketing decisions in the market economy. It is not limited exclusively to the problems of placement of manufactured goods. It is also important in terms of the long-term orientation of producers to those products that can bring the greatest profits. It is also necessary to adapt to the consumer's demand (Vlahović, 2004).

Table 3: Export of fish and fish products
Tabela 3: Izvoz ribe i proizvoda od ribe

(000 US dollars)

Year *Godina*	Export to EU *Izvoz u EU*	Index *Indeks*	Export total *Izvoz ukupno*	Index *Indeks*	Share of exports to EU in total exports *Zastupljenost izvoza u Eu u odnosu na ukupan izvoz* (%)
2006	626	100	863	100	72
2007	145	23	1289	149	11
2008	123	20	1361	158	9
2009	72	11	991	115	7
2010	291	47	1053	122	28

Source: Statistical Office of Serbia

The total export value of fish and fish products has increased in recent years. In 2006 it was 863,000 US dollars and in 2010 1.053 million US dollars (table 3). Potential exporters are facing many problems. One of them is that the Serbian Government did not include fish and fish products in the Decree on the use of funds to encourage exports of agricultural and food products. With the unilateral implementation of the SAA in the last four years it increased it portion in exports to the EU from 9% to 29%.

The export of the following products originating in Serbia to the EU will be subject to these concessions (table 4).

Table 4: EU concessions on the Serbian fish products
Tabela 4: Koncesije EU na srpske riblje proizvode

Tariff code *Tarifna oznaka*	Nomination *Naimenovanje*	Since the Agreement entry into force to 31 December of the same year *Od stupanja sporazuma na snagu do 31. Decembra iste godine* (n)	From 1 January to 31 December *Od 1. januara do 31. decembra* (n +1)	For each subsequent year from 01 January to 31 December *Za svaku narednu godinu od 01. januara do 31. decembra*
0301 91 10 0301 91 90 0302 11 10 0302 11 20 0302 11 80 ex 0304 19 911 0304 29 15	Trout (*Salmo trutta, Oncorhynchus mykiss, Oncorhynchus clarki,* alive, fresh or chilled;	Quota: 15 tons at a rate 0% Over the Q: 90% MFN rates	Quota: 15 tons at a rate 0% Over the Q: 80% MFN rates	Quota: 15 tons at a rate 0% Over the Q: 70% MFN rates
0301 93 00 0302 69 11 0303 79 11 ex 0304 19 19 ex 0304 19 91	Carp: live, fresh or chilled, frozen, dried, salted or in brine, smoked, fillets and other fish	Quota: 60 tons at a rate 0% Over the Q: 90% MFN rates	Quota: 60 tons at a rate 0% Over the Q: 80% MFN rates	Quota:60 tons at a rate 0% Over the Q: 70% MFN rates

Source: "Official Gazette of the Republic of Serbia", No. 90/2010

In tab. 4 concessions are given for fish originating from Serbia, primarily related to trout and carp, as well as their products. Quota of 15 t of trout and 60 t of carp should be filled with the support of the state and it has to help export with export subsidies or export additional resources. Large growers (primarily carp) complain about the extremely low duty free quota for this type of fish. However, they are expecting a major return to the markets of Germany, France, Italy, Greece and Israel (Tomić et al, 2010).

CONCLUSION

The Stabilisation and Association Agreement will have its full effect only when ratified by all EU member states. Fisheries are classified as a sensitive area and the protection of domestic production is expected. But the problem of competitiveness of our products on the EU market will still exist, because the fishery in Serbia is not supported by the state. Serbia needs a department for fisheries and an associated fund for fishery development as well as favourable loans to help fish producers to perform equally in the EU market. There are very large opportunities for building carp ponds because there are sufficient waters of acceptable quality, as well as land that is not used for crop production and can be bought at favourable prices.

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SPORAZUM O STABILIZACIJI I PRIDRUŽIVANJU: UTICAJ NA UVOZ I IZVOZ RIBE I PROIZVODA OD RIBE

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Izvod

Istraživanje problematike ekonomike ribarstva 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), ispitivani uvoz i izvoz ribe i proizvoda od ribe, kao i efekti Sporazuma o stabilizaciji i asocijaciji između EU i Srbije na uvoz i izvoz ribe i proizvoda od ribe. Za analizu su korišćene standardne statističke metode, kao što su: prosečna vrednost i indeksi.

Ključne reči: Sporazum o stabilizaciji i pridruživanju, riba, uvoz, izvoz.

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SECONDARY POPULATIONS OF TURKISH HAZELNUT (CORYLUS COLURNA L.)IN NOVI SAD

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SUMMARY: Results of nuts morphological analysis for selected genotypes of Turkish hazel (Corylus colurna L.) refer to secondary origin trees. Seed germination and graft acceptance on seedlings was established for examined genotypes. Dependence correlation of seed germination from examined morphological features was determined. These findings could have significant importance on process of selecting mother plants for production of seedlings and planting material. Selected genotypes from nursery Gradsko zelenilo (A4, A5, A8, A13, A14, A16, B7, C1, C2, C3, C4) had high seed germination and formed thick line composition during examination year. Grafting of genotypes was done at beginning of April year 2011 intent to get high grafted hazel trees. During vegetative period percentage of graft acceptance ranged from 70.00% (genotype C1) to 93.3% (genotype A4) which implies high compatibility of rootstocks with Tonda Gentile Romana cultivar.

Key words: *Corylus colurna, genotype, hazel grafting, population, seed germination, rootstocks.*

INTRODUCTION

Department of Pomology, Viticulture, Horticulture and Landscape Architecture in Faculty of Agriculture, University of Novi Sad, is engaged in hazel selection, breeding and planting material production. Three decades ago, in year 1983 started program of selecting Turkish hazel (*Corylus colurna* L.) genotypes from trees growing in natural populations and trees of secondary origin growing in green areas of Novi Sad. Selection undertook 45 individual Turkish hazels out of which several genotypes were selected.

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Genotypes are characterized with alternative fruiting, high morphological, technological and physiological features of nuts, as well as with excellent medical conditions (Ninić-Todorović, 1990). Biochemical compound of Turkish hazel seed is significant for confectionary industry as shown in publications Ninić-Todorović (1992; 2000).

Sowing material of Turkish hazel examined fruits was planted in Faculty of Agriculture nursery in Rimski Šančevi where ground germination was established, followed with development of seedlings in examination year. Three year old Turkish hazel seedlings were grafted with tongue grafting method, what led to determination of remarkable compatibility and acceptance percentage. High grafted hazel plants are suitable for growing plantations with hazel trees, where full application of mechanization and appropriate protection is possible.

Grafting of hazel cultivars on rootstock of Turkish hazel in Serbia is successfully done since 1989. Results are published in publications Ninić-Todorović et al. (1994; 2003; 2006, 2007), Korać et al. (1995; 1996), Cerović et al. (2007) and others.

Figure 1. Turkish hazel populations of secondary origin in area of Novi Sad
Slika 1. Populacije mečje leske sekundarnog porekla u Novom Sadu

In city area of Novi Sad totally 231 trees of Turkish hazel in park areas are recorded, planted in groups or solitary. On green lines of roads, as line planting, 501 tree of Turkish hazel is noted. Trees are from different age class and size depending on time

of roads building. Tendencies for growing new tree lines of Turkish hazel exist (streets Janka Veselinovića, Pasterova, Ive Andrića, Kolo srpskih sestara, Cankareva, Bulevar oslobođenja). In relation to the total length of tree lines in the city of Novi Sad, which amounts 80 km, Turkish hazel trees cover 5.3 km or 6.7% of the total tree lines length (Ninić-Todorović et al., 2011).

MATERIAL AND METHODS

Turkish hazel fruits were gathered in moment of physiological independence of cupule petiole from mother plant. Indicator for maturity determination is yellow-green color of cupule and light brown color of nuts visible part (Ninić-Todorović, 1990). Pomological testing of samples in groups of 30 fruits determined length, width, thickness and pericarps thickness with micrometer precision of 0.01mm, nut and kernel mass were determined on technical scale with precision of 0.01 g.

Ground nut germination from mother plants in Nursery Gradsko zelenilo was determined in June 2008, and expressed in percentages out of total nuts amount sowed in October year 2007. Graft acceptance of cultivar Tonda Gentile Romana is determined in June year 2011.

In table are presented measuring results, for nuts morphological features, processed by appropriate statistical methods. For variability determination, with each examined feature coefficient of variance (CV) is presented, importance of differences between genotypes was tested with LSD test of statistical program STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA). Internal dependencies correlations were determined between nuts examined features. In table are presented values and simple correlation coefficients (r) between examined morphological traits. Genotype groups were determined by Discriminant Analysis in relation to examined morphometric features and influence on germination.

RESULTS AND DISCUSSION

Turkish hazel genotypes of secondary origin, during testing showed variability of fruits morphological features. Shape of fruit is constant for certain genotypes and directly influenced with heritage features of mother plants. The size of nuts and fruits mass vary over the years, depending on fruiting amount. On fruits size and amount of fruiting influence tree age and volume of tree crown (Ninić-Todorović, 1990).

Significant individual diversity of Turkish hazel genotypes are shown in nuts morphological, technological and physiological features. Amount of trees fruiting is alternative. Extensive fruiting of trees from park surfaces and alley on average is every third year. In Table 1 are presented measuring results and variability of nuts basic morphological traits for selected genotypes from nursery Gradsko zelenilo in Novi Sad.

Table 1. Morfometric fruit traits of selected Turkish hazelnut (*Corylus colurna* L.) at locality Gradsko zelenilo - nursery

Tabela 1. Morfometrijske osobine plodova turske leske (Corylus colurna L.) obranih na području rasadnika Gradskog

Genotypes *Genotipovi*	Fruit length *Dužina orašica*		Fruit width *Širina orašica*		Fruit thickness *Debljina orašica*		Fruit mass *Masa orašica*		Kernel mass *Masa jezgra*		Pericarp thickness *Debljina perikarpa*		Kernel/ fruit mass ratio *Randman*	
	mm	CV (%)	mm	CV (%)	mm	CV (%)	g	CV (%)	g	CV (%)	mm	CV (%)	%	CV (%)
A_4	17.89	3.16	13.94	1.23	11.40	2.88	1.48	4.96	0.55	6.15	1.91	2.73	37.05	4.39
A_5	17.17	1.60	18.32	1.14	14.45	2.98	2.58	4.35	0.89	6.27	2.71	5.93	34.52	3.82
A_8	17.17	0.96	14.82	3.28	12.45	3.16	1.80	6.03	0.61	4.14	2.31	5.04	33.97	6.51
A_{13}	17.93	0.53	16.54	2.54	12.45	3.18	2.04	5.09	0.70	7.33	2.49	7.46	34.21	6.21
A_{14}	17.40	2.47	15.05	2.64	12.46	3.93	1.93	6.35	0.63	5.73	2.43	5.93	32.75	8.62
A_{16}	17.42	1.61	14.49	2.86	10.89	3.22	1.47	6.80	0.63	5.58	0.70	9.31	42.95	4.90
B_7	16.43	1.60	14.38	8.13	11.19	3.00	1.51	11.21	0.69	10.28	0.56	5.60	45.97	4.12
C_1	18.34	1.50	15.59	3.04	12.02	4.22	1.57	6.02	0.64	5.07	0.63	3.95	40.82	5.35
C_2	16.87	1.97	17.42	4.76	13.91	5.64	2.25	7.03	0.70	4.89	2.05	4.18	31.48	10.13
C_3	19.22	2.53	14.02	2.22	11.50	2.98	1.31	11.47	0.56	5.09	1.57	5.54	43.10	14.44
C_4	18.89	1.95	14.62	2.45	11.99	4.25	1.78	5.43	0.63	4.16	0.64	6.52	35.61	3.12
Mean	17.71		15.38		12.26		1.80		0.66		2.15		37.50	
SD	0.88		1.48		1.13		0.39		0.10		0.37		5.33	
$LSD_{0.05}$	0.44		0.70		0.59		0.15		0.05		0.16		3.54	
$LSD_{0.01}$	0.59		0.94		0.79		0.21		0.07		0.22		4.73	

Nuts length for examined genotypes ranged from 16.43 (B7) to 19.22 mm (C3), while the largest nuts length variability expressed genotype A4 (CV=3,16%). Genotype A5 had the largest width of nuts (18.32 mm), statistically highly important difference in relation to all other genotypes, whilst minimal value of nuts width was noticed with genotype A4 (13.94 mm). Genotype B7 showed largest variability of tested trait (CV=8.13%), and with genotype A5 (CV=1.14%) was noted lowest variance in nuts width. Statistically significant larger nuts thickness in relation to other genotypes had genotype A5 (14.45 mm), while smallest had genotype A16 (10.89 mm). Most consistent nuts thickness, with lowest CV is recorded for genotypes A4, A5 and C3. Examined nuts mass was in range from 1.31 g, for genotype C3, to 2.58 g for genotype A5. Supporting results were found by Erdogan and Aygun, (2005) and also by Srivastava et al. (2010), testing nuts morphological characteristics and chemical compound of seed in seven different Turkish hazel genotypes.

Variance in nuts mass was from 4.35% (A5) to 11.47% (C3). The largest kernel mass had genotype A5 (0.89 g), and smallest mass had genotype A4 (0.55 g). The smallest variation in kernel mass is noted for genotype A8 (CV=4.14%), while largest mass for genotype B7 (CV=10.28%). Individual A5 had largest measured pericarps' thickness (2.71 mm), statistically significant larger in relation to genotypes A16, B7, C1 and C4. Most consistent pericarps thickness had genotype A4 (CV=2.73%), while genotype A16 (CV=9.31%) had largest variability in this feature. Biggest kernel / fruit mass ratio had genotypes B7 (45.97%), C3 (43.10%) and A16 (42.95%). Biggest coefficient of kernel / fruit mass ratio variance is noted with genotype C3 (CV=14.44%), while lowest coef-

ficient of fluctuation is recorded for genotype C4 (CV=3.12%). (figure 2)

Noticed nuts morphological features are important for planning depth and distance of planting in nursery production. Coefficients of variation imply weak fluctuation in size of nuts, so the same depth and distance is applied (6cm, 7 cm respectively) in sowing of selected nuts genotypes, on ground type chernozem.

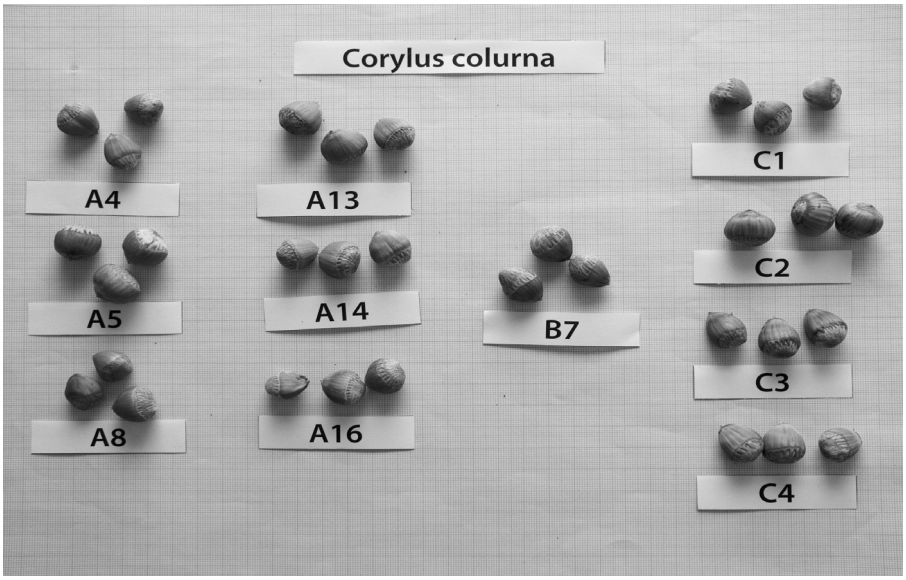

Figure 2. Genotypes of *Corylus colurna* L.
Slika 2. Genotipovi mečje leske

Correlation analysis included relations between morphometric features for selected genotypes of Turkish hazel nuts (Table 2). Significant negative correlations are noticed among length with width, thickness and mass of nuts, while correlation with thickness of pericarp is very significantly negative (r= - 0.557 **). Width and thickness of nuts had highly positive correlation between themselves, as well as with all examined features. Mass of nuts and kernel mass had highly significant positive correlation (r=0.783**). Relation of nuts mass, kernels mass and pericarps thickness are also in highly positive correlation. Kernel / fruit mass ratio, as significant economic entity, is in highly significant negative correlation with pericarps thickness as expected, since that with increase of pericarps thickness part of kernel mass is lowered.

Table 2. Simple correlation coefficients among investigated fruit traits of Turkish hazelnut genotypes (*Corylus culurna* L)
Tabela 2. Prosti koeficijenti korelacije između ispitivanih osobina osobina orašica genotipova mečje leske (Corylus colurna L.)

Trait *osobina*	Fruit width *Dužina orašica*	Fruit thickness *Debljina orašica*	Fruit mass *Masa orašica*	Kernel mass *Masa jezgra*	Pericarp thickness *Debljina perikarpa*	Kernel/fruit mass ratio *Randman*
Fruit length / *Dužina orašica*	-0.455*	-0.383*	-0.352*	-0.241	-0.557**	0.283

Fruit width / *Širina orašica*	0.878**	0.891**	0.779**	0.758**	-0.520**
Fruit thickness / *Debljina orašica*		0.913**	0.637**	0.763**	-0.709**
Fruit mass / *Masa orašica*			0.783**	0.852**	-0.713**
Kernel mass / *masa jezgra*				0.577**	-0.144
Pericarp thickness / *Debljina perikarpa*					-0.717**

* Significant correlations (p<0,05); ** High significant correlations (p<0,01).

Seed germination for examined Turkish hazel genotypes and percentage of graft acceptance are presented in Table 3. Based on presented data, very high values of seed germination in field conditions can be determined.

Table 3. Seed germination in terms of nursery and percentage of accepted grafts
Tabela 3. Klijavost semena u rasadniku i procenat primljenih kalemova

Genotype *Genotip*	Field germination *Klijavost u polju* (%)	Graft height *Visina kalemljenja* (cm)	Number of grafted rootstocks *Broj kalemljenih podloga*	Number of accepted grafts *Broj primljenih kalemova*	Percentage of acceptance *Udeo uspešnih kalemova* (%)
A_4	93.00	85	300	280	93.33
A_5	89.00	90	150	120	80.00
A_8	86.00	85	150	115	76.67
A_{13}	90.00	80	200	175	87.50
A_{14}	83.00	95	200	160	80.00
A_{16}	87.00	90	150	120	80.00
B_7	82.00	90	150	110	73.33
C_1	76.00	85	100	70	70.00
C_2	88.00	95	150	110	73.33
C_3	85.00	90	200	170	85.00
C_4	91.00	85	300	275	91.67

At beginning of April year 2011, hazel cultivars were grafted on rootstocks of Turkish hazel produced from nuts of mentioned genotypes. Three year old rootstocks in fourth vegetation period were used. Grafting height was from 80 cm on rootstock A13 to 95 cm on rootstocks A14 and C2, depending on rootstocks' thickness at grafting point.

Method used was tongue grafting. Percentage of grafting acceptance was in range from 70.00% (genotype C1) to 93.3% (genotype A4) at the end of vegetation period, which implies high scion / rootstock compatibility.

Figure 3. Hazelnuts grafted on *Corylus colurna* L. rootstocks
Slika 3. Leska kalemljena na podloge mečje leske

Applying Discriminant statistical analysis in relation, brought results connected to morphological characteristics. According to mentioned analysis most significant morphometric features of Turkish hazel nuts are length and nuts mass, and kernel mass (table 4.), influencing seed germination, or selection of best genotypes. In first group genotypes A5 and C2 outstand, with largest mass of nuts (2.58 and 2.25 g respectively), statistically significant larger than other genotypes. On chart, genotype B7, A16, A4, C4 and C3 have small nuts mass, but diversify on based on maximal values for nut length (16.43-19.22 mm). In third and fourth group are genotypes A8, A14, A13 and C1, with very similar values for morphological features and low variability in tested parameters.

Table 4. Discriminant Analysis on the basis of the selected parameters. Marked loadings are >0.7000 and significant for the axis.
Tabela 4. Diskriminantna analiza na bazi izabranih osobina.

Trait / *Osobina*	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6	Root 7
Fruit length / *dužina orašica*	0.758*	-0.380	0.626	0.315	0.288	-0.174	-0.125
Fruit width / *širina orašica*	-0.144	-0.086	-0.208	1.039*	0.608	0.600	-0.276
Fruit thickness / *debljina orašica*	-0.098	-0.234	-0.318	-1.122*	0.849*	-0.709*	-0.289
Fruit mass / *masa orašica*	-0.264	-1.252*	1.173*	-0.078	-0.908*	0.768*	2.734*

Kernel mass / *masa jezgra*	-0.563	0.343	-1.001*	-0.233	-0.402	-0.579	-2.274*
Pericarp thickness / *debljina perikapra*	-0.166	0.649	0.445	0.509	0.378	-0.671	0.014
Kernel/fruit mass ratio / randman	0.216	-0.166	0.342	0.228	0.298	-0.206	2.525*
Eigenval	21.973	5.077	3.838	1.598	1.1079	0.3869	0.219
Cumulative %	0.642	0.791	0.903	0.950	0.9823	0.9936	1.000

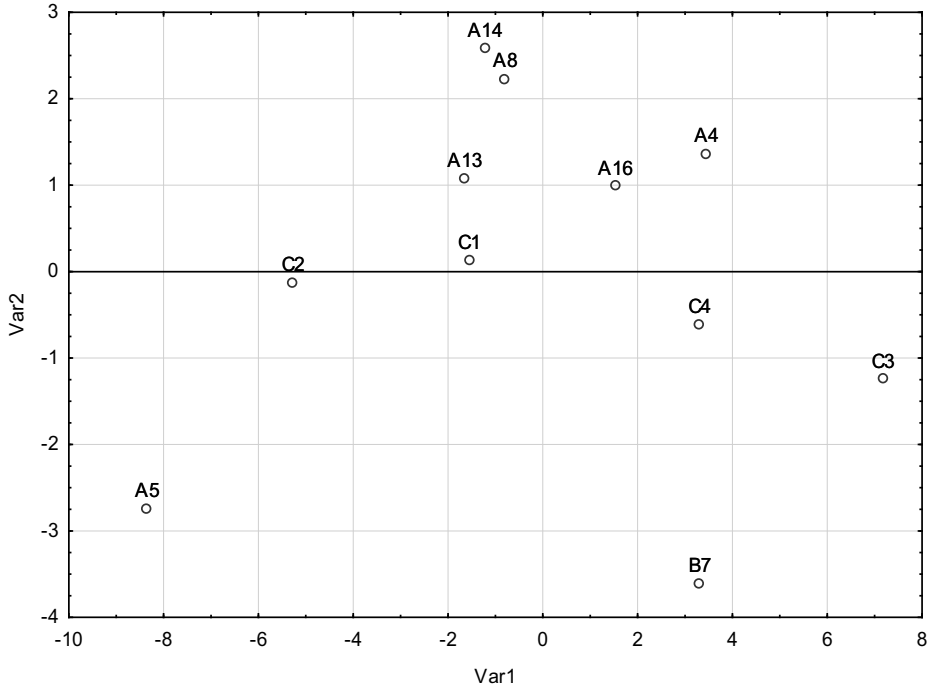

Figure 4. Discriminant Analysis Scatter plot based on investigated genotypes and parameters
Slika 4. Diskriminantna analiza (Scatter plot) bazirana na istraživanim genotipovima i parametrima

Stated findings draw conclusion that process of selecting nuts that is mother plants for production of generative rootstocks, should select genotypes with lengthier nuts, larger kernel / fruit mass ratio, and thinner pericarp. Considering that, larger fruit mass provides greater amount of available food to embryo, while with thinner pericarp enables easier germination, results are as expected. These findings however should be taken as guidelines, and not as specific determinants. Environmental conditions influence nuts germination in a year of gathering, sowing and follow up of seedling germination in nursery. As well, as manner and length of stratification, pests and disease resistance.

CONCLUSION

Large numbers of Turkish hazel trees in different age class are available in parks and tree lines in Novi Sad. Those secondary origin populations are used as a source of

sowing material for planting material production. Considering the range and alternative fruiting, recorded trees in park surfaces, depending on year and locality can provide enough nuts for planting material production for cities' green spaces needs.

Morphological and physiological traits of nuts, primarily germination, guide technological procedures toward achieving high productivity and profitability of producing Turkish hazel planting material. In nursery Rimski Šančevi, Turkish hazel is used as rootstock for hazel cultivars grafting.

Based on morphological measurements, most significant genotypes for nursery production in testing period are A4, A16, B7, C3 and C4 also confirmed with results of Discriminant Analysis.

Field germination of nuts implicates high percentage of technical germination, as especially showed in year 2007/2008. Reasons for high germination are affordable ecological conditions in period of seed production and germination readings, as well as successful protection of nuts from rodents in seed plot.

At beginning of April year 2011 tongue grafting was done on seedlings of examined hazel genotypes sowed in year 2007. Achieved graft acceptance, in range from 70.00% (genotype C1) to 93.3% (genotype A4), imply high affinity and compatibility of scion with rootstocks.

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SEKUNDARNE POPULACIJE TURSKE LESKE (*CORYLUS COLURNA L.*) U NOVOM SADU

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Izvod

Rezultati morfometrijske analize orašica odabranih genotipova mečje leske (*Corylus colurna* L.) odnose se na stabla sekundarnog porekla. Ustanovljena je klijavost semena i prijem kalemova na sejancima ispitivanih genotipova. Utvrđena je korelaciona zavisnost klijavosti semena od ispitivanih morfometrijskih osobina, što bi moglo imati značaja pri izboru matičnih stabala za proizvodnju sejanaca i sadnog materijala. Odabrani genotipovi sa lokaliteta rasadnika Gradsko zelenilo (A4, A5, A8, A13, A14, A16, B7, C1, C2, C3, C4) imali su visoku klijavost semena u godini ispitivanja i formirali su gust sklop u redovima. Kalemljenje genotipova obavljeno je početkom aprila 2011. godine u cilju dobijanja visokokalemljenih leski - stablašica. U toku vegetacionog perioda procenat prijema kalemova kretao se od 70.00% (genotip C1) do 93.3% (genotip A4) što ukazuje na visok afinitet podloga sa plemkama sorte Tonda Gentile Romana. Izdvojeni genotipovi pokazuju ujednačen rast i razvoj sejanaca, što je od značaja za primenu metoda kalemljenja engleskim spajanjem.

Ključne reči: *Corylus colurna*, populacija, genotip, klijavost semena, kalemljene leske, podloge.

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SURVIVAL AND VEGETATIVE GROWTH OF NURSERY GRAFTED WALNUT PLANTS

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SUMMARY: The survival and vegetative growth of nursery grafted walnut plants were observed in cv. Šeinovo, Ovčar, G-286, G-139 and Elit. The highest survival (74.2%) and the highest percentage of class I plants (63.4%) were obtained in Seinovo, and the lowest (63.3% and 50.9% respectively) in Elit. Vegetative growth in the first year was the highest in G-286 (15.5 cm) and the lowest in Elit (13.9 cm) on average. In the second year, Seinovo had the highest growth (178.0 cm) and Elit the lowest (165.7 cm).

Key words: *vegetative growth, walnut plants, nursery, cultivar.*

INTRODUCTION

The technology employed in grafted walnut production is rather complex. Particular attention should be given to grafting and stratification methods as well as to proper care of grafted walnut plants in the nursery in order to obtain quality planting material. The survival and growth of nursery walnut plants are much dependent upon the effect of climatic factors, primarily air temperature and rainfall. Paunović (2010) reported that temperature and rainfall directly affect survival and growth of nursery walnut plants, their winter frost resistance, lignification, apical bud development and root system growth. Korać (1987) and Korać et al. (1997) emphasised the suitability of vine growing regions for walnut production due to the higher temperature sum and the requirement for considerably higher air temperatures during foliation than in most other fruit species. Moreover, the same authors observed that walnut remains underdeveloped under soil water deficiency, becoming susceptible to parasite attacks and winter frosts. Similar results were obtained by Šoškić (2007) who underlined the importance of favourable rainfall distribution during the growing season, particularly in July, given the fact that early droughts reduce vegetative growth.

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The objective of this study was to evaluate the survival and vegetative growth of nursery walnut plants under natural temperate continental climate conditions in Serbia.

MATERIAL AND METHODS

The experiment was conducted during 2003-2005 in the nursery of the Fruit Research Institute, Cacak, Serbia, located in the Zapadna Morava river valley at an altitude of 242 m (43°53'17''N, 20°20'35''E). One cultivar (Seinovo-control) and four walnut selections, including Ovcar, G-286, Elit and G-139, were studied.

Walnut planting was conducted in the nursery on 20 May 2003 and 25 May 2004 following a randomised block design (5 cultivars x 4 replications), 34 grafted plants of each cultivar were included in replications, totalling 680 plants. According to its physical and chemical properties, the soil used in the study was a mildly acid (pH 6.35) loamy alluvial deposit that had a good supply of humus in the 0-20 cm layer (2.76%), a low humus content in deeper layers (1.50%), a moderate supply of N (0.13%), an abundant supply of P_2O_5 (17.80 mg/100 g air dry soil) and K_2O (28.25 mg/100 g air dry soil), and no content of $CaCO_3$.

At the end of the first and second growing seasons, the percentage of survived plants was visually determined. During both growing seasons, at 20-day intervals upon shoot emergence, plant height was measured from the graft union upwards.

The obtained results are presented in percentages. The results were subjected to Fisher's model of analysis of variance - ANOVA (Fisher, 1953). The significance of differences between the means of the control cultivar and the selections at $P \leq 0.01$ and $P \leq 0.05$ significance levels was determined using Dunnett's one-sided and two-sided comparison test (Dunnett, 1955). The significance of differences between particular seasons, and interaction means were tested using the LSD test at $P \leq 0.05$. The results are presented in tabular form.

RESULTS AND DISCUSSION

Cacak has a temperate continental climate. The mean annual air temperature in Čačak (Table 1) during 1992-2002 was 11.9°C. The average air temperature during the growing season (April-October) was 17.9°C. The coldest months were January and December, as evidenced by the mean monthly temperature of 0.5°C and 0.8°C, respectively, whereas the warmest months were July (22.6°C) and August (23.0°C). The average annual rainfall was 714.5 mm. Total rainfall was highest in June (88.4 mm) and lowest in January (30.7 mm). The amount of rainfall during the growing season was 462.7 mm, accounting for 66.6% of the total annual rainfall.

Table 1. Mean monthly air temperatures and average monthly rainfall in Čačak during 1992-2002
Tabela 1. Srednje mesečne temperature vazduha i prosečne količine padavina po mesecima u Čačku (1992-2002)

Months *Meseci*	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
t (°C)	0.5	3.1	7.6	11.7	17.9	21.3	22.6	23.0	16.8	12.2	6.1	0.8
mm m^{-2}	30.7	38.9	42.5	51.2	56.4	88.4	82.6	51.6	74.9	57.6	52.8	52.7

During the 2003-2005 period, the average air temperature was 9.7°C, and the average air temperature during the growing season was 16.0°C. Mean annual air temperatures varied 0.9°C over the three-year period, being 9.3°C in 2003, 10.2°C in 2004 and 9.7°C in 2005 (Table 2). During the growing season (April-October), the same mean air temperature variation (0.9°C) was observed. Mean temperatures during the growing season from April through October were 16.2°C in 2003, 16.4°C in 2004 and 15.5°C in 2005. The mean annual sum of sunshine duration was 1,956 hours.

Table 2. Mean monthly and annual temperatures (°C) during 2003-2005
Tabela 2. Srednje mesečne i godišnje temperature (°C) za period od 2003. - 2005. godine

Month / *Mesec*	Year / *Godina*			Average, mean monthly temperatures *Prosek, srednje mesečne temperature*
	2003	2004	2005	
I	-2.5	-3.5	0.1	-1.4
II	-4.7	0.1	-1.7	-1.5
III	3.3	4.8	3.9	4.0
IV	9.3	11.4	10.5	10.4
V	18.3	16.5	15.3	16.7
VI	21.4	18.8	17.0	19.1
VII	20.7	23.5	20.6	21.6
VIII	22.2	19.3	18.7	20.1
IX	13.6	13.5	15.9	14.3
X	7.7	12.1	10.2	10.0
XI	4.7	4.4	3.8	4.3
XII	-2.0	1.6	2.0	1.6
Average I–XII / *Prosek I–XII*	9.3	10.2	9.7	9.7
Average IV-X / *Prosek IV-X*	16.2	16.4	15.5	16.0

During the study period, different amounts of rainfall were recorded, particularly during the growing season (April-October). Total rainfall in 2003, 2004 and 2005 was 590.1 mm, 707.1 mm and 474.3 mm, respectively (Table 3). The average rainfall received was 590.5 mm during the study years and 342.8 mm during the growing season. The 2003 growing season received a total of 316.4 mm rainfall. Insufficient rainfall was recorded during June (23.0 mm) and August (6.0 mm), whereas July had satisfactory rainfall (60.8 mm). During the 2004 growing season, rainfall totalled 442.5 mm, with the summer rainfall amount being satisfactory (June - 112.5 mm, July - 84.4 mm and August - 70.7 mm). The 2005 growing season was characterised by a dry period with an amount of rainfall of 269.5 mm. Notably low rainfall was recorded during intensive growth of walnut (June - 35.4 mm, July - 6.8 mm and August - 22.7 mm).

Table 3. Total monthly rainfall and average rainfall during the growing season (mm m^{-2}) for the period 2003-2005

Tabela 3. Ukupna količina padavina po mesecima i prosečna količina padavina u toku vegetacije (mm m^{-2}) (2003.- 2005)

Month / *Mesec*	Year / *Godina*			Average rainfall *Prosečna količina padavina*
	2003	2004	2005	
I	76.8	111.1	10.8	66.2
II	42.5	48.6	74.6	55.2
III	2.5	31.7	33.4	22.5
IV	20.0	68.7	67.6	52.1
V	72.7	66.0	61.8	66.8
VI	23.0	112.5	35.4	56.9
VII	60.8	84.4	6.8	50.6
VIII	6.0	70.7	22.7	33.1
IX	41.7	22.4	63.6	42.6
X	92.2	17.8	11.6	40.5
XI	66.6	59.3	58.2	61.4
XII	85.3	13.9	27.8	42.3
Average I–XII / *Prosek I–XII*	590.1	707.1	474.3	590.5
Average IV-X / *Prosek IV-X*	316.4	442.5	269.5	342.8

At the end of the first growing season, Dunnett's test ($P \leq 0.01$ and $P \leq 0.05$) showed that the control cv. Seinovo had a highly significantly greater percentage of survived plants and as compared to the selections tested. As for years, the LSD test ($P \leq 0.05$) revealed a highly significantly greater percentage of survived plants (72.1%) in 2004 as compared to those in 2003 (62.3%) (Table 4).

The highest survival of plants on average during both years was obtained with cv. Seinovo (69.9% in 2003 and 78.6% in 2004), followed by selections G-286 (63.4% - 2003 and 73.5% - 2004), Ovcar (60.2% - 2003 and 71.9% - 2004), G-139 (59.3% - 2003 and 68.6% - 2004) and Elit (58.9% - 2003 and 67.8% - 2004).

Table 4. Survival of nursery walnut plants at the end of the first growing season

Tabela 4. Prijem sadnica oraha na kraju prve vegetacione sezone

		Procenat prijema sadnica na kraju I vegetacione sezone / *Percentage of survival plants at the end of the 1st growing season*
Sorta / *Cultivar (A)*	Ovcar	66.0±0.92 **
	Elit	63.3±0.68 **
	G-139	63.9±0.92 **
	G-286	68.4±0.64 **
	Seinovo	74.2±0.58
Godina / *Year (B)*	2003	62.3±0.47 b
	2004	72.1±0.43 a
ANOVA		
Sorta / *Cultivar (A)*		**
Godina / *Year (B)*		**
A x B		**

A and B represent cultivar and years, respectively */ A i B predstavljaju sorte i godine.*

Asterisks in vertical columns represent significant differences between the means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test); ns- non-significant */ Zvezde u vertikalnim kolonama obeležavaju značajne razlike između sredina za P≤0.05 i P≤0.01 na osnovu Dunnett testa i rezultata ANOVA (F-test); ns- nije značajno.*

The values designated with same small letters within columns for years and interaction means do not differ significantly at P≤0.05 according to LSD test */ Vrednost u kolonama za godine i interakcijske sredine označene istim malim slovima značajno se ne razlikuju za P≤0.05 na osnovu LSD-testa.*

At the end of the second growing season, Dunnett's test (P≤0.01 and P≤0.05) showed a highly significantly greater percentage of class I plants in cv. Seinovo than in the selections tested. No significant difference was observed in the percentage of class II and unclassed plants between the control cultivar and the selections tested. LSD test (P≤0.05) indicated a highly significant difference in the percentage of class I plants between 2004 and 2005, and no significant difference in the percentage of class II and unclassed plants (Table 5).

The survival percentage of cv. Seinovo in 2004 was 65.9% - (58.4% class I plants, 7.5% class II plants) and 4.0% of unclassed plants, and that in 2005 - 75.2% (68.4% class I, 6.8% class II) and 3.4% of unclassed plants. The selection Ovcar had 55.6% of plant survivals (46.8% class I, 8.8% class II) and 4.6% of unclassed plants in 2004, and 67.8% (60.4% class I, 7.4% class II) and 4.1% of unclassed plants in 2005. The survival percentage of Elit in 2004 was 54.3% (46.0% class I, 8.3% class II) and 4.6% of unclassed plants, and that in 2005 was 63.3% - (55.9% class I, 7.4% class II) and 4.5% of unclassed plants. The selection G-139 showed a survival percentage of 54.8% (46.6% class I, 8.2% class II) and 4.5% of unclassed plants in 2004, and 64.7% (57.4% class I, 7.3% class II) and 3.9% of unclassed plants in 2005. The survival percentage in the selection G-286 was 59.1% (51.2% class I, 7.9% class II) and 4.3% of unclassed plants in 2004, and 69.7% - (62.5% class I, 7.2% class II) and 3.8% of unclassed plants in 2005.

Table 5. . Survival of nursery walnut plants at the end of the second growing season
Tabela 5. Prijem sadnica oraha na kraju druge vegetacione sezone

		Percentage of class I plants *Procenat sadnica I klase*	Percentage of class II plants *Procenat sadnica II klase*	Percentage of unclassed plants *Procenat sadnica van klase*
Sorta / *Cultivar* (A)	Ovcar	53.6±0.99**	8.10±0.21ns	4.35±0.20ns
	Elit	50.9±0.81**	7.85±0.26ns	4.55±0.26ns
	G-139	52.0±1.02**	7.75±0.25ns	4.20±0.20ns
	G-286	56.8±0.96**	7.55±0.25ns	4.05±0.26ns
	Seinovo	63.4±0.78	7.15±0.16	3.70±0.24
Godina / *Year* (B)	2004	49.8 ±054 b	8.14±0.15 a	4.40±0.17 a
	2005	60.9±0.59a	7.22±0.16 a	3.94±0.13 a
ANOVA				
Cultivar / *Sorta* (A)		**	ns	ns
Year / *Godina* (B)		**	ns	ns
A x B		**	ns	ns

A and B represent cultivar and years, respectively */ A i B predstavljaju sorte i godine.*

Asterisks in vertical columns represent significant differences between the means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test) results; ns - non-significant */ Zvezde u vertikalnim kolonama obeležavaju značajne razlike između sredina za P≤0.05 i P≤0.01 na osnovu Dunnett testa i rezultata ANOVA (F-test); ns- nije značajno.*

The values designated with same small letters within columns for years and interaction means do not differ significantly at P≤0.05 according to LSD test */ Vrednost u kolonama za godine i interakcijske sredine označene istim malim slovima značajno se ne razlikuju za P≤0.05 na osnovu LSD-testa.*

Dunnett's test (P≤0.01 and P≤0.05) used at the end of the first and second growing seasons (Table 6) showed that cv. Seinovo had highly significantly greater plant growth as compared to the selections Elit and G-139, whereas no significant difference was observed between the control cultivar and the selections Ovcar and G-286. LSD test (P≤0.05) revealed highly significantly lower plant growth at the end of the first growing season in 2003 than in 2004.

At the end of the second growing season, growth was found to be highly significantly greater during 2004 than in 2005. Vegetative growth of nursery walnut plants in 2003 was 13.4 cm in cv. Seinovo, 13.0 cm in Ovcar, 12.1 cm in Elit, 12.2 cm in G-139, and 13.9 cm in G-286. Plant growth during 2004 was 3.7 cm higher on average than in 2003, being 16.8 cm in cv. Seinovo, 17.0 cm in Ovcar, 15.8 cm in Elit, 16.1 cm in G-139, and 17.2 cm in G-286.

At the end of the second growing season in 2004, plant height was as follows: cv. Seinovo - 186.6 cm, Ovcar - 187.1 cm, Elit - 178.9 cm, G-139 - 179.4 cm and G-286 - 189.6 cm. In 2005, when the lowest average rainfall was recorded, the average plant growth was 24.0 cm lower, being 169.4 cm in cv. Seinovo, 162.8 cm in Ovcar, 152.5 cm in Elit, 157.6 cm in G-139, and 159.1 cm in G-286.

Table 6. Vegetative growth of nursery walnut plants
Tabela 6. Vegetativni porast sadnica oraha

		Vegetative growth of plants at the end of the 1st growing season *Vegetativni porast sadnica na kraju I vegetacione sezone* (cm)	Vegetative growth of plants at the end of the 2nd growing season *Vegetativni porast sadnica na kraju II vegetacione sezone (cm)*
Sorta / *Cultivar* (A)	Ovcar	15.0±0.69 ns	174.9±6.72 ns
	Elit	13.9±0.66 **	165.7±6.82 **
	G-139	14.1±0.75 **	168.5±5.58 **
	G-286	15.5±0.80ns	174.3±8.52 ns
	Seinovo	15.1±0.60	178.0±8.86
Godina / *Year* (B)			
	2003	12.9±0.39 b	
	2004	16.6±0.25 a	184.3±5.09 a
	2005		160.3±2.29 b
ANOVA			
Cultivar / *Sorta* (A)		**	**
Year / *Godina* (B)		**	**
A x B		**	**

A and B represent cultivars and years, respectively */ A i B predstavljaju sorte i godine.*

Asterisks in vertical columns represent significant differences between the means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test) results; ns - non-significant / *Zvezde u vertikalnim kolonama obeležavaju značajne razlike između sredina za P≤0.05 i P≤0.01 na osnovu Dunnett testa i rezultata ANOVA (F-test); ns- nije značajno.*

The values designated with same small letters within columns for years and interaction means do not differ significantly at P≤0.05 according to LSD test / *Vrednost u kolonama za godine i interakcijske sredine označene istim malim slovima značajno se ne razlikuju za P≤0.05 na osnovu LSD-testa.*

An analysis of both air temperatures during the 2003-2005 study period and the long-term average (1992-2002) suggests that the mean annual air temperature and growing season air temperature were 2.2°C and 1.9°C lower, respectively. No early autumn and late spring frosts occurred during the period under study. Bulatović (1985) reports that regions with mean air temperatures during the growing season exceeding 16.5°C are most favourable for normal growth of walnut plants. This is in agreement with Šoškić (2007). Ogašanović et al. (1991) found that winter frosts occurring at a temperature of -23°C damage buds, insufficiently lignified upper plant portions or whole plants above the snow cover. Less severe autumn frosts occurring at a temperature of -3°C result in leaf damage, thus forcing early termination of foliation and leading to complete or partial defoliation.

An analysis of both average annual rainfall during 2003-2005 and the long-term average (1992-2002) suggests that the average annual rainfall during the experiment and over the growing season was 124.0 mm and 119.9 mm lower, respectively. Rainfall showed significant variations during the period under study. As compared to the long-term average, a lower amount of rainfall was recorded in 2003 and 2005, being 65.4 and 53.0 mm in June, 21.8 mm and 75.8 mm in July, and 45.6 mm and 28.9 mm in August, respectively. Total rainfall in 2004 was higher than the long-term average - 24.1 mm in June, 1.8 mm in July and 19.1 mm in August. During the first year after grafted walnut planting, a total of 121.8 mm of rainfall was recorded for the January-March period, with March receiving only 2.5 mm rainfall, at an average air temperature during the period (January-March) of -0.3°C. During the summer period of 2003 (June-August), total rainfall was 89.8 mm. The lowest average rainfall during the period was in August (6.0 mm) when the highest average monthly air temperature (22.2°C) was recorded, which induced a stress effect on young plants. During the second year after planting of grafted walnut plants in the nursery, the climatic conditions were observed to be much more favourable. The period prior to planting was characterised by sufficient rainfall and moderate temperature. The summer months had sufficient rainfall which was evenly distributed. During the initial growth of grafted walnut plants in June, July and August, a total of 267.6 mm of rainfall and an average temperature of 20.5°C were recorded. Total rainfall in 2003 was 126.1 mm lower than in 2004. The rainfall deficiency in this year induced a 9.8% decrease in plant survival and 3.7 cm lower vegetative growth, as compared to the survival and growth of plants in 2004. The lowest average amount of rainfall during the experiment was recorded in 2005 (269.5 mm). Namely, dry periods were particularly characteristic of the summer months receiving rainfall totals of 64.9 mm (June 35.4 mm, July 6.8 mm and August 22.7 mm). The rainfall amount during the

2005 growing season was 173.0 mm lower than in 2004, and resulted in the 23.5 cm lower vegetative growth of plants.

Some authors report different water requirements of walnut plants as dependent upon the agro-environmental characteristics of a particular region. Karadeniz (2005) emphasises the dependence of walnut nursery tree production on the climatic conditions of the soil used for walnut cultivation. The author reveals that number of survived plants in the second year of cultivation is governed not only by temperature and relative air humidity during August and September but also much more by climatic conditions during winter. Bulatović (1985) reports the rainfall requirement for walnut growth of over 600 mm. Šoškić (2007) reports variations in water requirements of walnut plants depending on agro-environmental conditions of a particular region, viz. about 750 mm water is required in California (USA) and somewhat lower amounts in Serbia due to lower summer temperatures than in California. The mean annual rainfall received in the walnut growing region in Bulgaria ranges from 400 to 600 mm (Dzuvinov et al., 2009).

Walnut plants show substantial variations in survival rate, depending on the effect of climatic factors. Stanisavljević and Mitrović (1997) studied plant survival of a large number of walnut cultivars and selections, and reported the total survival percentage at the end of the first year of walnut cultivation of 86.5%. The survival percentage of the selection Elit obtained by Solar et al. (2001) was within the 43.0-73.0% range. Korać (1987) and Korać et al. (1997) deem it realistic to produce 40.0 to 50.0% of class I walnut plants at the end of the second year of walnut production.

The vegetative growth analysed in this study was lower during the first year of walnut cultivation. At the end of the second year, the results on vegetative growth were in agreement with those obtained by other authors. Under the agro-environmental conditions of Cacak, Stanisavljević and Mitrović (1997) produced plant growth at the end of the first year of 23.4 cm in cv. Seinovo, 24.1 cm in Ovcar, 11.2 cm in Elit, 17.6 cm in G-286, and 21.4 cm in G-139. In the same study, plant growth during the second year was highest in Ovcar - 187 cm, followed by cv. Seinovo - 185 cm, G-286 - 172 cm, G-139 - 167 cm, and Elit - 152 cm. Korać (1987) and Korać et al. (1997) report that walnut plants generally reach 10-25 cm in height at the end of the first growing season and about 150 cm at the end of the second. These data are in agreement with the results of the present study. Cultivar-dependent plant height in the second year of walnut cultivation ranged from 150-180 cm in Bulgaria (Gandev, 2009), 168-172 cm in Romania (Achim and Botu, 2001) and 179.7-244.5 cm in Turkey (Ozkan et al., 2001).

CONCLUSION

In conclusion, the results presented in this study show that the survival and vegetative growth of nursery walnut plants are dependent upon the effect of climatic factors, primarily air temperature and rainfall.

The adverse effect of climatic factors on walnut production under partially altered temperate continental climate in Western Serbia, notably rainfall and air temperature variations during summer months (June-August), should be overcome through the use of different methods of irrigation and conservation of soil moisture from autumn and winter rainfall.

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PRIJEM I VEGETATIVNI PORAST OKALEMLJENIH SADNICA ORAHA U RASTILU

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Izvod

Ispitivan je prijem i vegetativni porast okalemljenih sadnica oraha u rastilu kod sorte Šeinovo i selekcija Ovčar, G-286, G-139 i Elit. Najveći prijem (74,2%) i najveći procenat sadnica I klase (63,4%) imala je sorta Šeinovo, a najmanji prijem (63,3%) i najmanji procenat sadnica I klase (50,9%) selekcija Elit. Najveći porast u prvoj godini imala je selekcija G-286 (15,0 cm) a najmanji Elit (13,6 cm). U drugoj godini najveći porast bio je kod sorte Šeinovo (177,6 cm), a najmanji kod Elita (165,5 cm).

Ključne reči: vegetativni porast, sadnice oraha, rastilo, sorta.

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CHARACTERIZATION OF DROUGHTS IN SERBIA USING STANDARDIZED PRECIPITATION INDEX AND MARKOV CHAINS[1]

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SUMMARY: This paper presents an application of homogeneous Markov chain model to time series of SPI in order to characterize droughts in Serbia. The long-term probabilities of occurrence of different drought severity classes, recurrence times, expected residence time in each drought class and expected first passage time to reach the non-drought state were computed. Results are presented in a form of maps and the drought-prone areas are identified. Results of the performed analysis are useful in the context of drought early warning systems and in a planning of drought mitigation practices.

Key words: drought vulnerability map, SPI, Markov chain.

INTRODUCTION

Agricultural production in Serbia over the last decades has been affected by frequent occurrence of drought periods. In the most part of Serbia, climate can be characterized as a moderate continental climate with varying meteorological conditions, particularly quantity and temporal distribution of precipitation. Since Serbia is predominantly an agricultural region, especially Vojvodina region with over 75% of arable land, drought can have considerably negative economic impact. As a consequence of the insufficiently developed irrigation systems, the atmospheric precipitations are still the major factor in providing water to the soil and crops. In a greater or lesser intensity drought occurs regularly in different parts of Serbia and it is the limiting factor of high yields (Dragović, 2001; Spasov, 2003; Dodig et al., 2006; Benka et al., 2010; Rajić et al., 2010).

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Drought is a complex natural disaster that causes serious environmental, social, and economic consequences worldwide (Wilhite et al., 2000). Complex drought phenomenon is usually explained by drought index, which identifies drought characteristics like magnitude, duration, severity and spatial extent of drought. Drought indices are used in analysis of occurrence probability and predictability characteristics of drought events. 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 drought conditions (such as soil water balance) it was recommended also by Commission of Agrometeorology of the World Meteorological Organization to implement SPI worldwide (WMO, 2009). Analysis of drought occurrences and identification of drought prone regions are necessary for developing long-term water resources management policies and drought mitigation strategies. The Markov chains are commonly used in climatology to evaluate predictability characteristic of meteorological droughts (Lohani et al., 1998; Steinemann, 2003; Abreu et al., 2008). In recent years Paulo et al. (2005) and Paulo and Pereira (2007) presented a Markov chain approach, using both homogeneous and nonhomogeneous formulations, to characterize the stochastic nature of drought and to evaluate the predictability characteristics of drought represented by the SPI. Khalili et al. (2011), in their research, presented a comparability analysis of predictability characteristics of the SPI and more recently developed reconnaissance drought index (RDI).

In this paper the Markov chain approach was applied to time series of SPI in order to characterize droughts in Serbia. Markov chains are used to estimate: the probability of occurrence of different drought severity classes, recurrence time of drought classes, expected residence time in each class of severity and expected first passage time to reach non-drought class.

MATERIAL AND METHODS

The Standardized Precipitation Index (SPI) was developed by McKee et al. (1993) for the purpose of defining and monitoring drought. For its calculation only precipitation data are needed. Its main feature is that it could be computed at different time scales (1, 3, 6, 9, 12, 24 and 48 months) to monitor droughts with respect to different usable water resources. Short-term SPI could be used to detect agricultural drought, and long-term SPI could be used for water supply management. The SPI is computed by fitting historical precipitation data to a probability density function for a specific time period and location. Typically, the gamma distribution is applied. The cumulative distribution function of a Gamma distribution is then transformed to a normal distribution with a mean of zero and standard deviation of one. This procedure enables 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. Since distribution of SPI fits normal distribution, SPI values between -1 and 1 have 68% probability of occurrence, between -2 and 2 have 95% probability of occurrence and between -3 and 3 have 99%

probability of occurrence. The categories of the SPI, according to McKee et al. (1993) are shown in Table 1.

Table 1. Categorization of moisture condition by SPI, McKee et al. (1993)
Tabela 1. Kategorizacija uslova vlažnosti na osnovu SPI, McKee et al. (1993)

SPI value *Vrednost SPI*	Moisture conditions *Uslovi vlažnosti*	Cumulative probability *Kumulativna verovatnoća*
+2,00 ≤ SPI	Extreamly wet / *Ekstremno kišno*	0.000 - 0.023
+1,50 ≤ SPI ≤ +1,99	Severely wet / *Vrlo kišno*	0.023 - 0.067
+1,00 ≤ SPI ≤ +1,49	Moderately wet / *Umereno kišno*	0.067 - 0.159
-0,99 ≤ SPI ≤ +0,99	Near normal / *U granicama normale*	0.159 - 0.841
-1,00 ≤ SPI ≤ -1,49	Moderate drought / *Umereno sušno*	0.841 - 0.933
-1,50 ≤ SPI ≤ -1,99	Severe drought / *Vrlo sušno*	0.933 - 0.977
SPI ≤ -2,00	Extreme drought / *Ekstremno sušno*	0.977 - 1.000

Monthly precipitation data, period from 1971 to 2010, were used for calculation of the SPI. Data were collected from 27 principal meteorological stations in Serbia. Calibration period from 1971 to 2000 was used to determine parameters of the gamma distribution. The SPI was calculated at 3 month scale (SPI3) for each month.

The Markov chain model was used to investigate the stochastic behavior of drought. A Markov chain is a stochastic process $\{X_t : t \in T\}$ in which the probability distribution of the random variable X_t at the time $t=t_n$ depends only on the value x_{n-1} at time t_{n-1}, and not on the values of the process at previous times (Winston, 2003). Since the current value is fully determined by the knowledge of only one past period, this Markov chain is said to be of order one. The first-order Markov chain can be expressed as:

$$P[X_{t_n} \le x_n \mid X_{t_{n-1}} = x_{n-1},..., X_{t_1} = x_1] = P[X_{t_n} \le x_n \mid X_{t_{n-1}} = x_{n-1}] \quad (1)$$

A Markov chain can be viewed as system of states which are describing the status of each random variable. The changes of state of the system are called transitions, and the probabilities associated with various state-changes are called transition probabilities. The set of all states and transition probabilities completely characterizes a Markov chain. The transition probability p_{ij} is the probability of going to state j for the next time period, given that the present state is i. It can be mathematically expressed as:

$$P = [p_{ij}] = P\{X_{t+1} = j \mid X_t = i\} \quad (2)$$

Estimation of the transition probabilities can be done by calculating the conditional relative frequencies of the transition counts, i.e. counting the number of times that SPI passes from state i to state j as follows:

$$\hat{p}_{ij} = \frac{n_j}{\sum_j n_j} \quad (3)$$

where, $\hat{p}_{ij}$ is transition probability, n_{ij} is number of transitions from state i to state j and $\sum_j n_{ij}$ is number of occurrences of state i.

If the transition probabilities are independent of the time period under

consideration, then the Markov chain is stationary or homogeneous in time and if the transition probabilities depend on the time period then the Markov chain is nonstationary (nonhomogeneous). The transition probabilities are most conveniently represented by a matrix P, called the transition matrix. The transition matrix of homogeneous first-order Markov chain is:

$$P = \begin{pmatrix} p_{11} & p_{12} & \cdots & p_{1j} & \cdots \\ p_{21} & p_{22} & \cdots & p_{2j} & \cdots \\ \vdots & \vdots & \ddots & \vdots & \ddots \\ p_{i1} & p_{i2} & \cdots & p_{ij} & \cdots \\ \vdots & \vdots & \ddots & \vdots & \ddots \end{pmatrix} \tag{4}$$

The transition matrix has a property of the right stochastic matrix since each row of the transition matrix P sums to one and all elements are non-negative. Number of elements of the transition matrix depends on the number of states.

In this study long-term probabilities (steady state), recurrence time, expected residence time and expected first passage time to reach non-drought class were analyzed.

Steady state probabilities are the long-term probabilities of each drought class and they are independent of the initial state of the Markov chain. Steady state probabilities, π_j, are computed as the non-negative solution of the system of linear equations:

$$\pi_j = \sum_{k=1}^{S} \pi_k p_{kj} \quad , \quad \sum_{j=1}^{S} \pi_j = 1 \ , \quad \forall k, j \in S \tag{5}$$

where π_j is the steady state probability of drought class j and S is number of drought classes.

The mean recurrence time, t_{ii}, which is the average time to return to the same drought class, can be computed from the steady state probability π_i as:

$$t_{ii} = \frac{1}{\pi_i} \tag{6}$$

The probability of uninterrupted residence time, duration of drought class i in m month, can be computed as:

$$P(X_1 = i \mid X_0 = i)P(X_2 = i \mid X_1 = i)\cdots P(X_{m-2} = i \mid X_{m-1} = i)P(X_m \neq i \mid X_{m-1} = i) =$$
$$= P_{ii}^{m-1}(1 - P_{ii}) \tag{7}$$

The expected uninterrupted residence time for class i is given by:

$$E(T_i \mid X_0) = \sum_k k P\ (m = k \mid X_0 = 1) \tag{8}$$

The expected first passage time is the average time period taken for the process to move for the first time from some class i to a class j. It is computed as the non-negative

solution of the system of linear equations:

$$t_{ij} = 1 + \sum_{k \neq j} p_k t_{kj} \quad , \quad \forall i, j \in S \tag{9}$$

RESULTS

Values of SPI3 for every month, period from 1971 to 2010, were computed for 27 principal meteorological stations in Serbia. SPI3 was chosen because research conducted by Szalai (2000) showed that considering agricultural drought, there is a significant correlation between 3 month SPI and ground layer soil moisture. Also, Labedzki (2007) stated that SPI3 well reflect agricultural drought.

The four state first-order homogenous Markov chains were applied to the SPI. Four drought categories (states) were considered: non-drought – "state 1" (SPI>0), mild drought – "state 2" (0≥SPI≥-0.99), moderate drought – "state 3" (-1≥SPI≥-1.5) and severe/extreme drought – "state 4" (SPI<-1.5). Steady state probabilities, recurrence times and residence times were computed for each drought category for every meteorological station. Expected times to reach non-drought state from any drought state are also computed. Results are presented in Table 2.

In order to test the adequacy of application of Markov chain model, the Nash-Sutcliffe efficiency test of hydrological model was performed between empirical steady state probabilities and Markovian steady state probabilities. Test result was 0.999, which is very close to the optimum value of 1.0, confirming the adequacy of using Markov chains.

Table 2. Steady state probabilities, residence times and mean first passage times
Tabela 2. Stacionarne verovatnoće stanja, vremena trajanja i vremena prvog relaza u normalno stanje vlažnosti

Station *Stanica*	Steady state probability *Stacionarne verovatnoće stanja*				Residence time (month) *Vreme trajanja (mesec)*				Mean first passage time to state 1 (month) *Vreme prvog prelaza u stanje 1 (mesec)*		
	1	2	3	4	1	2	3	4	2	3	4
Becej	0.55	0.29	0.09	0.07	3.8	2.1	1.2	1.8	3.8	5.6	5.8
Beograd	0.55	0.30	0.08	0.07	3.5	1.7	1.1	1.8	3.2	4.6	5.2
Crni Vrh	0.55	0.30	0.08	0.07	3.8	2.0	1.1	1.6	3.9	4.5	5.6
Cuprija	0.55	0.31	0.09	0.06	3.5	1.8	1.2	1.7	3.2	4.2	4.8
Dimitrovgrad	0.61	0.24	0.08	0.07	3.7	1.6	1.1	2.4	2.6	3.5	4.8
Kikinda	0.57	0.28	0.08	0.08	3.8	2.1	1.1	1.7	3.3	4.6	5.1
Kragujevac	0.53	0.32	0.08	0.07	3.6	2.0	1.2	2.0	3.8	4.9	5.3
Kraljevo	0.53	0.32	0.09	0.07	3.4	1.8	1.1	1.5	3.3	3.8	5.0
Krusevac	0.56	0.28	0.09	0.07	3.7	1.7	1.2	1.6	3.5	4.5	5.3
Leskovac	0.56	0.30	0.05	0.08	3.6	1.7	1.1	1.8	3.1	4.1	4.5
Loznica	0.57	0.27	0.09	0.08	3.6	1.6	1.1	1.8	3.0	4.1	5.2
Negotin	0.52	0.31	0.10	0.07	3.7	1.9	1.1	1.8	3.9	4.9	6.1
Nis	0.55	0.31	0.07	0.07	3.7	1.8	1.1	1.3	3.6	4.7	5.1
Palic	0.53	0.34	0.06	0.08	3.7	2.5	1.1	2.0	4.0	5.0	5.5

Pozega	0.52	0.32	0.09	0.07	3.4	1.7	1.2	1.5	3.3	4.5	5.0
Rimski Sancevi	0.55	0.28	0.10	0.07	3.8	1.9	1.3	2.1	4.0	5.5	6.6
Sjenica	0.53	0.32	0.10	0.05	3.2	1.7	1.2	1.2	3.0	3.9	4.0
Sremska Mitrovica	0.54	0.31	0.07	0.08	3.7	2.0	1.1	1.9	3.7	4.9	5.7
Sombor	0.52	0.32	0.09	0.06	3.6	2.0	1.1	1.8	3.7	4.3	5.6
Smederevska Palanka	0.53	0.32	0.08	0.07	3.4	1.9	1.0	1.6	3.2	4.5	5.6
Valjevo	0.56	0.30	0.07	0.08	3.6	1.7	1.1	1.8	3.2	4.0	4.5
Veliko Gradiste	0.55	0.29	0.10	0.07	3.8	2.0	1.1	1.8	3.7	4.9	5.7
Vranje	0.55	0.29	0.10	0.06	3.8	1.8	1.2	1.6	3.7	4.9	5.0
Vrsac	0.54	0.30	0.08	0.08	3.6	1.7	1.2	2.0	3.4	4.8	5.9
Zajecar	0.53	0.33	0.06	0.08	3.6	2.1	1.1	2.4	3.5	4.5	5.6
Zlatibor	0.55	0.32	0.07	0.07	3.4	1.7	1.0	1.6	3.0	4.4	5.1
Zrenjanin	0.52	0.32	0.08	0.08	3.6	2.0	1.1	2.2	3.7	4.6	6.4

Spatial distributions of the results of Markov chain modeling, regarding severe droughts, are presented on Figures 1 - 3. Apart from result values from Markov chain modeling, it was necessary to spatially define station's position. For each station, coordinates are determined in state coordinate system applying Gauss–Krüger projection. Spatial interpolation was performed using Inverse Distance Weighting method. Recurrence times of the severe droughts vary from 12.2 to 19.1 months. Regions of most frequent drought occurrences are Banat, North Backa, West part of Srem, Macva and Kolubara district and East and South-East Serbia. Residence times of severe drought are ranging from 1.2 to 2.4 months. Longest durations of severe droughts are in Central Banat and South-East Serbia. Period needed to reach non-drought state from state of severe drought is from 4 to 6.6 month. Most time needed to reach non-drought state is in Central Vojvodina and in East Serbia surrounding Negotin. Drought vulnerability map, presented on Figure 4, was created based on previous three maps. In the first step, maps of recurrence times, residence times and passage times from drought state 4 to state1 were reclassified into five classes and values from 1 to 5 were assigned to each class. Value 1 represents non-vulnerable class, value 5 represents most vulnerable class. Then, all three maps were added together and reclassified again into five classes. Results showed that most vulnerable regions to drought are North and Central Vojvodina and Eastern Serbia.

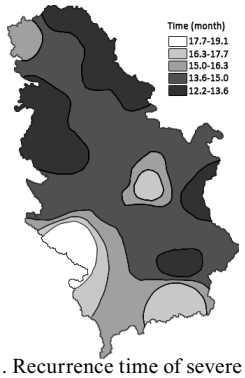

Figure 1. Recurrence time of severe drought
Slika 1. Povratni period jake suše

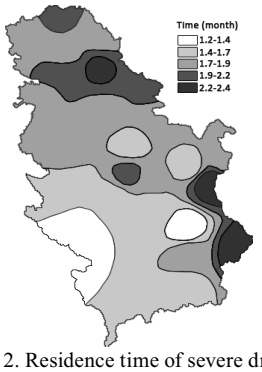

Figure 2. Residence time of severe drought
Slika 2. Vreme trajanja jake suše

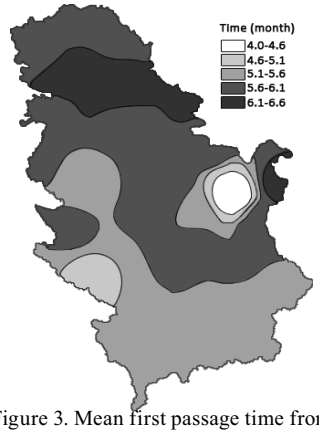

Figure 3. Mean first passage time from severe drought to non-drought state
Slika 3. Potrebno vreme za prelaz iz jake suše u normalno stanje

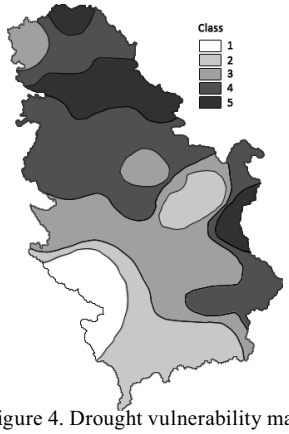

Figure 4. Drought vulnerability map
Slika 4. Karta ugroženosti od suše

DISCUSSION

As in presented paper, the homogeneous formulation of Markov chain has been successfully used by other authors to evaluate predictability characteristics of droughts (Khalili et al., 2011; Abreu et al. 2008; Steinemann, 2003). Non-homogeneous formulation of Markov chains has been used by Lohani et al. (1998), Paulo et al. (2005) and Paulo and Pereira (2007) in order to account the nonstationarity, i.e. dependency of transition probabilities on the initial month. In general, results of application of both formulations agree well, except in short-time forecast of drought classes where the non-homogeneous formulation has some advantages.

Drought occurrences in Serbia have been examined by many authors. Spasov and Zelenhasić (1990) concluded that longest durations of dry spells are in Eastern Serbia. Also, Dodig et al. (2006) stated that drought in Serbia occurs most frequently and it is the most intensive in the region of Eastern Serbia. Those results are in agreement with result presented in this paper. However, results in this paper also show that Central and North part of Vojvodina are equally vulnerable to drought as region of Eastern Serbia and that the spatial extent of drought is much larger in Vojvodina region.

CONCLUSION

This paper presents an application of Markov chains to time series of SPI3 in order to characterize droughts in Serbia. The four state first-order homogenous Markov chains were used to compute long-term probabilities of occurrence of different drought severity classes, recurrence times, expected residence time in each drought class and expected first passage time to reach the non-drought state. Historical time series of precipitation data collected form 27 principal meteorological stations in Serbia were analyzed and results are presented in a form of thematic maps which are convenient to examine the spatial extent of drought. Results indicate that the most drought-prone areas are regions of North and Central Vojvodina and Eastern Serbia.

Drought analysis based on stochastic methods, such as Markov chain model, are

quite useful in the context of drought early warning systems, identification of drought prone areas and in a planning of drought mitigation practices.

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KARAKTERIZACIJA SUŠA U SRBIJI KORIŠĆENJEM STANDARDIZOVANOG INDEKSA PADAVINA I MARKOVLJEVIH LANACA

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Izvod

U radu je prikazana primena modela homogenih Markovljevih lanaca na vremenske serije indeksa SPI za potrebe karakterizacije suša u Srbiji. Proračunate su verovatnoće pojave različitih kategorija jačine suše, povratni periodi, vreme trajanja određene kategorije suše i trajanje prvog prelaza u stanje normalne vlažnosti. Rezultati su predstavljeni u formi karata i identifikovane su oblasti ugrožene sušom. Rezultati sprovedene analize su korisni za potrebe sistema rane najave suše i za potrebe planiranja mera za ublažavanje posledica od suše.

Ključne reči: karta ugroženosti sušom, SPI, Markovljevi lanci.

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INFLUENCE OF SELENIUM ON MAMMARY GLANDS AND MILK SOMATIC CELLS IN DAIRY COWS

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SUMMARY: The proper functioning of the mammary gland requires high-quality diet, which is based on the presence of macro- and micro-nutrients, as well as proper sanitary conditions. One of the essential and most important micro-nutrients is selenium, which is part of the enzyme glutathione peroxidase, and has antioxidant effects. The research was conducted on two groups of cows, where group I was a control group, and group II received via food 50 mg / day of selenium. According to the blood test and blood serum analysis, we noticed that in the group I selenium levels were below the physiological limits, while in group II the level of selenium was within the margin of physiological values. Also, after conducting milk somatic cell count in groups I and II, we found that the majority of cows in the group I had a somatic cell count between 310.000 and 500.000/ ml and in the group II the majority of cows had somatic cell count between 210.000 and 300.000/ ml. According to the analysis of the correlation test, we noticed that there was a negative correlation with the second group of cows, because the increasing levels of selenium in blood serum cause a decline in the number of milk somatic cells. On the basis of these results we conclude that selenium is of great importance in the preservation and proper functioning of the mammary glands of cows.

Key wards: *selenium, somatic cell count, mammary gland, cow.*

INTRODUCTION

Selenium functions primarily as an antioxidant. Selenium is an integral component of the enzyme, glutathione peroxidase (Cortinhas et al., 2010; Joksimović-Todorović et al., 2007). This enzyme is an important part of the cellular antioxidant system, but glutathione peroxidase is water soluble and it is found in the cytosol of cells, not in cellular

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membranes. Selenium as a micronutrient is involved in the cellular antioxidant system (Engle, 2001; Spears and Weiss, 2008).

Cell processes, environmental insults and inflammatory responses produce compounds called free radicals. The major free radicals found in biological systems are superoxide, hydrogen peroxide, hydroxyl radical and fatty acid radicals. Free radicals are highly reactive compounds because they are missing an electron. Free radicals can react with nucleic acids causing mutations, with enzymes and render them inactive, and with fatty acids in membranes causing membrane instability. Free radicals can eventually kill cells and damage tissues (Knaapenet et al., 1999; Mukherjee, 2008).

In healthy dairy cows, about one-third of selenium in blood is in serum and two-third is in red cells. Selenium is incorporated into red cells only when the cell is made (Andrieu, 2008; Engle, 2001). Therefore, selenium content of the red cells reflects selenium intake 1 to 3 months ago. The selenium in serum mainly represents a transport pool and reflects the current status. Plasma or serum selenium will increase shortly after selenium is injected but the selenium content of red cells will not change for several weeks. Blood as a whole reflects longer term status but is somewhat sensitive to recent changes in selenium nutrition. The recommended level of selenium in blood serum of dairy cows is 8 to 10 mg/100 ml (Erdeljan et al., 2011; Gunter et al., 2003; Juniper et al., 2006).

Dairy cows bred on the soil where concentration of selenium is very low, should be fed with supplemental selenium (Arvidson et al., 2005; Joksimović-Todorović et al., 2007). Potential benefits include reduced clinical mastitis and reduced milk somatic cells (Barbano et al., 2006; Davidov et al., 2011; Weiss, 2002). Diets fed to cows should be supplemented with 0,3 ppm of selenium (NRC, 2001). In most situations, feeding 0,3 ppm provides adequate selenium, but occasionally that amount is not adequate. Certain conditions reduce the availability of selenium or increase its requirement.

The aim of our investigation is to determine influence of selenium on mammary glands and milk somatic cells in dairy cows.

MATERIAL AND METHODS

The examinations were performed on 30 cows of Holstein-Friesian breed. The cows were in similar physical fitness, lactating and giving approximately the same amount of milk. During the experiment, cows were living under the same conditions and were divided into two groups of 15 cows. The first group of cows was the control group and this group was not treated during the experiment, while the other group received 50 mg of selenium via food per day. Blood samples were taken after the morning milking from the caudal vein by applying the principles of asepsis and antisepsis. Tubes with appropriate needle were used for taking blood. The blood in tubes was left at room temperature for 24 hours to separate the serum. The level of selenium in blood serum was determined using gas-chromatography.

Milk samples were taken during the morning milking from each district. Milk somatic cell count of each udder quarter was determined by using Draminski device.

RESULTS

After morning milking we took milk from all four quarters of each cow and counted milk somatic cells. The results of milk somatic cell count are shown in Table 1.

Table 1. The distribution of cows in group I and II according to somatic cell count
Tabela 1. Distribucija krava u I i II grupi prema broju somatskih ćelija u mleku

Milk somatic cell count /ml *Broj somatskih ćelija u mleku/ml*	Group / *Grupa*	
	I (n=15)	II (n=15)
210.000-300.000	(20.00%) 3/15	(60.00%) 9/15
310.000-400.000	(53.33%) 8/15	(33.33%) 5/15
410.000-500.000	(26.67%) 4/15	(6.67%) 1/15

The results in table 1 show the value of somatic cells in milk of both groups: in group I 4 cows had milk somatic cell count between 410.000-500.000/ml, while the same value of somatic cells in group II had only one cow. A significant decrease of somatic cells was recorded in the second group that received food supplement of selenium. The results indicate the possibility of positive effects of selenium supplement in food to reduce the number of milk somatic cells, and thus to reduce the incidence of subclinical mastitis. Table 2 shows the average value of selenium in the blood serum in the first and second groups of cows.

Table 2. The average value of blood serum selenium in dairy cows I and II group
Tabela 2. Prosečna vrednost selena krvnog seruma krava I i II grupe

Group / *Grupa*	Average amount of selenium in blood serum(mg/100 ml) *Prosečnan saržaj Se u krvnom serumu (mg/100 ml)*
I	5.016
II	8.881

The average value of selenium in the blood serum differed between groups I and II of cows, where the group I had a level of selenium in blood serum below the physiological limits, as opposed to group II which had a level of selenium in blood serum in the lower margins of physiological value. However, cows from group II, which received 50 mg / day of selenium via food, had an increase in the concentration of selenium in blood serum and had a mammary gland in better functioning then cows from group I, which can be seen in Table 2. For better understanding of the influence of selenium on mammary gland and on milk somatic cell count, we made a statistical test of correlation, which provided us with important information. That statistical information is shown in Table 3.

Table 3. Test correlation between value of blood serum selenium and milk somatic cell count
Tabela 3. Test korelacije prosečne vrednosti selena u krvnom serumu I i II grupe i broja somatskih ćelija mleka

Milk somatic cell count/ml *Br. somatskih ćelija mleka/ ml*	Group I / *I grupa*	Group II / *II grupa*
210.000-300.000	3	9
310.000-400.000	8	5
410.000-500.000	4	1
	0.188982*	-1**

* Positive correlation, ** Negative correlation.

From the statistical analysis of data in table 3, it can be seen that the existence of positive correlation in group I indicates that the level of selenium in blood serum under physiological conditions causes growth of somatic cells in cow's milk. Unlike group I, in group II, which received selenium as a supplement in foods, we noticed the existence of negative correlations, indicating that increasing levels of selenium in blood serum cause a decline of the number of somatic cells in cow's milk.

DISCUSSION

Selenium is found in grass, tinned products and home-grown feeds, and it can be governed by geological/geographical situations and may be inadequate throughout the whole year. The cows from group I, in our examination, were fed with that quality of food. Our results indicate that cows from group I had a level of selenium in blood serum below the physiological limits, which indicate that they need to be fed with supplements of selenium. Our result is similar with authors (Arvidson et al. (2005) and Joksimović-Todorović et al. (2007). Erdeljan et al. (2011), Gunter et al. (2003) and Juniper et al. (2006)) who reported that the recommended level of selenium in blood serum of dairy cows is 8 to 10 mg/100 ml. In our examination, cows from group I had an average value of selenium in the blood serum 5.016 mg/100 ml, and in group II 8.881 mg/100 ml. Also, cows from group I, 12 of 15, had milk somatic cell count between 310.000 to 500.000and ml, which indicate subclincal mastitis. Only one cow of 15 from group II who received supplement of selenium in 50 mg/ day in food, had milk somatic cell count between 410.000 to 500.000/ml. Davidov et al. (2011), Barbano et al. (2006) and Weiss (2002) reported reducing subclinical mastitis and milk somatic cell count in cows who received supplements of selenium via food. The same was noticed in our examination, because the group II had received selenium supplements in food and milk somatic cell count was between 210.000 and 300.000/ ml. By using test of correlation we notice that group I had positive correlation, which indicate that level of blood serum selenium had negative influence on mammary gland and on milk somatic cells as well. In group II which received 50 mg/ day selenium in food, we noticed negative correlation, which indicated that physiological level of blood serum selenium had a positive influence on mammary gland and on milk somatic cell as well.

CONCLUSION

Selenium as a natural antioxidant plays an important role in maintaining the health of the mammary glands of cows. It enhances the activity of neutrophils, improves its ability to engulf and phagocyte pathogenic microorganisms. Adding selenium to cow's feed can prevent the occurrence of clinical and subclinical mastitis, and based on our results we can conclude that selenium has a large impact on the proper functioning of the mammary glands of cows.

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UTICAJ SELENA NA MLEČNU ŽLEZDU I BROJ SOMATSKIH ĆELIJA MLEKA KRAVA

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Izvod

Za pravilno funkcionisanje mlečne žlezde, pored pridržavanja zoohigijenskih mera, neophodna je i kvalitena iskrana prvenstveno u makro i mikro nutritientima. Jedan od esencijalnih mikro nutritienta je selen, koji ulazi u sastav enzima glutation peroksidaze i ima antioksidativni efekat. Istraživanje smo sproveli na na visoko mlečnim kravama, koje su bile podeljene u dve grupe. I grupa je bila kontrolna, dok je II grupa dobijala u hrani 50 mg /danu selena. Nakon vađenja krvi i izdvajanja seruma, krvni serum je analiziran. Uočili smo da je nivo selena u krvnom serumu I grupe bio ispod fizioloških granica, dok je kod II grupe bio na donjoj margini fizioloških vrednosti. Takođe, nakon brojanja somatskih ćelija mleka I i II grupe, uočili smo da je najveći broj krava I grupe imao broj somatskih ćelija između 310.000 i 500.000/ ml, dok je najveći broj krava II grupe imao broj somatskih ćelija između 210.000 i 300.000/ ml. Analizom testa korelacije uočili smo da postoji negativna korelacija kod krava II grupe, jer sa povećanjem nivoa selena u krvnom serumu dolazi do pada broja somatskih ćelija mleka. Na osnovu dobijenih rezultata možemo da zaključimo da selen ima veliki uticaj na pravilno funkcionisanje mlečne žlezde krava.

Ključne reči: selen, somatske ćelije, mlečna žlezda, krava.

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RESULTS OF FEED CONVERSION, DAILY GAIN AVERAGE THICKNESS OF THE BACK AND SIDE BACON AND PERCENT OF MEAT IN PERFORMANCE TEST OF BOARS LANDRACE RACE (PHENOTYPIC PARAMETERS)*

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SUMMARY: To achieve a larger and more profitable production, breeding goal in swine production has to be focused on the repair of all economically important traits. It is important that in addition to good nutrition, care and keeping conditions, use of the best breeding animals,which is achieved by proper selection of the organization of work, selection of animals base d on test results and assessments of breeding values with modern methods to ensure highaccuracy. Analyzed the performance characteristics of the test boars Landrace breeds such as: Dutch, Swedish, German and Danish, originating from several farms in Vojvodina, in order to analyze the variability of different traits. Phenotypic variability properties of the aggregate genotype is high and within the quoted literature.Phenotypic variability properties of the aggregate genotype is high and within the quoted literature. Identified a statistically significant difference in the thickness of the side of bacon and meat percentage ($p<0.05$) and Duncan test were statistically significant differences in the thickness of back fat between Danish Landrace and other (Dutch, Swedish and German 8,60:14,37, 13.22 and 13.18 mm, $p <0.05$), then the thickness of the side fat between the Dutch and Danish (14,78:10,60, $p <0.05$) and the percentage of meat between the Danish and other Landrace (Dutch, Swedish and German 64,29:58,27, 59.62 and 58.79; $p <0.05$). Duncan test were not statistically significant differences in feed conversion and daily gain.

Keywords: *Landrace, boars, performance test, daily gain, backfat thickness, percentage of meat.*

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INTRODUCTION

To achieve greater and more cost-effective production, breeding goal must be focused on the repair of all economically important traits. It is essential to improving nutrition, care and housing conditions and applying theproper selection, based on the selection of animals by test results and evaluation of breeding values with modern methods of high accuracy, which will allow us to select the best breeding animals. A significant factor in increasing production efficiency is improved pig fattening properties, which include weight gain, feed conversion and percentage of meat (Imboonta et al., 2007). These properties can be measured directly or indirectly on animals and they have medium to high heritability, which is their genetic improvement can be achieved based on test results. To select a boar, we dispose of data on the results of his test (weight gain, feed conversion, back fat thickness, meat percente, number of teats, constitution, litter size and parity from which derived) (Vidovic and Kosarcic, 1998). Candidates for selection, ie, the next generation of parents should be selected based on several characteristics (Petrovic, 1991). Merx and Oijen, (1994) show that in the Netherlands at the nucleus of selection forgrowth traits and carcass quality based on information obtained in performance and sib testing.

Greater intensity of selection, requires an increasing number of animals tested and therefore, in many countries use the so-called. Farm test. One of the reasons is very expensive and capacity limited testing only in the central test stations (Sckolling et al., 1981; Hudson and Kennedy, 1985; Merx, 1987; Vidovic et al., 1993). At the level of commercial gilts and sows F1 generation is only phenotypic selection, without tests and experiments (Vidovic et al., 2011). Since the beginning of testing of breeding pigs to date, we were there more test methodologies, the recommendations of Markovic et al., 1963, Markovic et al., 1976, Drobnjakovic et al., 1988, Vidovic et al., 1993.

MATERIALS AND METHODS

Investigations were made on four breeds Landrace boars from Vojvodina in the period since 2007. through 2011, during the performance test. Included are farms(total18) which are registered at the Department of Animal Husbandry, Faculty of Agriculture in Novi Sad, as the Main breeding organization for APVojvodina. The study included a total of 699 tested Landrace boars of four breeds: Dutch (51); Sweden (337), Germany (306) and Danish Landrace (5). During the performance test, the aggregate genotype boars consisted of the following features: feed conversion (FC, kg) daily gain (DP, kg), average back fat thickness, (LS, mm), average thickness of the side of bacon, (BS, mm) and the percentage of meat, (PM, %).

Rating phenotypic parameters was performed in the program “Statistics 10” as follows: mean, standard deviation, coefficient of variation, minimum and maximum values, analysis of variance and Duncanov test.

RESULTS AND DISCUSSION

Average values and variability of phenotypic expression of traits in the aggregate genotype in 699 boars performance tested, originating from 18 farms in Vojvodina are shown in Table 1.

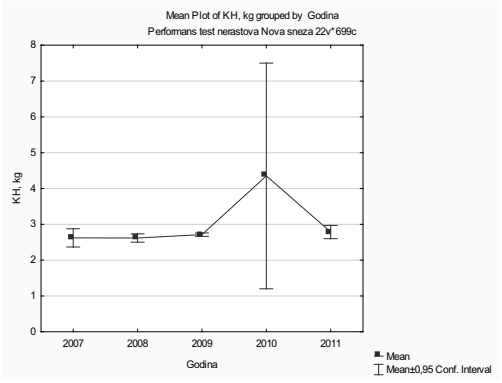
Table 1. Average values and variability of the traits of boars tested (699 boars tested)
Tabela 1. Prosečne vrednosti i varijabilnost ispitivanih osobina kod 699 testiranih nerastova

Traits / *Osobina*	$\overline{X}$	σ_X	σ	σ^2	CV%	Min	Max
FC,kg	2,28	0,01	0,33	0,12	14,86	1,45	4,83
DG, kg	0,827	0,005	0,136	0,0186	16,48	0,328	1,365
LS, mm	13,25	0,18	4,85	23,55	36,60	5,00	28,00
BS, mm	12,94	0,18	4,80	23,06	37,09	5,00	26,00
PM, %	59,19	0,17	4,62	21,37	7,80	44,07	72,33

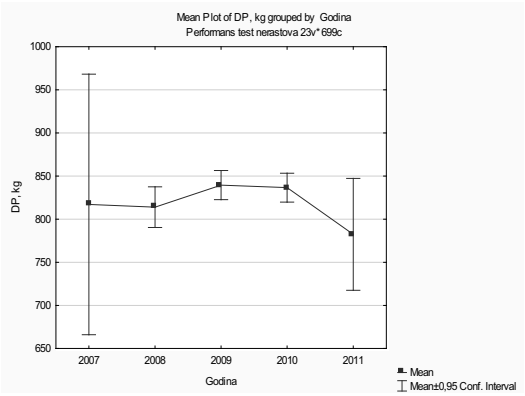
In Table 1 we see that the average feed conversion of all boars in the performance test was 2.28±0.01 kg, daily gain of 0.827±0.005 kg, back fat thickness of 13.25±0.18mm, thick side of bacon 12.94±0,18 mm and the percentage of meat in carcass 59.19±0.17%. Examining the performance test boars from Vojvodina, Jakovljev et al., (1987) found that the feed conversion in the test since 1982. to 1986. was reduced from 2.89 kg to 2.80 kg, an average of more test race and combinations. If we look at our results, we see that the conversion is drastically reduced (2.28) compared to the conversion in 1987. year.

According to the Short et al., (1994), the mean value for daily weight gain ranged from 770.4 to 868.2 g, which is consistent with our results (0.827 kg). The same author states that the average back fat thickness ranged from 11.6 mm to 14.7 mm, while in our data the average value was 13.25 mm and feed conversion of 2.16 kg to 2.31 kg, while in our results, feed conversion was 2.28 kg. Approximate results and other authors (Trivunović, 1996; Brkic et al., 2000; Mijatovic et al., 2006).

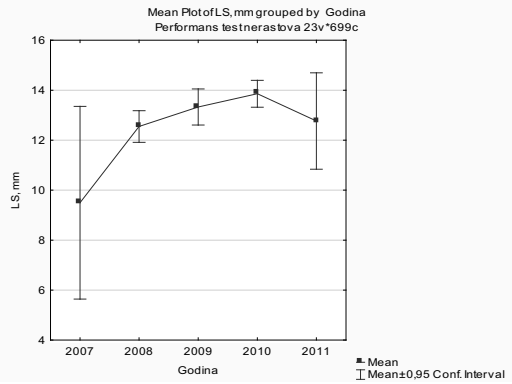
In Graph 1, 2, 3, 4 and 5 is shown a trend KH, DP, LS, BS and PM since 2007. - 2011. year.



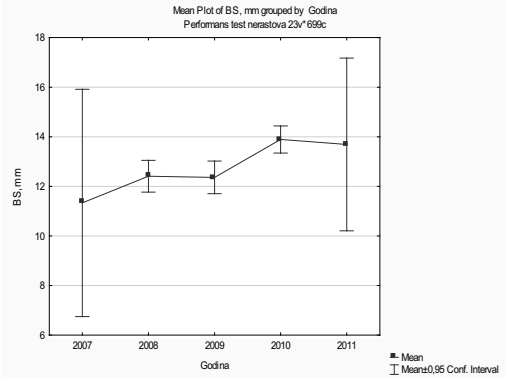
Graph. 1.The average feed conversion performance test
Graf. 1. Prosečna konverzija hrane u performans testu



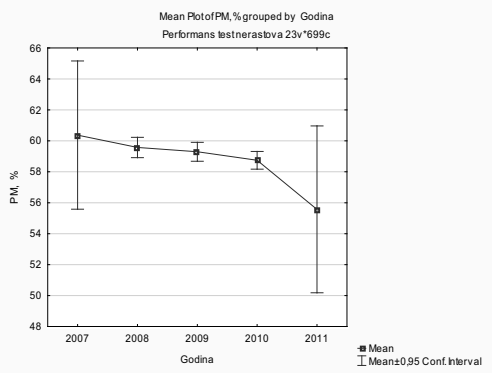
Graph. 2. Average daily gain in performance test Landrace since 2007-2011
Graf. 2. Prosečan dnevni prirast u performans testu landrasa od 2007.-2011.



Graf 3. Prosečna debljina leđne slanine u performans
Graph. 3. The average thickness of back fat Landrace



Graf 4. Prosečna debljina bočne slanine u performans testu landrasa od 2007-2011.
Graph. 4. The average thickness of the side of bacon Landrace performance test since 2007-2011.



Graph. 5. The average percentage of meat in the performance test Landrace since 2007.- 2011.
Graf. 5. Prosečan procenat mesa u performans testu landrasa od 2007. – 2011.

In Graph. 2. we see a slight decrease DP since 2009. to 2010. sharply declining in 2011. years with extreme variability. The research Mijatovic et al., (2006), according to average daily gain in Swedish Landrace, Large White and Duroc in the period since 1995. to 2011.year had adeclining trend. Shown in graph 3 and 4 is observed a slight increase in the average value of LS and BS by 2010. with a decrease in 2011. while the PM had a steady decrease (Graph 5). With all the traits expressed the large variability in 2011. year. Mijatovic et al., (2006), indicate that the early years of the test was statistically highly significant (P<0.001) and resulted in the variation of all traits of food utilization and almost all the investigated fattening properties, except for the duration of the test and the average daily gain

The content of meat in the carcass is one of the main indicators of quality of carcass. Timanovic, (2003), Tomovic, (2000), Petrovic, Milica and Pusic, (2004), Petrovic, Milica et al., (2006), Radovic et al., (2007) indicate a fairly consistent results on the content of meat in carcass (from 41.71 to 43.32%) which does not agree with the results of this study, where he established a general average content in the carcass meat for all breeds of Landrace 59.19 % with variation of 44.07 to 72.33%.

Average values and variability of phenotypic expression of traits in the aggregate genotype in Dutch and Swedish Landrace is shown in Table 2 and 3.

Table 2. Average values and variability of the traits of Dutch Landrace breed of 51-th tested boars
Tabela 2. Prosečne vrednosti i varijabilnost ispitivanih osobina holandskog landrasa (51 nerast)

Traits / *Osobina*	$\overline{X}$	σ_X	σ	σ^2	CV,%	Min	Max
FC,kg	2,31	0,03	0,28	0,07	12,11	1,78	2,88
DG, kg	0,792	0,013	0,096	0,0092	12,20	0,554	0,993
LS, mm	14,37	0,72	5,21	27,15	36,25	5,00	28,00
BS, mm	14,78	0,75	5,39	29,09	36,48	5,00	26,00
PM, %	58,27	0,80	5,78	33,41	9,91	47,97	72,33

Table 3. Average values and variability of the traits in the breed Swedish Landrace (337 boars tested)
Tabela 3. Prosečne vrednosti i varijabilnost ispitivanih osobina za rasu švedski landras (337 nerastova)

Traits / *Osobina*	$\overline{X}$	σ_X	σ	σ^2	CV,%	Min	Max
FC,kg	2,24	0,02	0,36	0,14	16,47	1,50	4,83
DG, kg	0,836	0,007	0,142	0,0201	16,98	0,328	1,365
LS, mm	13,22	0,27	4,99	24,96	37,76	5,00	28,00
BS, mm	12,73	0,26	4,88	23,82	38,33	5,00	26,00
PM, %	59,62	0,24	4,50	20,26	7,54	44,07	72,19

The average feed conversion in Dutch and Swedish Landrace amounted to 2.31 ± 0.03 and 2.24 ± 0.02 kg. The standard deviation for this trait was 0.28 and 0.36 kg, with a coefficient of variation of 12.11 and 16.47%.%. Average daily gain for the Dutch and Swedish Landrace was 0.792 ± 0.013, 0.836 ±0.007 kg, the absolute variations of the Dutch from 0.554 to 0.993 and 0.328 kg to 1.365 kg in Swedish Landrace. The average thickness of back fat in Dutch Landrace was 14.37± 0.72 and 13.22 in Sweden ± 0.27 mm, the coefficient of variation of 36.25 and 37.76%. The average thickness of the side of bacon in Dutch Landrace was 14.78 ± 0.75 mm and 12.73 ± 0.26 for the Swedish Landrace, with coefficient of variation of 34.48% and 38.33%. The resulting average feed conversion in the Swedish ladnas is lower than the results they get Teodorovic and Ninkov, (1972; 3.50 kg); Brkic et al., (2000; 2.74 kg); Mijatovic et al., (2005; 2.42 kg). Average daily gain was lower than in daily gain that received Brkic et al., (2000; 0.871 kg), Johnson et al., (2002) and Mijatovic et al., (2005; 0.865 kg). The average percentage of meat in the carcass of the Dutch Landrace, was 58.27 ±0.80%, with coefficient of variation of 9.91% and 59.62 S±0.24% Swedish Landrace, with coefficient of variation of 7.54%.

Average values and variability of phenotypic expression of traits in the aggregate genotype in German and Danish Landrace is shown in Table 4 and 5.

Table 4. Average values and variability of the traits in the breed German Landrace (306 boars tested)
Tabela 4. Prosečne vrednosti i varijabilnost ispitivanih osobina nemačkog landrasa (306 nerastova)

Traits / *Osobina*	$\overline{X}$	σ_X	σ	σ^2	CV%	Min	Max
FC,kg	2,31	0,01	0,31	0,10	13,38	1,45	3,04
DG, kg	0,824	0,007	0,1341	0,0179	16,27	0,418	1,197
LS, mm	13,18	0,26	4,61	21,32	35,02	5,00	27,00
BS, mm	12,91	0,26	4,58	21,03	35,50	5,00	26,00
PM, %	58,79	0,25	4,47	19,99	7,06	47,04	71,98

Table 5. Average values and variability of the traits of the Danish Landrace breed (5 boars tested)
Tabela 5. Prosečne vrednosti i varijabilnost ispitivanih osobina danskog landrasa (5 nerastova)

Traits / *Osobina*	$\overline{X}$	σ_X	σ	σ^2	CV%	Min	Max
FC,kg	2,41	0,07	0,17	0,03	7,35	2,21	2,63
DG, kg	0,824	0,091	0,205	0,0421	24,90	0,460	0,934
LS, mm	8,60	0,97	2,19	4,80	25,47	5,00	10,00
BS, mm	10,0	0,74	1,67	2,80	15,78	8,00	12,00
PM, %	64,29	1,63	3,65	13,39	5,69	59,25	68,25

The average feed conversion in the German and Danish Landrace was 2.31±0.01 and 2.41 ± 0.07 kg. The standard deviation for this trait was 0.31 and 0.17 kg, with a coefficient of variation of 13.38 and 7.35%. Average daily gain in the German and Danish Landrace was 0.824 ± 0.007; 0.824 ± 0.091 kg, with absolute variations in German Landrace from 0.418 to 1.197 and 0.460 to 0.934 kg in the Danish Landrace.

The average thickness of back fat in German Landrace was 13.18 ±0.26 mm, and the Danish 8.60 ±0.97 mm, with coefficient of variation of 35.02 and 25.47%. The average thickness of the side of bacon was 12.91 ± 0.26 mm for German and 10.00 ± 0.74 mm for the Danish Landrace, with coefficient of variation of 35.50% and 15.78%. The average percentage of meat in the carcass of German Landrace was 58.79 ± 0.25%, the coefficient of variation of 7.06% and in the Danish Landrace 64.29 ± 1.63%, the coefficient of variation of 5.69%.

Table 6. Analysis of variance of the traits in the aggregate genotype
Tabela 6. Analiza varijanse ispitivanih osobina u agregatnom genotipu

Traits / *Osobina*	SS - race *SS – rase*	df-race *df-rase*	MS - race *MS – rase*	SS - erors *SS – greške*	df- erors *df – greške*	MS – erors *MS - greške*	F	P
FC, kg	296,5432	3	98,84773	115511,2	695	166,2032	0,594740	0,618594
DG, kg	117218,2	3	39072,7	15178741	695	21839,9	1,789052	0,147867
LS, mm	173,9	3	58,0	16264	695	23,4	2,476328	0,060322
BS, mm	215,9	3	72,0	15882	695	22,9	3,149103	0,02453*
PM, %	277,0	3	92,3	15848	695	22,8	4,048626	0,00721*

* Marked effects are significant at p<0.050, *Vrednosti su statistički značajne p<0,050

**SS-sums of squares df-degrees of freedom, MS-square environment **SS-sume kvadrata; df-stepeni slobode; MS-sredine kvadrata

Analysis of variance (Table 6) were found statistically significant differences in BS, PM (p <0.05) and Duncan test were statistically significant differences in LS (Table 7) between the Danish and Dutch, Swedish, German Landrace (8.60 : 14.37, 13.22 and 13.18 mm, p<0.05), the BS (Table 7), between the Dutch and Danish Landrace (14,78:10,60, p<0.05) and PM (Table 9) between the Danish and Dutch, Swedish, German Landrace (64,29:58,27, 59.62 and 58.79, p<0.05). Duncan test were not statistically significant differences in the FC and DG.

Table 7. Duncan test for LS, BS and PM
Tabela 7. Duncanov test za LS, BS i PM

LS / *LS*	**Dutch / *Holandski*** $\overline{X}$ **14,37**	**Swedish / *Švedski*** $\overline{X}$ **13,22**	**German / *Nemački*** $\overline{X}$ **13,18**	**Danish / *Danski*** $\overline{X}$ **8,60**
Dutch / *Holandski*		0,481577	0,494649	0,000729*
Swedish / *Švedski*	0,481577		0,977698	0,006154*
German / *Nemački*	0,494649	0,977698		0,004830*
Danish / *Danski*	0,000729*	0,006154*	0,004830*	
BS / BS	**Dutch / *Holandski*** $\overline{X}$ **14,78**	**Swedish / *Švedski*** $\overline{X}$ **12,73**	**German / *Nemački*** $\overline{X}$ **12,91**	**Danish / *Danski*** $\overline{X}$ **10,60**
Holandski/Dutch		0,230019	0,244567	0,015383*
Švedski/Swedish	0,230019		0,908293	0,184865
Nemački/German	0,244567	0,908293		0,175245
Danski/Danish	0,015383*	0,184865	0,175245	
PM / *PM*	**Dutch / *Holandski*** $\overline{X}$ **58,27**	**Swedish / *Švedski*** $\overline{X}$ **59,54**	**German / *Nemački*** $\overline{X}$ **58,72**	**Danish / *Danski*** $\overline{X}$ **64,29**
Holandski/Dutch		0,462113	0,779219	0,000346*
Švedski/Swedish	0,462113		0,611988	0,003068*
Nemački/German	0,779219	0,611988		0,000765*
Danski/Danish	0,000346*	0,003068*	0,000765*	

* Marked effects are significant at p <0.050 * Vrednosti su statistički značajne p <0,050

The difference that is established between the Danish Landrace and others into the LS, BS and PM is the result of a small number of boars performance tested.

Lundeheim and Eriksson, (1984) found that the genetic improvement and the annual average daily gain in the Landrace breed test amounted to 6.0 g and feed consumption per kilogram of weight gain was reduced by 0.032 g, as a result of the direction and intensity of selection. Suzuki et al., (1991) analyzed data for 141 and 136 are not castrated boars of Landrace breeds, selected for 10 generations on the basis of the index to increase daily gain and back fat thickness reduction. The results showed that the increase in daily gain 19.47 g, and reduce back fat thickness of 0.04 mm per generation.

CONCLUSION

Analysis of performance test results and traits of boars included in the aggregate genotype, and based on quoted litetature may be drawn the following conclusions. Average daily gain was 0.827 kg, the absolute variations that range from 0.328 to 1.365 kg. Average back fat thickness was 13.25 mm, with the absolute variation of 5.0 to 28.0 mm. Average thickness of the side of bacon was 12.94 mm, with variations from 5.00 to 26.00 mm. Established a general average content in the carcass meat for all breeds Landrace was 59.19%, with variations from 44.07 to 72.33%. Comparing the average values of medium traits in the aggregate genotype was not a statistically significant difference between the four breeds Landrace. Phenotypic trait variability is high and within the quoted literature. Such high variability suggests that it is possible to apply an intensive selection work to improve the traits in the study population.

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REZULTATI KONVERZIJE HRANE, DNEVNOG PRIRASTA, PROSEČNE DEBLJINE LEĐNE I BOČNE SLANINE I PROCENTA MESA U PERFORMANS TESTU NERASTOVA RASE LANDRAS (FENOTIPSKI PARAMETRI)

IVAN V. RADOVIC; SNEŽANA TRIVUNOVIĆ, NENAD GVOZDENOVIĆ

Izvod

Oplemenjivački cilj u proizvodnji svinja usmeren je na popravku svih ekonomski važnih osobina, da bi ukupna proizvodnja bila veća i rentabilnija. Veoma važno je da pored poboljšanja ishrane, nege i uslova držanja, iz matičnih zapata iskoristiti najbolja priplodna grla, što se postiže primenom pravilne organizacije selekcijskog rada, izborom grla zasnovanog na rezultatima testiranja i ocenama oplemenjivačkih vrednosti savremenim metodama, visoke tačnosti. U radu su analizirane osobine iz performans testa nerastova rase landras i to: holandski, švedski. nemački i danski, poreklom sa farmi na teritoriji AP Vojvodine, kako bi se doneo zaključak o postojanju varijabilnosti različitih osobina. Fenotipsku varijabilnost osobina iz agregatnog genotipa je visoka i u granicama citirane literature. Utvrđene su statistički značajne razlike između landrasa u debljini bočne slanine i procentu mesa ($p<0,05$), a Duncanovim testom su utvrđene statistički značajne razlike u debljini leđne slanine između danskog i ostalih landrasa (holandski, švedski i nemački 8,60:14,37, 13,22 i 13,18 mm; $p<0,05$), u debljini bočne slanine između holandskog i danskog (14,78:10,60; $p<0,05$) i u procentu mesa između danskog i ostalih landrasa (holandski, švedski i nemački 64,29:58,27; 59,62 i 58,79; $p<0,05$). Duncanovim testom nisu utvrđene statistički značajne razlike u konverziji hrane i dnevnom prirast.

Ključne reči: landras, nerastovi, performans test, dnevni prirast, debljina slanine, procenat mesa.

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DETERMINATION OF CAPTAN, CHLORPYRIPHOS AND CYPERMETHRIN RESIDUES IN CHERRIES*

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SUMMARY: The paper deals with the determination of the residues of captan, chlorpyriphos and cypermethrin in the samples of cherry species Aida, Margit and Šandor grown in the orchards of Vojvodina according to Good Agricultural Practice. By the analysis of cherry samples with the application of GC-ECD, the detected values of captan and cypermethrin range from 0.39 to 1.44 mg/kg i.e. 0.01 to 0.08 mg/kg and do not exceed the MRLs of 5.0 i.e. 3.0 mg/kg respectively. The detected values of chlorpyriphos range from 0.21 to 0.29 mg/kg but taking into account the relative standard deviation the detected values may be above the MRLs of 0.3 mg/kg.

Key words: *cherry, pesticide residues, captan, chlorpyriphos, cypermethrin, GC-ECD.*

INTRODUCTION

The production of fruit in Serbia is economically important due to the favourable climatic and soil conditions for growing numerous fruit species among which cherries tend to be more and more significant. Serbia is 16th in the world for cherry production and in the total production it participates with only 2.7% (Ognjanov et al., 2011; FAO, 2010). Cherry fruits are predominantly used for table consumption and to a lesser extent for home-made or industrial processing into: stewed fruit, jam, fruit salad and juices (Milatović and Nikolić, 2011). In order to prevent the cherry production from being endangered by plant deseases agents *Blumeriella jaapii*, *Monilinia spp.*, *Stigmina carpophila,* phytopathogenic bacteria, viruses and pests *Rhagoletiscerasi* (cherry fly),

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Myzus cerasi (black cherry louse), *Aculus fockeui* (sour cherrypink rust mite) and *Rynchites auratus* (sour cherry weevil), it is necessary to use, besides agrotechnical measures, the chemical measures of protection as well (Miletić and Tamaš, 2011).

It is known that in the conditions of the contemporary agricultural production over 30% of the produce does not find its way to the consumers, whereas with the application of the chemical agents the loss will be doubled (Bursić et al., 2006; Bursić et al., 2009). There have been eight insecticide, one acaricide, eleven fungicide and one herbicide active compounds registered in our country (Sekulić and Jeličić, 2011). The pesticides affect the quality of the product and the yield increase but on the other hand they are dangerous because of the possible pesticide residues in products and as such a potential threat to consumers (Pucarević et al., 2010). The residues of pesticides in fruit are the consequence of their direct application in the agricultural production (Lazić et al., 2009). Due to their biocide activity and potential risk to the consumers, the concentration of pesticides must be kept at the minimum in fruits and has to be below the maximum residue limits (MRLs).

For the analysis of pesticides in fruit, simple treatment is required in order to isolate the compounds from the complex matrices (Vuković et al., 2010). Liqud-liquid extraction with organic solvents such as acetone, ethyl acetate, cyclohexane, acetonitrile and dichlormethane (Sannino, 2007; Wang et al., 2006) or solid phase extraction – SPE (Zroslikova et al., 2003; Topuz, 2005) were used to determine captan, chlorpyriphos and cypermethrin in fruit.

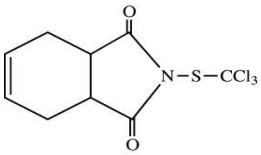
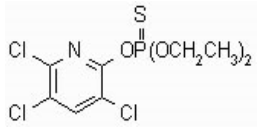
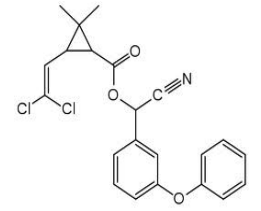
This paper will present the insight into the health safety of the analyzed cherry samples of the species Aida, Alex, Margit and Šandor, based on the residues of captan, chlorpyriphos and cypermethrin and it will certify whether the pesticide residues are within the regulated MRLs, which for captan is 5.0 mg/kg, for chlorpyriphos 0.3 mg/kg and for cypermethrin 1.0 mg/kg (Gazzete RS 25/2010). The pesticide residues determination will be carried out by gas chromatography with electron capture detector, GC-ECD.

MATERIAL AND METHODS

Chemicals and Apparatus. The analytical standards of captan (99.1%), chlorpyriphos (99.5%) and cypermethrin (95.8%) by Dr. Ehrenstorfer GmbH, Germany, were used. Table 1 shows the chemical characteristics of the compounds studied. The stock standard solutions in the concentration of 100 μg/ml of captan were prepared in acetone, chlorpyriphos in acetonitrile (ACN) (J.T. Baker) and cypermethrin in petroleum ethar (PE) (J.T. Baker). The 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 acetone, ACN and PE. The pesticide determination was carried out by the use of a GC-ECD (Hewlett Packerd Gas Chromatograph 5890 Series II) with splitless injection (2 μl) and capillary column SPB-5 (30mx0.32mmx0.25μm, Supelco, No 18441-03A). The carrier gas flow was 1 ml/min. The temperature of the injector was 250 ºC and of the detector 300 ºC. The temperature programme of captan and chlorpyriphos determination: the column temperature of 160 ºC maintained for 1 minute, the rise of 7 ºC/min to 250 ºC; of cypermethrin determination: the column temperature of 150 ºC maintained for 1 minute, the rise of 20 ºC/min to 260 ºC hold for 20 min. The total run time for captan was 7 min, for chlorpyriphos was

15 min, and for cypermethrin was 20 min. The retention time of captan was 3.37 min, of chlorpyriphos 12.95 min and of cypermethrin 10.82 min.

Table 1. Chemical characteristics of the pesticides studied
Tabela 1. Hemijske karakteristike ispitivanih pesticida

Name *Ime*	Chemical structure *Hemijska struktura*	Molecular weight *Molekulska težina*	Molecular formula *Molekulska formula*	K_{ow}* K_{ow}*	ADI* (mg/ kg b.w. daily) *ADI (mg/ kg t.m. dnevno)*	Oral LD* (mg/kg) *Oralna LD* (mg/ kg)*
Captan *Kaptan*		300.6	$C_9H_8Cl_3NO_2$	2.8	0.1	9000, rats
Chlorpyriphos *Hlorpirifos*		350.6	$C_9H_{11}Cl_3NO_3PS$	4.7	0.01	490, duck
Cypermethrin *Cipermetrin*		416.3	$C_{22}H_{19}Cl_2NO_3$	6.6	0.05	>10000, duck

*Tomlin (2006).

Validation. The detection limit (LOD) was determined as the lowest concentration giving a response of three times the average of baseline (SHI, 2009; SANCO, 10232/2006). 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 untreated cherry samples spiked with 0.03 μg/ml of captan, 0.001 μg/ml of chlorpyriphos and 0.01 μg/ml of cypermethrin were used. The linearity in the response was studied with a standard solution of captan ranging from 0.05 to 5.5 μg/ml, for chlorpyriphos from 0.05 to 0.5 μg/ml and for cypermethrin from 0.05 do 1.25 μg/ml. The recovery assays were performed by spiking untreated cherry samples with captan, chlorpyriphos and cypermethrin, each (separately) with the concentration of 0.05, 0.25, 0.5 and 1.0 μg/ml in three replicates.

Sampling. The treatment of cherries with the compounds based on captan, chlorpyriphos and cypermethrin was carried out at the end of April 2011 in accordance with GAP (Good Agricultural Practice) and the sampling was carried out at the end of June on the expiry of the pre-harvest interval of all the pesticides (PHI for captan is 21 days, for chlorpyriphos is 28 days and for cypermethrin is 28 days as well). The basic samples

of cherry species Aida, Alex, Margit and Šandor, 2 kg each, were collected from various orchards in Vojvodina. All the samples were kept in polyethilene black bags in deep-freeze until being analyzed.

RESULTS

Analytical method. By means of the ANOVA Single Factor and Regression Statistics all the validation parametars were calculated and shown in Table 2.

Tabe 2. Validation parameters
Tabela 2. Parametri validacije

	LOD (mg/kg) *LOD (mg/kg)*	LOQ (mg/kg) *LOQ (mg/kg)*	Prosečna vrednost prinosa ekstrakcije±RSD (%) *Average Recovery±RSD (%)*
Kaptan/*Captan*	0.030	0.090	94±3.57
Hlorpirifos/*Chlorpyriphos*	0.001	0.003	97±3.45
Cipermetrin/*Cypermethrin*	0.010	0.030	85±7.29

By checking the linearity of the detector response, the correlation coefficients of 0.994 for the captan, 0.998 for the chlorpyriphos and 0.991 for the cypermethrin were obtained. By meeting the basic validation parameters, the conditions for the reliable chromatographic analysis of the pesticides of interest in the samples of cherry species Aida, Alex, Margit and Šandor were checked and defined.

The obtained LOD and LOQ were low enough for the adequate determination of the captan, chlorpyriphos and cypermethrin residues in cherry samples.

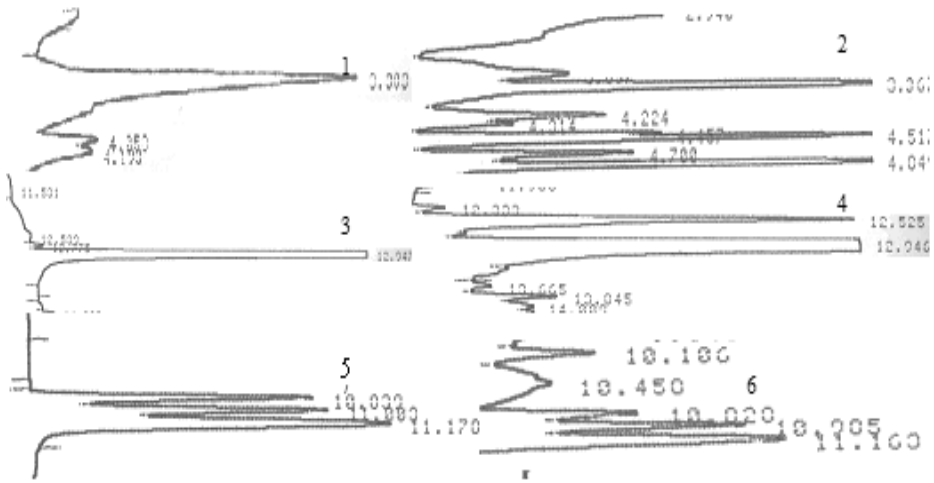


Figure 1. Chromatograms of captan (1), chlorpyriphos (3) and cypermethrin (5) standards and samples with detected captan (2), chlorpyriphos (4) and cypermethrin (6).
Slika 1. Hromatogrami standarda kaptana (1), hlorpirifosa (3) i cipermetrina (5) i uzoraka trešanja sa detekcijama kaptana (2), hlorpirifosa (4) i cipermetrina (6).

The detection and quantification of the pesticide residues were carried out based on the gas chromatographic analysis of standard captan, chlorpyriphos and cypermethrin and then by injecting cherry samples when, based on the retention times of the standards, the detection of captan, chlorpyriphos and cypermethrin in the samples was carried out.

Table 3. Captan, chlorpyriphos and cypermethrin content in cherry samples
Tabela 3.Ostaci kaptana, hlorpirifosa i cipermetrina u uzorcima trešanja

Sorte *Species*	Prosečne vtrednosti detektovanih ostataka (mg/kg) ±RSD(%)* *Mean value of residues (mg/kg) ±RSD(%)***		
	Kaptan *Captan*	Hlorpirifos *Chlorpyriphos*	Cipermetrin *Cypermethrin*
Aida	0.39 ±13.49	0.21±11.64	0.08±13.01
Alex	0.85±9.14	0.23±14.11	0.01±11.42
Šandor	1.33±7.95	0.24±12.50	0.01±7.69
Margit	1.44±5.19	0.29± 8.97	0.01±11.11

*Svaki uzorak je analiziran tri puta u dva ponavljanja
**Each sample was individually analized three times in duplicates*

DISCUSSION

By the statistical analysis of the studied validation parameters (LOD, LOQ, linearity and recovery) the LODs for captan, chlorpyriphos and cypermethrin were 0.030, 0.001 and 0.010 mg/kg with the LOQs of 0.090, 0.003 and 0.030 mg/kg, respectively. The linearity of detector response with the regression coefficient in the range of 0.991-0.998, confirms the linearity. The recovery for the analyzed pesticides ranges from 85.0-97.0% (RSD<8.0%).

Based on the presented values of captan in the analyzed cherry samples, the detected values ranged from 0.39 do 1.44 mg/kg and do not exceed the maximum residue value of 5 mg/kg. The detected values of chlorpyriphos range from 0.21 to 0.29 mg/kg but taking into account the relative standard deviation the detected values may be above the MRLs of 0,3 mg/kg. The residue values of cypermethrin are from 0.01 to 0.08 mg/kg and do not exceed the MRLs of 3.0 mg/kg, as regulated (Gazette RS 25/2010). It is interesting to note that the MRLs of captan in cherries according to Codex Alimentarius is 25 mg/kg and according to the Canadian and Australian regulations 5 and 15 mg/kg respectively (Hugh, 2011).

According to the available data in Slovenija out of the total 391 analyzed samples (10 cherries), 3.3% exceeded the MRLs for acetamiprid, captan, chlorothalonil, cyprodinil, fludioxonil and folpet (Česnik et al., 2008). Based on the results of the study of pesticide residues in fruit carried out by Pucarević et al. (2010), the most frequently detected pesticide residues in different fruit samples were exactly captan, chlorpyriphos and cypermethrin.

CONCLUSION

By the statistical analysis of the studied limit of detection, limit of quantification, linearity and recovery, as the basic validation parameters needed for this kind of labora-

tory certification of the method, the LODs for captan, chlorpyriphos and cypermethrin were 0.030, 0.001 and 0.010 mg/kg with the LOQs of 0.090, 0.003 and 0.030 mg/kg, respectively, which enabled the detection of very low amounts, below the MRLs, of the studied analytes. The linearity of detector response with the regression coefficient in the range of 0.991-0.998, confirms the linearity. The recovery for the analyzed pesticides ranges from 85.0 to 97.0% (RSD < 8.0%). The obtained validation parameters prove that the qualitative analysis of the treated samples of cherry species Aida, Alex, Margit and Šandor, grown in various orchards throughout Vojvodina according to Good Agricultural Practice – GAP. In the analysis of cherry samples with the application of GC-ECD, the detected values of captan range from 0.39 to 1.44 mg/kg and do not exceed the maximum residue level of 5 mg/kg. The detected values of chlorpyriphos range from 0.21 to 0.29 mg/kg but taking into acount the relative standard deviation the detected values may be above the MRLs of 0.3 mg/kg. The values of cypermethrin are from 0.01 to 0.08 mg/kg and do not exceed the MRLs of 3.0 mg/kg, as regulated (Gazette RS 25/2010).

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ODREĐIVANJE OSTATAKA KAPTANA, HLORPIRIFOSA I CIPERMETRINA U UZORCIMA TREŠANJA

VOJISLAVA BURSIĆ, SANJA LAZIĆ, MIRA PUCAREVIĆ,
FERENC BAGI, SLAVICA VUKOVIĆ

Izvod

U radu je prikazano određivanje ostataka kaptana, hlorpirifosa i cipermetrina u uzorcima trešanja sorti Aida, Alex, Margit i Šandor, gajenih u voćnjacima u Vojvodini u skladu sa dobrom poljoprivrednom praksom. Analizom uzoraka trešanja primenom GC-ECD, detektovane vrednosti kaptana i cipermetrina se kreću u intervalu od 0,39 do 1,44 mg/kg, odnosno 0,01 do 0,08 mg/kg i ne prelaze MDK od 5,0, odnosno 3,0 mg/kg. Detektovane vrednosti hlorpirifosa, kreću se u intervalu od 0,21 do 0,29 mg/kg, ali uzimajući u obzir relativnu standardnu devijaciju, detektovane vrednosti mogu biti iznad MDK od 0,3 mg/kg.

Ključne reči*:* trešnje, ostaci pesticida, kaptan, hlorpirifos, cipermetrin, GC-ECD.

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VEGETATION AS AN BIOLOGICAL MEASURE FOR FLOOD CONTROL

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SUMMARY: The aim of research is improvement of the quality of the landscape structure of Jaša Tomić village for adequate flood protection. A multidisciplinary approach to the researched problem was contributed to the economic, social and environmental perspective. Based on analysis of existing conditions, and guided by the experiences of international examples, it has been proposed a biological method of flood control for the banks of Jaša Tomić village. This method of defense is implemented by connecting hydrophilic fragments of native forests, but also by improving vegetative fund of non-indigenous species adaptable to the environmental conditions.

Key words: *flood, the biological form of defense, floodplain vegetation.*

INTRODUCTION

Due to extremely high water levels in a rivers, water from their beds raises over the coast and flows surrounding area. Floods are natural large-scale phenomena that can endanger human life or cause damage to a large extent. They can be viewed as a natural hazards and as a natural risks. Natural hazard means any natural phenomenon that could be a danger to human life, property or human interests. If flooding occurs in a limited duration, in a clearly defined geographic area, with accurate consequences and if the interactions between humans and natural processes resulting in significant property damage, injury or loss of life, then a natural hazard becomes a natural risk.

The essence of understanding the natural phenomena like floods is dealing with their history. These are the events that come over and again, and thus the study of their past will provide appropriate information for reducing a degree of an impact that they can bring to people and environment in the present or future. Floods can be regulated in several ways. First of all, by legal policies, monitoring of meteorological forecast, by

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raising the flood protection facilities, and by biological methods. Raising the protective forest belts along the streams brings positive effects, because does not allow the local snow accumulation and soil erosion; it can improve air mode, and it could implement a surface water runoff into the interior of the land (Lješević, 2002). At the same time, these protective forest belts could reduce the hazardous effects of flooding consequences to the environment.

Serbia has been struggling with flood hazard far away in the past. The largest floods were recorded in the basin of Tisa, Danube and Sava (Gavrilović, 1981) - the three largest rivers in Vojvodina (Table 1.). The first observations of water levels on rivers in Serbia started in the first half of the nineteenth century and the first one was in the territory of Vojvodina. The oldest hydrological station was founded in the 1819 in the city of Novi Sad, then in the city of Novi Bečej (began its work after 36 years). As Gavrilović (1981) said, in the period of thirty years, since 1955 to 1985, in Serbia the floods with catastrophic proportions has been occurred every two years. One of the major flood occurred in 1955 when the total damage amounted to about 9.3 million old dinars, of which only in Vojvodina the damage was estimated at 7.3 billion old dinars. Ten years later in 1965 the major watercourses affected another floods of large-scale.

Table 1. Built constructions of the embankment of important river basins in Vojvodina
Tabela 1. Izgrađenost nasipa važnih rečnih slivova u Vojvodini

river/*reka*	length of the constructed embankments/*dužina izgrađenog nasipa (km)*
Dunav	304,16
Tisa	289,63
Sava	119,77
Tamiš	86,04
Stari i Plovni Begej	127,80
other enbankments	532,62

Tamiš with Begej belongs to the categories of the rivers, which were the first one that started with reclamation works in the Pannonian Plain since 1728 in the Austro-Hungarian period. Large floods in the
past were associated with Tamiš river, and in the year of 2005 this river has experienced perhaps the largest ever recorded water spills on its banks. The catastrophic flood completely flooded the village Jaša Tomić, located in the far east of central Banat, in the municipality Sečanj (Grupa autora, 1998).

MATHERIAL AND METHOD

As Kandilioti and Makropoulos (2011) pointed out, an appropriate method for effective study of the area potentially affected by floods, should provide a detailed analysis of a researched space, according to the size of the problem, and such methods, that require extensive data and considerably effort, can not be rely on modeling. Also, the same authors suggest that the most appropriate approach to this issue is an analysis of multiple criteria, which this paper seeks.

In order to adequately regulate the floods in the village of Jaša Tomić, also to built a defensive model against the flooding and applicable to the entire territory of Vojvodina, it has beeen joined multidisciplinary to the study of the regulation of this problem through a social, an economic and ecological aspects. On the one hand, through the

comparative method, there have been compared examples of the world practice (biological methods of flood protection), with local experience and it has been given a guideline for further activities in the study area. On the other hand, in order to improve future planning and space it was analyzed the current state of landscape structure within the village Jaša Tomić by making an unique check lists. Making a check lists serve as the method of assessment and the method of evaluation of existing space conditions, whether it is for an inventory of existing conditions, the development of the planning ideas or simply for the state of environmental impact assessment. Check list conducted in this study was done on the basis of the relevant examples, which were given by Kandilioti and Makropoulos (2011). Investigated parameters and criteria were adapted to the issue of the work involved. In particular, the selection criteria for evaluating the natural complex planning, were based on general theoretical knowledge, intuition, experience, attributes fields for which the plan is working, based on current needs and planning practice in Serbia.

The survey was conducted during 2010 and 2011 year. In order to encourage biological methods of flood protection, the work gravitated to examination of the present state of the vegetation, so the check list that was used for the analysis was the check list of the space greenery (Table 2.) throughout the evaluation of the composition, form, color, mosaic elements and so on.

Analysis of the greenery, carried out in the relation to the examinated criteria, which were based on the desire for the formation of high landscape architectural quality, environmental and spatial composition. By observing the vegetation analysis, the criteria were evaluated in order to obtain biodiversity and bio-engineering diverse composition. There have been set out following positive parameters: a balanced relationship between broadleaves and conifers, expressed vegetation floors and composition in accordance with the natural characteristics of the space, good maintenance of open space, its wealth of colors and smooth shadows and light ratio, as well as physical and visual barriers. As a negative remaining parameters there have been assessed values within the stated test of the checklist.

Table 2. Check list for analyzing the space greenery
Tabela 2. Lista procene postojećeg stanja zelenila

Category of the greenery/ *Kategorija zelenila*	Number/ *Broj*		(%)		General mark/ *Opšta ocena*
conifers	-		-		positive negative
broadleaves	-		100%		
note	It is hard to speak about exactly number of trees because they are in the form of masses.				
Greenery floor/ *Spratnost zelenila*	<10%	10-40%	40-70%	100%	General mark/ *Opšta ocena*

high greenery	-	-	x	-	positive negative
middle greenery	x	-	-	-	
low greenery	-	-	-	-	
ground flowering greenery	-	-	-	-	
lawn	-	x	-	-	
note	Middle greenery is a wild, and direct reflects to the greenery floor.				
Composition of the greenery/ *Kompozicija zelenila*	<10%	10-40%	40-70%	100%	General mark/ *Opšta ocena*
groups	-	-	-	x	positive negative
tree lines	-	-	-	-	
soliter trees - soliters	-	-	-	-	
note	The composition is monotonous.				
Mark/ *Ocena*	Bad/ *Loše*	Good/ *Dobro*	Excellent/ *Odlično*		General mark/ *Opšta ocena*
maintaining	-	x	-		positive negative
note	The environment is not too degraded, and the maintenance is solid.				
Space feeling/ *Osećaj u prostoru*	Monochrome/ *Monohrom*		Reach in colours/ *Bogat bojama*		General mark/ *Opšta ocena*
couloring/ kolorit	x		-		positive negative
	Sunned areas/ *Osunčanost*		Shadowed areas/ *Senka*		
light	20% 40% 60% 80% 100%		20% 40% 60% 80% 100%		
	Geometric/ *Geometrijska*		Organic/ *Organska*		
form	-		x		
note	A dominance of green colour is emphasized.				
Position/ *Pozicija*	Positive contribution/ *Pozitivan doprinos-opis*		Negative contribution/ *Negativan doprinos-opis*		
физичке и визуелне баријере	The protective role of greenery is highlighted.		Physical separation of the settlements and the river.		
GENERAL MARK OF THE PRESENT STATE OF SPACE GREENERY/ *OPŠTA OCENA STANJA ZELENILA U PROSTORU*					
Vegetation is balanced. There is a uniform relation between the mass and open space. The space is very monotonous, mainly represented by poplars and invasive species in the first floor of vegetation. There is a lot of open grass areas, which is evaluated relatively positively, because it opens up pleasant visions to the Romanian coast.					

RESULTS

By analyzing the the present state of the space and by conducting the check list, it was observed an absolute representation of broadleaves. The presence of conifers and other relevant plant species was not observed. In this way it was created one monotonous and monochrome space, and the estimation of this parameter is negative. The composition of vegetation in the area is very modest - one hundred percentage of the groups'

vegetative form presence, so it could be said that there are the positive values of this area in the terms of composition. The form is organic character, where forests and meadows alternating, at the same time light and shadow (Figure 2). The form was evaluated positively. The floors of greenery (Figure 1) are not clearly expressed. There is a domination of high vegetation and the score is negative. In the area of landscape structure it has been noticed the fragmentation of hydrophilic forests (Figure 4.A), which reflect negatively on the existing biodiversity. The existing vegetation is very good potential for the creation of coastal water protection greenery. On the other hand vegetation alone gives the impression of physical space barriers, by surrounding the settlement, which was assessed as negative effect.

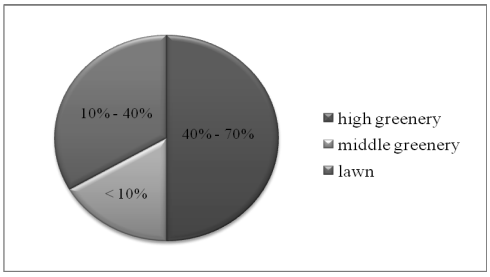

Figure 1. Percentages of green floors
Slika.1. Procentualno izražena spratnost zelenila

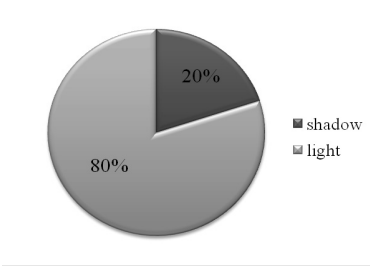

Figure 2. Space in the terms of percentages of sunshine
Slika 2. Procentualno izražena osunčanost područja

In the rational use and protection of river water areas and streams, one of the main measure is the regulation of their water-protective vegetation (Vujković, 2003). Vegetation affects the reduction of groundwater levels in the lower flows, thanks to absorptive power of the underground parts of the plants. It affects that the water slowly drains to streams, thus reducing the likelihood of spring flooding, and creates more abundant waterways (Vujković, 2003). According to Vujković (2003) it is also pointed out that the forest and parks plantations on the banks of river waters, fully express their capabilities to chain the ground and river enbankment, so their waterproof function is very valuable. Precisely, in these areas that are clearly expressed soil erosion.

Since the coast is exposed to the strong intensive water-blows and to changing the water level, field afforestation is desirable, in order to provide stability and faster absorption in the period of the increasing the flood waters. On the other hand, it is necessary to pursue the connection of forest fragments (Figure 4.B) with the aim of restoring

and improving the biodiversity habitats (Turner, 2003). In this process, presence of the monocultures should be avoided and the work should be direct to diversity of the vegetative composition, in biological terms, but in the floors also.

The studied area belongs to alluvial - hydrophilic forest, type of wetland forests of alliance *Alnion glutinosae* (Table 3), forests of white willow and poplar alliance *Salicion albae* (Table 4) and oak forests association *Genisto elatae - Quercetum roboris* (Table 5), so their planting is recommending. However, in order to create a harmonious composition if it proves as feasible, it should strive to introduction of the non-indigenous species, resistant to the natural space conditions. In the case of Jaša Tomić those are species resistant to wet areas, salt and clay soils (Table 6) (Jović et al., 1996).

Table 3. Swamp forests of alliance *Alnion glutinosae*
Tabela 3. Močvarne šume crne jove sveze Alnion glutinosa

woody forms/ *drvenaste forme*	herbaceous forms/ *zeljaste forme*
Salix cinerea L., *Fraxinus angustifolia* Vahl., *Alnus glutinosa* L.	*Phragmites communis* Trin., *Glyceria maxima* (Hartm.) Holmb., *Eleocharis palustris* L., *Carex elongate* L., *Carex leporine* L., *Juncus effuses* L., *Galium palustre* L.

Table 6. Potential forest and shrub speaces tolerante on salts in the ground
Tabela 6. Izbor potencijalnih vrsta drveća i žbunja tolerantnih na soli

species tolerant to the presence of salts in the soil/ *vrste tolerantne na prisustvo soli u zemljištu*	tree and shrub species/ *vrste drveća i žbunja*
very tolerant	*Fraxinus angustifolia* Vahl., *Chamaecyparis lawsoniana* (A. Murray) Parl., *Thuja orientalis* L., *Tamarix tetrandra* L.
middle tolerant	*Acer platanoides* L., *Acer campestre* L., *Sophora japonica* L., *Robinia pseudoacacia* L., *Pyrus piraster* L.
poorly tolerant	*Morus alba* L., *Gleditsia triachantos* L., *Ribes aureum* Pursh., *Caragana arborescens* Lam., *Rhus typifina* L.
tolerant to the salt in soil	*Platanus acerifolia* (Aiton) Willd., *Malus silvestris* L., *Maclura aurantiaca* Nutt., *Crataegus monogyna* Jacq.

The research has shown that the space itself is very rich in monocultures and these are mostly species of willows and poplars in 90% of the examined territory. The fragments are observed with *Alnus glutinosa* L. and some other species adaptive to occasional flooded areas.

At water flows, largely made up from broadleaved forests, the most favorable planting is with *Alnus glutinosa* L. However, in order to avoid the already mentioned danger of monocultures, but also to achieve biodiversity and ecological effects (changes of the light and darkness, heat flow of various air streams etc.) besides planting *Alnus glutinosa* L. it is recommending the planting of other plant species as so woody as herbaceous. So called "living material" involves the useage of *Alnus glutinosa* L., *Quercus robur* L., *Salix alba* L. etc., depending on space conditions (Cvejić, 1999).

Alnus glutinosa L. root penetrating up to 1.5 m deep in the ground water in the form of a dense palisade, ensuring lower parts of slopes from erosion. Fine roots in the zone of water level changes, providing the upper surface of erosion, affect at the formation of biotopes and eventually make the image of landscape beautifully. Young plants are planted at a distance 100cm - 150cm, holes are up to 20cm - 40cm above the middle summer waters. If the planting is done in a row, then striking the coast should be planted with *Alnus glutinosa* L. and the interior could be planted with some other species. But if it is planted in two rows, then the internal row should exclusively be from *Alnus glutinosa* L., and the external from the other species (Figure 3) (Cvejić, 1999).

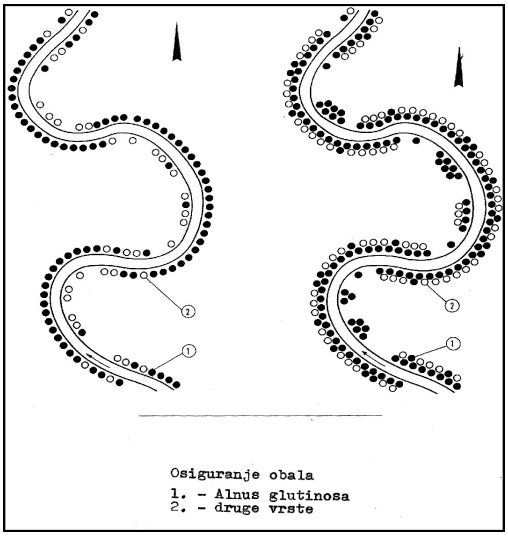

Figure 3. Protecting the river's coast with the roots of state of the research area Alnus glutinosa L. (1.- Alnus glutinosa L., 2.- other species)
Slika 3. Osiguranje obala korenom Alnus glutinosa L. istraživanog područja (1.- Alnus glutinosa L., *2.- druge vrste)*

A.

B.

Figure 4. A. Map of the present state of the research area
B. Map of the planning state of the research area
Slika 4. A. Karta postojećeg stanja istraživanog područja
B. Karta planiranog stanja istraživanog područja

Also, for the purpose of flood control and avoiding the formation of single crops, and according to natural conditions it is proposed a plantation of a certain types of clones such as clones *Populus x euramericana* Dode (Guinier) and *Populus deltoides* W.Bartram ex Humphry Marshall (Orlović et al., 2006).

In order to create a dynamic, functional (in the purpose of flood control), and above all colorful composition, which in its current condition rated negatively, it must not be neglected the aquatic flora as well. In the case of Jaša Tomić it is suggested an emerzion vegetation of alliance *Phragmition communis*, which proved to be resistant in this area. On the other hand, one should carefully choose the species of this alliance, in order not to cover whole rivers' bed due to an expansive spreading (Džigurski et al., 2011). The consequence of it is directly related to the flooding of river banks.

CONCLUSION

The floods in Serbia have increase ratio, as well as water levels. Almost every year some waterways leave their beds and flooding larger or smaller areas, thus endangering people and their material wealth. Funds invested now in the protection and flood defense can not be even remotely comparable to the effects that the foods cause, whether there are a visible consequences or with a prolonged negative effects on the environ-

ment. Technology and organization of flood protection must be constantly improved, and this work shows a little neglected way of flood control - biological methods.

Formation of landscape composition must be first in accordance with certain natural characteristics considered to be appropriate and acceptable for the environment and its users. Priority should be directed towards the restoration of native vegetation. However in the process of designing landscape composition it may be used non-indigenous species as well, resistant to the natural characteristics of the relevant settlement. These are figures that should form the basis for further planning and project structure of the landscape composition.

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VEGETACIJA KAO BIOLOŠKA MERA ODBRANE OD POPLAVA

IVANA BLAGOJEVIĆ, ANA GAČIĆ, JELENA ČUKANOVIĆ,
EMINA MLADENOVIĆ

Izvod

Cilj rada je unapređenje kvaliteta predeone strukture naselja Jaša Tomić radi adekvatne odbrane od poplava. Multidisciplinarni pristup istraživanom problemu doprineo je sagledavanju problema iz ekonomskog, sociološkog i ekološkog ugla. Na osnovu analize postojećeg stanja, a vođeni iskustvima svetskih primera, na obalama naselja Jaša Tomić predlaže se biološki način odbrane od poplava. Ovaj način odbrane se sprovodi povezivanjem fragmenata autohtonih higrofilnih šuma, ali i unapređenjem vegetacijskog fonda alohtonim biljnim vrstama prilagodljivim uslovima sredine.

Ključne reči: poplave, biološki vid odbrane, vegetacija plavnih područja.

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SOME EXPERIENCES AND CONCERNS ABOUT THE PRIVATIZATION OF AGRICULTURAL LAND

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SUMMARY: Privatization has been a term frequently mentioned in the past decades by both governments of transition countries and governments of developed countries. Privatization is a socio-economic process, which involves changes and as such becomes a world trend. The privatization process is usually initiated by the difficult economic situation, high debts, or a process initiated by the global trend of moving from one economic system to another. Privatization should, together with other transition reforms, accelerate the change of economic structure, foster entrepreneurship, improve competitiveness, all of which should result in the creation of an appropriate market environment in which it would be possible to implement the transition to a market economy. Privatization should provide clear and open ownership structure that will provide the entry of strategic investors into state economy. The authors of this paper discuss the current issue of privatization of agricultural land from the viewpoint of countries in transition. The authors also point out some issues and current views regarding the privatization of land in the Republic of Serbia and the new agreement with the EU.

Keywords: *privatization of agricultural land, transition, agriculture, agro-industry, the Republic of Serbia.*

INTRODUCTION

In Serbia, 87 percent of 4.2 million hectares of total arable land is privately owned and only 13 percent belongs to the state and agricultural companies. The average size of a household in Serbia is three, in Denmark, for example, 43 and in UK 69ha. Even more dramatic is the fact that only 2.9 percent of arable land in Serbia accounts for households bigger than 5.000 ha. There are 700.000 households, of which just about

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90.000 are registered. The ownership structure is quite diverse, and owners are predominantly ex-merchants, craftsmen, economists, doctors, who all make so-called new *agrobusinessman*. In transition countries in our region the privatization of agro-industry was carried out in different ways and the chosen method of privatization depended on the size and strategic importance of processing capacities. The privatization of small processing facilities in most countries was carried out through the sale, while the more complex procedure was applied in large systems or enterprises whose value is turned into equity. A transfer of shares was carried out in different ways.

In the Eastern countries, agricultural land was partly owned by the state, and in some countries of the Soviet Union completely, so that in the transition process as a central element of the reform of the agricultural sector of these countries there was a need for restructuring and privatization. This segment of reform has the greatest impact on the transition of agricultural sector, having in mind not only the economic but also strong social implications. The process of transition of agricultural sector has caused ownership, production and organizational structure changes in this countries. The expropriation in Eastern Europe was conducted differently and it demanded the implementation of restitution or compensation. The common characteristic of de-collectivization of the land is re-privatization (restitution) of land in collective ownership and sales of state enterprises. In cases where the land that they owned before collectivization could not be restored, the previous owners were given ownership rights to the land surface compatible.

The former socialist countries are far from us. They have transformed from kolkhoz – sovhozna systems into modern agricultural structure modelled on the EU concept. The experiences of former socialist countries show that that in the first five years of transition (1989-1994) there was a drop of performance, and subsequently, over the next five years (1994-1999) came to revive of the economy.

MODELS OF LAND PRIVATIZATION

Privatization of agricultural land is different from privatization of building land. Privatization of agricultural land is primarily released from the complications of connecting buildings related to land, as is the case with urban land. Also, the state land is not indispensably preconditioned for the survival of agricultural and agro-industrial companies that use it. Such features of agricultural land in state ownership certainly facilitate its privatization (Begović i sar., 2006). The process of privatization of agricultural land was carried out in different ways in countries in transition and using different models of privatization.

In the **Baltic countries** (Lithuania, Latvia, Estonia), where 100% of the land was taken away, the restitution or compensation was carried out. For these countries it is characteristic that in the process of agricultural policy reforms was implemented so-called "Shock therapy". At the beginning of the transition period agricultural support and import protection have dramatically reduced, national economy was opened to foreign competition and holdings were more taxed than encouraged, and in the second phase (late 1990's) support level increased and reached level similar to other countries (Pejanović, 2005).

In **Romania, Bulgaria, The Czech Republic and Slovakia**, where it was taken

around 90% of the land, have also made full restitution, but in stages (the assignment of land in small quantities). The reforms of agricultural policy in the Czech Republic and Slovakia were carried out gradually, while the level of support to agriculture in Romania and Bulgaria was very unstable. In restoring the land to the farmers in Romania there was a huge fragmentation of tenure, most of the new owners remained in the cities and never engaged in agriculture, the aging processes of villages and 'deagrarization' spread to the villages. The process of enlargement of agricultural properties in Romania has been very slow.

During the Soviet Union, the agriculture in Ukraine was organized through two centrally controlled sectors – kolkhoz and sovhoz. The reorganization of kolkhoz/ sovhozn sector began in 1992 year. Till year 2000, almost 36 000 farms were created, with 1.162 million hectares of agricultural land, with an average size of farm of 32ha (Bondar, 2002). The regulation of the Ministry of Ukraine "On Privatization of land parcels" from December of 1992 played a significant role at the beginning of the privatization of land. In Ukraine, about 70% of agricultural land transferred from state ownership into the ownership of 6.6 million inhabitants of rural areas with no charge (Bondar, 2002). These new owners exercised their right in the form of owners of land shares. The regulation of the land from January 2002 used this land shares as a basis for further privatization of agricultural land. According to this regulation, the citizens of Ukraine who wanted to buy agricultural land had to have education in agriculture, experience in agricultural business, or be associated with agricultural production. The Regulation contains important restrictions on agricultural land, such as: to 2010 the citizens of Ukraine may possess a maximum of 100 hectares of agricultural land; by 2005 the holders of land shares are prohibited from selling or donating their share to someone else; the owner of agricultural land can be either a citizen of Ukraine or company, whether is a citizen of either a foreigner, with two constraints - foreign citizens and foreign legal entities cannot have any agricultural or other land if it is outside of the village or there are no pre-made objects.

In 2002, **Russia** has passed a new law on land, which allows the sale of agricultural land, with certain restrictions.

The privatization in **Poland** was conducted by direct sale of state land. In pre-transition period Poland had a large share of privately owned land in total area, and there has been an increase in the size of private farms, as a result of land sale of state firms.

The process of land privatization has led to the establishment of a new ownership structure, with a growing number of farms and their significant participation in the farmland fund. The common characteristic of the CEE countries during the transition period was that these countries did not foresee the possibility of returning the land to foreigners, and that at first was not possible land purchase to foreigners (it was the result of negotiations).

The exception to the restitution is the voucher privatization and distribution to the employees in collective farms in **Hungary** (in this way was privatized about 2/3 of the cooperative land).

Table 1. Modles of privatization of agricultural land in CEE countries (Central and Eastern Europe)
Tabela 1. Modeli privatizacije poljoprivrednog zemljišta u zemljama CIE – Centralne i istočne Evrope

Country	Collective farms	State farms
Bulgaria	Restitution	Others
Czech Republic	Restitution	Sale / lease
Hungary	Restitution Distribution Sales for compensation bonds	Sales for compensation bonds Sale / lease
Latvia	Restitution	Restitution
Lithuania	Restitution	Restitution
Poland		Sale / lease
Romania	Restitution Distribution	Restitution Not decided
Slovakia	Restitution	Sale / lease
Slovenia		Restitution
Estonia	Restitution	Restitution

Source: Zekić, S., 2003.

CEE countries have begun the transition from very different initial levels and the effects of these initial differences are shown in Table 2.

Table 2. The structure of agriculture in the CEE according to use of land in pre and post transition period
Tabela 2. Struktura poljoprivrede u zemljama CIE prema korišćenom zemljištu u pred i post tranzicionom periodu

Country	Share of total agricultural area (%)							
	Cooperatives		State-owned enterprises		Other corporate farms		Private farms	
	Period before transition	Period after transition	Period before transition	Period after transition	Period before transition	Period after transition	Period before transition	Period after transition
Hungary	80	28	14	4	-	14	6	54
Czech Republic	61	43	38	2	-	32	0	23
Poland	4	-	19	7	-	8	77	82
Estonia	57	-	37	-	-	37	6	63
Slovenia	-	-	8	4	-	-	92	96
Latvia	54	-	41	1	-	4	5	95
Lithuania	-	-	91	33	-	-	9	67
Bulgaria	58	42	29	6	-	-	13	52
Romania	59	12	29	21	-	-	12	67
Slovakia	69	60	26	15	-	20	5	5

Source: EU Commissiona (1998).

Slovenia, in contrast to other transition countries, retained their traditional ownership structure of agriculture. More than 99% of farms remained in private ownership and just a smaller part of farms were nationalized. These characteristics caused minimal changes in the ownership structure in agriculture. From 1991 and with the process of denationalization and privatization in Slovenia and the adoption of the Low of decentralization, all agricultural land and all forests, with which conglomerates and state farms were disposed, were transferred into state ownership. The Fund of agricultural land and forests of the Republic of Slovenia was established, which had the function of managing the land, deciding to return the land to their original owners in the process of denationalization and giving land to lease agricultural enterprises.

Taking into account the previously mentioned fact that in Slovenia in pre-transition period the dominant share of the total land area was in private ownership, structural reforms have been a less important goal. In Slovenia, the emphasis was placed on promoting multi-activities of individual farms and the development of multifunctional agriculture (Pejanović, 2005). Slovenia, unlike all other countries in transition, had a relatively high rate of growth of agricultural production in this period.

In **Hungary**, after the mistakes made at the beginning of the privatization of large complex of land, all land without an owner was declared as state land. This land is without auction (auction) given to the large agricultural enterprises.

In Slovenia and Hungary, agricultural producers had the advantage in the distribution, which had a double positive effect: reducing the monopoly power of processing sector, while on the other hand, providing more stable raw material base (the verticality of the market structure), thus preserving the reproduction unit, from primary agricultural production (crop and livestock production) to the processing industry.

In some countries, the company was completely sold to employees and governed as part of the opportunity to purchase shares on preferential terms or free distribution.

In a **new agrarian structure** transition countries as responsible for the development of agriculture **medium-sized commercial farms**, owned by the younger, educated, energetic farmer, which are slowly extracted from a large group of family farms, although still hampered by significant financial, technological, administrative and other restrictions (Pejanović, 2005).

The issue of **foreign investments** had a very important role in securing the initial development capital and new technologies in agro-industry in transition countries. New investments which were expected through the privatization process should contribute to the restructuring, revitalization and modernization of processing facilities. It is often argued that this foreign investments / companies, after privatization, had the impact on the introduction of new health and hygiene standards in processing facilities necessary for EU accession processes. In particular a high amount of foreign investment in the sector of food and tobacco had Hungary. Estimates have shown that until 1996 about 60% of Hungarian agro-industrial capacities were controlled by foreign companies. In addition to Hungary, a high proportion of foreign investments in agro-industrial sector during the transition period, was recorded in Bulgaria and Poland (25%).

This is also characteristic of the privatization of this sector in Serbia, where in the individual sectors the share of foreign capital was almost one hundred percent (breweries, dairies, tobacco, etc.). Although the public has a fear of foreign control of strategic sectors of agriculture, there are also opinions that the investment has a positive impact on improving competitiveness. The fact is that foreign investors in food production in

transition countries may be motivated primarily with lower-cost resources and cheap labour. There are opinions that foreign investments in the industrial sector in transition countries were motivated by re-export products to the EU. There are doubts that in the production of sugar and milk foreign investments are used as a strategy for establishing control of production quotas. Analyses show that in 1999 the share of production capacities with foreign capital in the sugar industry was 100% in Hungary, Slovenia and Lithuania 95%, 90% in Slovakia, in the Czech Republic over 75% and in Poland 50%. In addition to the processing sector of sugar and milk, thoughts are that attraction for foreign investments are production of tobacco and alcohol.

In **Montenegro**, privatization was carried out by the so-called voucher model. The first division was carried out giving vouchers only to citizens of Montenegro. Later it was allowed also to the foreigners to become owners of the land.

In **Macedonia**, the privatization carried out by the stock model, where only Macedonians got shares (actions). Later the same was allowed to the foreigners.

Croatia has made privatization through stock model and the model of sale only to the citizens of Croatia, and in **Serbia** by combining several models: sales, stock and restitution model. The owners of the land became only the citizens of Serbia or the companies registered in Serbia. Privatization of agricultural land based on the sale has several advantages compared to the gift of land when the user is a private agricultural company, because it avoids favouring one at the expense of others.

The dilemmas that have occurred in the process of privatization of agricultural land in Serbia through the sale are the following: whether to give to existing user an advantage over others or to use a competitive method of sale "who offers more"; whether to sell by auction or solicitation of tenders; and whether to sell large land complexes of several thousand hectares, which occur in the possession of certain companies in Vojvodina, or divided into smaller parcels and sold separately (Begović i sar., 2006). It is believed that the competitive method of sale is best because, above all, provide the best offer/price, is the most transparent and is provided with the most efficient offer (presumably the most economically efficient is one who is able to offer the most). In the theory of auctions it is the same whether the competition is achieved by collecting auction or sealed bids, except that the transparency in auction is bigger. If the bigger land complex divides into smaller and thus sales, increases the number of interested potential buyers, thereby increasing the selling price and total revenue for the state.

According to the FAO data since 1994 in Albania about 94% of the total land has been de-collectivized and privatized; in Macedonia approximately 85% of agricultural land has been privately owned; in Bosnia and Herzegovina about 94% of agricultural land is privately owned, 86% in Serbia and 83% in Croatia Statistical data confirm that there is a large percentage of agricultural land in private ownership in the Balkan countries, but that the land market turnover is low, unstable, with a high degree of risk and inefficiency.

In contrast to these countries, many countries, including China and Vietnam, according to its current law did not allow the privatization of agricultural land, believing that this would be an obstacle to the successful realization of the set of defined goals and strategies.

SOME CONCERNS ABOUT THE PRIVATIZATION OF AGRICULTURAL LAND

In Serbia discussion was initiated on whether if it is necessary to make a change to the agreement with the EU which allows the sale of land to foreigners. Many believe that the concluded agreement should change and the period when non-residents will be able, without limitation, to buy our land should be extended. It is anticipated that the local "tycoons" will buy agricultural land cheaply and then they will sell it with higher prices to foreigners. One of the grounds for concern is the fact that a hectare of land can be bought in Serbia for 5.000 Euros, while hectare in EU cost from 50.000 to 70.000 Euros.

The land, like capital and labour, is the condition for business and production. Prices and availability of requirements and resources are different between Serbia and the EU and within the EU. Concerning the differences, Serbia has the leading roll. Our credit prices are several times higher than in the EU and since we do not have enough savings we sell assets and get into debt. Price of labour in Serbia is still several times lower, because there is no prosperity and employment. Subsidies to farmers and average yields of most crops are much lower here. Agriculture is not competitive, and there are other conditions that contribute to this poor state of this branch of economy.

On the other hand, the soil is a natural resource of any state, but we cannot keep it by choosing customers but we have to establish the conditions of its use. Our forest land is state-owned, but prescribed standards of rational maintenance of forest reserves are not used, as it is the case in some countries concerning private forests. At construction sites illegal construction is very common, and construction regulations make it difficult to work. The main problem of the regime of land as a public good is how to use it for economic growth. Foreigners are now indirectly owners or co-owners of agricultural land through their respective legal entities. Prices of capital and land are determinate with the agreement between buyers and sellers. The Foreign Investment Law allows that "a foreign physical or legal person, as a foreign investor, can acquire property over real estate", and the Law on Basic Property Relations define the conditional of reciprocity that a foreign country gives us the same right. Foreigners can obtain property on arable land and forest through legacy, also. With interstate agreements the equality of foreigners and domestic investors is protected.

Providing opportunities to foreigners to buy agricultural land in Serbia could be in the interest of "tycoon" who bought a part of that land and the other land owners, because it increases the number of potential buyers. On the other hand, the closing of the agricultural land market for foreigners could obviously be in the best interests of local "strong players" - in which case they will have more time to buy cheaper land from the state or small proprietors.

Some advocate the view that no serious country allows speculative trade with the land. Estimates are that the future will depend on how farmers, whose fields are first, bought by the "big", and now they buy products from small producers with over 180 day delay in payment, will behave. In Serbia, the most vulnerable will be small farmers, and they are majority. To survive they will have to join each other or to subscribe to large systems in Serbia.

Prohibition of sale of agricultural land to foreigners in Lithuania and Slovakia, which have long joined the EU, and will soon be able to fully make use of the European

agricultural budget, was recently extended until 2014. Baltic republics have managed to postpone the moment of the sale of land to their full influence in the EU agriculture. This issue was delayed in Bulgaria and Romania that are also EU members. Hungary decided during negotiations that the foreigners will be able to buy land after more than 10 years of its membership. Denmark, for example, allows the sale of agricultural land to foreigners, but only to those who have lived at least two years in that country. Croatia will offer land to foreigners after 15 years of their candidature for the EU.

Serbia, however, has negotiated that the foreigners will be able to buy our land after four years of full implementation of the Interim Trade Agreement. It must be borne in mind that Serbia is a country with high land quality, among the best in Europe. The land is not contaminated and it can be easily translated in to the soil for organic food. Today the best hectare of agricultural land in Serbia can be purchased for 5,000 Euros. Hectares of such land or worse in Europe will cost minimum from 50,000 to 100,000 Euros.

There are opinions that when it comes to ownership of agricultural land, the Government must urgently implement restitution, which is actually quite simple and does not threaten any country or "tycoons". State land is as much the state owes it to those from whom he was cruelly and unjustly taken away (about 350,000 hectares), and restitution in kind can be immediately implemented.

There are opinions that when it comes to the ownership of agricultural land, the Government must urgently implement restitution, which is actually quite simple and does not threaten to the country or the "tycoons". State land is as much the state owes it to those from whom it was cruelly and unjustly taken away (about 350,000 hectares), and restitution can be immediately implemented.

The second issue raised relates to the manner in which the state has disposed of the land that is one way or another in her possession. Strengthening the presence of the state as land owner may have its justification, but it certainly opens up new opportunities for corruption - the management of assets, lease, sell or trade information on future change of use. Corruption is always associated with the process of decision making. The most dangerous is the one that occurs when decisions on different acts are made (international treaties, laws and regulations). So the first task is to illuminate that process - to enable all interested parties to give their opinion (public debate), reveal the influences on decision makers (lobbying), obligate the decision makers to give a reasonable and detailed explanation of what they attempted to achieve and to present the regulatory impact analysis that were made for that purpose.

The fact that the process of decision-makers is closely guarded secret in Serbia is helping to hide corruption where it exists, but sometimes it can be seen where it does not exist.

CONCLUSION

The privatization process, as already stated, is a current problem that has existed for two decades in Europe and is a form of ownership structuring. For the success of privatization it is necessary to make several important preconditions: the selection of appropriate methods and efficient organizational activities related to its implementation; stable macroeconomic environment, the appropriate economic policies; transparent and stable system of rules and policies governing the rules of the game in the economy. The

goal of privatization should be to establish clear ownership structure, concentration of ownership in the hands of the relevant investor and restructure of the economy and companies.

The issue of foreign investment had a very important role in providing the initial development capital and new technologies in agro-industry in transition countries. With the analysis of the privatization process in all parts of the agro-economy in the countries in the region and in Serbia it can be concluded that the decisive role in the privatization had the foreign investment in processing capacity. In some privatizations of agro-industry in Eastern Bloc countries, it is characteristic that the whole companies are sold to foreign investors. New investments that were expected through the privatization process were to contribute to the restructuring, revitalization and modernization of processing facilities.

The process of land privatization was carried out in various ways and using different models of privatization across Europe. This process led to the establishment of a new ownership structure, with a growing number of farms and their significant participation in the farmland. The common characteristic of the CEE countries during the transition period was that these countries did not foresee the possibility of returning the land to foreigners, and initially the foreigners were not able to purchase land.

Serbia now has a low productivity and lack of competitiveness and this is a big problem for the Serbian agriculture. Inclusion of Serbian agriculture in European integration is one of the priorities of the current state administration. However, promised speed in this area still needs to be taken with great caution. In Serbia, two important issues related to privatization of land are raised, namely: whether to change the agreement with the EU that allows land sales to foreigners (with us earlier than in other countries), the question of how the state has disposed of land that is in one way or another in her possession.

Small areas do not allow the use of modern agro-technical measures and appropriate solutions for higher yields. Merging of property arises as a priority that cannot be achieved without a comprehensive national program and adhering to its implementation. If we aspire to be part of a large family of EU agricultural deeper cuts are inevitable in a land policy. The state must take responsibility for the food security of the nation, but it should not sell natural resources.

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NEKA ISKUSTVA I DILEME OKO PRIVATIZACIJE POLJOPRIVREDNOG ZEMLJIŠTA

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Izvod

Privatizacija je poslednjih decenija termin koji se često spominje, kako u vladama država u tranziciji, tako i u vladama razvijenih zemalja. Privatizacija je društveno-ekonomski proces koji znači promene i kao takav postaje i svetski trend. Proces privatizacije je najčešće iniciran nepovoljnom ekonomskom situacijom, koja za posledicu ima visoke dugove, ili je sam proces iniciran globalnim trendom prelaska sa jednog na drugi sistem ekonomije. Privatizacija bi trebalo da, zajedno sa drugim tranzicionim reformama, ubrza promene privredne strukture, podstakne razvoj preduzetništva, poboljša konkurentnost, što sve treba da rezultira u kreiranju odgovarajućeg tržišnog okruženja u kome bi bilo moguće sprovesti proces tranzicije u tržišnu privredu. Privatizacija treba da omogući jasnu i otvorenu vlasničku strukturu, koja će obezbediti ulazak strateških investitiora u agroprivredu. Autori u ovom radu razmatraju aktuelnu problematiku privatizacije poljoprivrednog zemljišta, dajući prikaz iskustava zemalja u tranziciji po tom pitanju. Autori takođe ističu neke dileme i aktuelne stavove vezano za privatizaciju zemljišta u Republici Srbiji i novog sporazuma sa EU.

Ključne reči: privatizacija poljoprivrednog zemljišta, tranzicija, poljoprivreda, agroindustrija, Republika Srbija.

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THE EFFECT OF TREHALOSE, CAFFEINE AND GLUTATHIONE ON BOVINE SPERMATOZOA: 1. MOTILITY *IN VITRO**

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SUMMARY: Results of this study show that trehalose, caffeine and glutathione supplementations have stimulating effects on spermatozoa motility parameters. Stimulating effects of trehalose were lasting over a longer time period, with recommendation of trehalose supplementation for a long-time spermatozoa cultivation. Caffeine and glutathione have the most markedly stimulating effects especially over the first 24 hours of cultivation, and they could be used as additives for a short-term in vitro spermatozoa preservation.

Key words: *trehalose, caffeine, glutathione, spermatozoa, bulls, motility.*

INTRODUCTION

Artificial insemination (AI) has become one of the most important pillars in animal biotechnology. Especially in the cattle AI, bull semen quality is highly important to ensure a good biological material for breeding as well as a certain biodiversity protection (Ibrahim et al., 2000).

Progress in the use of AI has been related to search for semen extenders with a potential ability to stimulate motility and to enhance the fertilizing ability of animal and human spermatozoa (Pivko et al., 2009; Spalekova et al., 2011). A special attention is dedicated to substances with antioxidant properties, as sperm cell membranes contain high concentrations of polyunsaturated fatty acids susceptible to lipid peroxidation. Inversely, the seminal plasma possesses a wide antioxidant system to prevent oxidative cellular damage (Kefer et al., 2009) Nevertheless, antioxidants present in the seminal plasma, are usually attenuated by dilution of the semen during the preparation of in-

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semination doses (Pivko et al., 2009).

It was confirmed that the addition of glutathione, as an antioxidant and trehalose, a nonpermeant cryoprotectant has elicited beneficial effects in many facets of AI and *in vitro* fertilization: increase of semen quality, spermatozoa motility and viability. Furthermore, it was documented that caffeine as a cyclic nucleotide phosphodiesterase inhibitor markedly increased and maintained the respiration and motility of ejaculated spermatozoa, which resulted in a higher fertilization rate of oocytes (Tatham et al, 2003; Spalekova et al, 2011).

The aim of this study was to examine the effects of glutathione, trehalose and caffeine on selected bovine spermatozoa motility parameters following an *in vitro* cultivation at different temperatures and time periods.

MATERIAL AND METHODS

Bovine semen samples (n=48) were obtained from 6 randomly selected adult breeding bulls (Slovak Biological Services, Nitra, Slovakia) on a regular collection schedule using an artificial vagina. The semen was cooled down to 4˚C and transported to the laboratory, where the samples were divided into four main groups, according to the concentration of the used experimental supplement and the cultivation temperature together with the time intervals of analysis.

The basic spermatozoa diluent medium consisted of Triladyl® *(*250 mL; Minitüb, Tiefenbach Germany*),* distilled water (750 mL) and egg yolk (62.5 mL). The treatment was based on the addition of trehalose (Sigma-Aldrich, St. Louis, USA) caffeine (Sigma-Aldrich, St. Louis, USA) and glutathione (Sigma-Aldrich, St. Louis, USA) into the semen diluent medium at dosages of 0 (Control), 1 (Group 1) and 2 (Group 2) mg/mL. Fresh semen was added to each medium with a final dilution rate of 1:50.

Group A was cultured at 37 °C and analyzed at time intervals of 0h, 1h, 2h, 3h and 4h after the experiment had started. Group B was cultured at 5°C and analyzed at 24h, 48h, 72h and 168h after the experiment had begun.

Spermatozoa motility analysis was carried out using the Computer Assisted Sperm Analysis (CASA) system – SpermVision (MiniTüb, Tiefenbach, Germany) with Olympus BX 51 phase contrast microscope (Olympus, Japan). Each sample was placed into Makler Counting Chamber (depth 10 µm, 37±1°C; Sefi–Medical Instruments, Haifa, Israel) and the following parameters were evaluated: percentage of motile spermatozoa (motility>5 μm/s), percentage of progressive motile spermatozoa (motility > 20 μm/s), VCL (velocity curved line, μm/s) ALH (amplitude of lateral head displacement, μm) and BCF (beat cross frequency, Hz) with minimum 1000 cells examined in each sample (Massanyi et al., 2008).

Statistical analysis of the obtained data was carried out using the SAS statistical program (SAS Institute Inc., Cary, USA). Basic statistical parameters (mean, standard deviation, coefficient of variation) were calculated at first. Subsequently, a paired t–test and Scheffe's test were used to compare the results between the control and experimental groups. The level of significance was set at *** ($p < 0.001$); ** ($p < 0.01$); * ($p < 0.05$).

RESULTS AND DISCUSSION

The overall motility of spermatozoa cultured at 37°C and 5 °C is shown in Table 1. In all of the groups the highest motility was detected at time 0 and decreased over the course of time.

The overall spermatozoa motility was recorded as 78.56 – 46.08% in the C1 A/B groups and 86.34 – 43.94% in the C2 A/B groups.

The motility varied from 84.02% to 43.52% in the T1 groups and from 89.00% to 56.04% in the T2 groups. When comparing the motility between T1 and T2, no significant differences ($p>0.05$) were observed. Compared to the control groups, higher motility parameters were found in groups T2A and T2B during all of the time periods, as well as in the T1A group from 0h to 4 h.

The overall motility in the experimental caffeine groups decreased with time, however no significance was proved ($p>0.05$). An interesting observation was made in the motility development in both of the KA groups as it decreased after 1h and 2 h of culture, then surprisingly increased at 3h and 4h, with subsequent decrease.

Controversial results were detected in the experimental groups with glutathione addition. Insignificantly ($p>0.05$) higher motility parameters were detected in the G2A group at a time period from 0h to 4h when compared with the control. However, a significant decrease was recorded in the G2B group at 48h and 72h ($p<0.01$). No significant differences ($p>0.05$) were observed in the G1 groups compared to the controls, however relatively higher, even though insignificant values ($p>0.05$) were observed at timeframes from 0h to 4h at 37°C as well as at 24h/5°C.

Table1. Spermatozoa motility (in %) in groups and time periods (mean±SD)

Group	Time 0h, 37°C	Time 1h, 37°C	Time 2h, 37°C	Time 3h, 37°C	Time 4h, 37°C
			Time 0h, 37°C		
C1A	78.56±16.07	76.81±9.23	76.07±8.38	78.20±6.46	77.14±12.08
T1A	84.02±10.87	79.77±11.61	81.86±9.66	76.16±8.67	76.25±7.18
K1A	79.49±14.81	75.36±10.86	71.97±11.10	77.96±9.33	78.58±10.45
G1A	80.66±14.64	82.94±6.97	81.18±7.13	79.26±9.92	77.75±7.90
C2A	86.34±9.03	79.58±7.64	77.38±7.79	79.74±11.32	79.27±10.47
T2A	89.00±4.98	84.43±8.07	80.01±8.79	81.21±8.17	79.01±13.08
K2A	75.41±17.05	78.54±6.95	78.94±7.86	78.48±7.87	75.04±13.31
G2A	85.46±10.24	83.53±7.00	80.48±7.65	80.91±9.47	83.76±6.42

Group	Time 24h, 5°C	Time 48h, 5°C	Time 72h, 5°C	Time 168h, 5°C
C1B	76.60±11.66	75.03±13.64	75.75±14.61	46.08±14.93
T1B	71.72±9.96	72.74±16.75	75.84±13.68	43.52±15.92
K1B	69.80±12.26	71.82±16.57	68.81±17.05	28.33±16.48
G1B	77.48±10.34	70.61±18.76	65.76±18.04	42.35±14.85
C2B	76.72±9.77	79.11±12.65	77.30±11.07	43.94±27.06
T2B	78.30±10.12	81.03±8.84	74.78±15.73	56.04±25.05
K2B	74.66±10.98	69.47±17.60	68.35±14.57	33.49±12.54
G2B	72.50±14.00	58.13^{B}±22.92	57.34^{B}±17.10	40.47±10.96

C – control group; T – trehalose; K – caffeine; G – glutathione; 1 – 1 mg/mL of the supplement; 2 – 2 mg/mL of the supplement; $^{A}p<0.05$; $^{B}p<0.01$; ^{C} – $p<0.001$; X – mean; SD – standard deviation.

The overall progressive spermatozoa motility in the C2A/B groups (79.09 – 34.29%) was generally slightly higher than in the C1A/B groups (68.76 – 32.00%; Table 2).

No significant differences ($p>0.05$) were observed in medias with trehalose supplementation. In all cases, the highest progressive motility was recorded at time 0. The lowest progressive motility in the group T2 was detected after 168h. Nevertheless, comparing the data with the control, insignificantly ($p>0.05$) higher values were observed in all of the time periods, except for 4h and 72h. A similar decrease of progressive motility was observed in the T1 group. The progressive motility was relatively ($p>0.05$) higher at times 0h – 4h and at 72h.

Considering caffeine as a culture medium supplement, the K2 progressive motility decreased during the first two hours, subsequently it increased at time 3h, however it decreased afterwards, and the lowest progressive motility was detected at time 168h. No significant differences ($p>0.05$) were observed in this group. Equally, the lowest progressive motility rates were detected in the K1 groups at 168h, with a significant decrease ($p<0.05$) when compared with the control.

Similarly as in previous groups, the progressive motility rates in the glutathione groups were the highest at time 0h. A relatively higher progressive motility was detected in the T2 groups at times 1h, 2h, 3h and 4h. However afterwards, the progressive motility started to decrease radically, with a significant reduction ($p<0.001$) at time 48h. Later on, the decrease was non-significant ($p>0.05$). No significant increase or decrease of progressive motility was recorded in the T1 groups, even though the rates were relatively increased in the timeframe from 0h to 4h. As in the T2 groups, the progressive motility visibly decreased from time 24h and the lowest rates were observed at 168h.

Table 2. Progressive spermatozoa motility (in %) in groups and time periods (mean±SD)

Group	Time 0h, 37°C	Time 1h, 37°C	Time 2h, 37°C	Time 3h, 37°C	Time 4h, 37°C
C1A	68.76±17.70	68.17±9.96	69.10±7.59	67.09±8.09	66.88±14.29
T1A	75.83±11.06	73.61±12.95	71.77±10.88	67.88±10.86	67.13±9.29
K1A	71.70±13.72	67.71±11.55	64.40±10.53	68.93±8.66	67.98±12.91
G1A	73.96±13.45	74.45±8.32	71.94±7.00	69.76±10.43	67.47±9.51
C2A	79.07±11.12	71.68±7.51	66.73±9.88	71.48±12.66	71.52±8.92
T2A	80.80±5.00	76.50±7.85	70.84±10.83	71.85±8.87	69.91±15.28
K2A	68.48±16.96	69.44±7.76	69.58±9.02	70.67±7.61	65.62±14.02
G2A	76.98±10.65	74.85±8.72	70.61±9.64	72.43±10.10	72.00±8.76

Group	Time 24h, 5°C	Time 48h, 5°C	Time 72h, 5°C	Time 168h, 5°C
C1B	65.79±13.29	62.72±15.16	63.83±15.91	32.00±14.00
T1B	60.40±11.31	60.87±16.39	64.43±15.75	28.78±14.03
K1B	58.87±12.91	61.07±17.05	58.77±17.58	13.45^{A}±9.67
G1B	63.91±11.42	54.09±17.46	53.72±18.30	22.70±7.11
C2B	66.51±11.55	67.37±12.85	64.37±13.10	34.29±25.68

T2B	67.19±9.96	68.07±13.17	63.48±17.57	41.26±23.51
K2B	65.29±11.39	55.27±16.61	54.17±14.74	19.15±11.96
G2B	59.80±15.56	44.05^{C}±22.28	40.68±18.59	19.96±10.96

C – control group; T – trehalose; K – caffeine; G – glutathione; 1 – 1 mg/mL of the supplement; 2 – 2 mg/mL of the supplement; $^{A}p<0.05$; $^{B}p<0.01$; $^{C}p<0.001$; X – mean; SD – standard deviation.

The VCL was elevated in all of the T groups with a significant increase ($p<0.001$) in the T2A group at time 4h, as compared to the control. A relative VCL increase was observed in the C2 and G2 media at times 2h, 3h and 4h, however a significant ($p<0.05$) decrease was recorded in both experimental media at time 72h. Regarding groups C1 and G1, a significant difference was not detected, even though a relative increase was observed in all of the experimental groups, especially at times 0h, 1h, 3h, 4h and 72h (Table 3).

Table3. Spermatozoa velocity curved line (VCL; in μm/s) in groups and time periods (mean±SD)

Group	Time 0h, 37°C	Time 1h, 37°C	Time 2h, 37°C	Time 3h, 37°C	Time 4h, 37°C
C1A	98.00±13.75	119.72±16.66	121.54±18.41	109.07±9.90	109.41±15.28
T1A	95.38±10.97	130.41±24.27	118.43±20.49	117.20±16.02	119.21±22.51
K1A	99.52±17.86	124.10±22.45	121.20±17.29	113.22±15.74	114.29±16.61
G1A	106.16±23.31	131.24±15.42	128.43±18.48	124.83±16.29	118.89±16.96
C2A	117.44±15.99	131.04±19.90	121.44±19.19	121.32±27.93	119.93±24.49
T2A	117.69±28.69	128.73±10.55	130.92±17.62	130.17±21.16	122.35^{C}±24.96
K2A	104.74±18.73	119.74±11.82	127.44±14.30	130.65±21.20	123.44±24.27
G2A	103.75±13.73	128.01±14.28	130.07±13.62	132.23±12.29	126.91±17.81

Group	Time 24h, 5°C	Time 48h, 5°C	Time 72h, 5°C	Time 168h, 5°C
C1B	118.66±17.21	27.70±24.02	18.11±2.64	15.94±17.56
T1B	116.70±17.19	25.86±21.38	19.58±2.83	16.36±18.11
K1B	116.53±15.29	16.98±16.33	13.22±1.91	28.14±37.21
G1B	111.25±13.67	20.28±19.01	16.34±2.36	14.51±17.67
C2B	127.37±18.85	14.20±11.47	14.71±2.10	23.22±24.81
T2B	124.24±26.15	18.15±15.44	22.04±3.18	12.09±12.96
K2B	117.37±21.92	15.87±15.72	15.54±2.24	17.61±24.00
G2B	124.64±27.09	20.86±20.31	24.02±3.47	12.59±16.76

C – control group; T – trehalose; K – caffeine; G – glutathione; 1 – 1 mg/mL of the supplement; 2 – 2 mg/mL of the supplement; $^{A}p<0.05$; $^{B}p<0.01$; $^{C}p<0.001$; X – mean; SD – standard deviation.

Analyzing the ALH, significant ($p<0.05$ and $p<0.01$ respectively) increases in all of the experimental groups A at time 2h was detected. Other important, but not significant differences ($p>0.05$) were detected at times 0h, 1h, 3h, 4h and 24h. Focusing on the B group we observed no significant differences ($p>0.05$), however a relative increase was observed in all of the media over the first 24 hours (Table 4).

Table 4. Spermatozoa amplitude of lateral head displacement (ALH; in μm) in groups and time periods (mean±SD)

Group	Time 0h, 37°C	Time 1h, 37°C	Time 2h, 37°C	Time 3h, 37°C	Time 4h, 37°C
C1A	4.57±1.15	4.78±0.94	4.76±0.97	4.51±0.84	4.57±0.84
T1A	4.90±0.73	5.35±0.95	5.01±1.00	4.67±0.88	4.67±0.87
K1A	4.90±1.00	5.17±0.99	4.73±0.86	4.69±0.98	4.59±0.81
G1A	4.63±0.87	5.51±0.70	5.26±0.78	5.18±1.13	5.08±0.96
C2A	5.37±0.70	5.15±1.16	4.78±1.07	4.91±1.31	4.84±0.93
T2A	5.39±0.43	5.47±1.01	5.66^{A}±0.41	5.31±0.61	5.01±0.70
K2A	5.14±0.82	5.46±0.88	5.70^{A}±0.92	5.40±0.93	5.20±0.94
G2A	4.93±0.78	5.30±0.84	5.81^{B}±0.62	5.64±0.74	5.66±0.65

Group	Time 24h, 5°C	Time 48h, 5°C	Time 72h, 5°C	Time 168h, 5°C
C1B	5.24±0.78	5.51±0.95	5.65±0.83	4.88±0.47
T1B	5.37±0.72	5.74±1.04	5.83±0.80	4.75±0.54
K1B	5.31±1.18	5.13±0.94	5.27±0.69	3.84±1.29
G1B	5.67±0.52	5.45±0.81	5.63±0.72	4.28±0.62
C2B	5.72±0.85	5.97±0.74	5.96±0.75	4.91±1.10
T2B	5.90±0.71	5.95±0.80	6.09±0.86	5.17±0.50
K2B	5.73±0.94	5.06±0.72	5.11±0.75	4.11±0.65
G2B	6.13±0.94	5.18±0.97	5.11±0.93	4.25±0.57

C – control group; T – trehalose; K – caffeine; G – glutathione; 1 – 1 mg/mL of the supplement; 2 – 2 mg/mL of the supplement; $^{A}p<0.05$; $^{B}p<0.01$; $^{C}p<0.001$; X – mean; SD – standard deviation.

Relatively controversial results were obtained for the BCF parameter in the A group. No significant differences ($p>0.05$) were observed in the T2 group. Nevertheless, a significant decrease was recorded in the K2 group at 48h ($p<0.05$) and in the G1 group at 2h ($p<0.05$). No significant differences ($p>0.05$) in the BCL parameter were observed in any of the experimental B groups when compared to the control (Table 5).

Table 5. Spermatozoa beat cross frequency (BCF; in Hz) in groups and time periods (mean±SD)

Group	Time 0h, 37°C	Time 1h, 37°C	Time 2h, 37°C	Time 3h, 37°C	Time 4h, 37°C
C1A	26.50±2.74	27.93±4.81	27.53±5.13	28.02±4.55	28.74±5.96
T1A	25.36±1.63	25.99±3.54	26.74±4.86	27.48±5.10	28.28±4.47
K1A	24.56±2.36	26.51±4.11	28.89±4.67	28.22±4.13	29.55±4.80
G1A	27.07±3.65	25.94±4.08	25.91±4.25	26.72±4.99	26.72±5.54
C2A	26.47±2.15	27.63±5.18	27.82±5.21	26.79±4.48	27.07±5.04
T2A	26.41±3.51	26.03±4.57	23.94±1.51	24.79±2.23	25.79±3.36
K2A	24.28±2.43	25.59±3.57	24.84±3.84	26.32±4.86	26.74±4.29
G2A	26.19±2.91	25.84±3.10	23.60^{A}±1.53	24.41±2.91	25.04±3.53

Group	Time 24h, 5°C	Time 48h, 5°C	Time 72h, 5°C	Time 168h, 5°C
C1B	25.62±4.17	23.54±3.34	23.24±3.65	20.20±2.31
T1B	23.05±3.41	23.20±3.85	22.36±3.46	21.41±3.30

K1B	24.38±5.79	24.09±3.40	24.18±3.76	19.99±5.93
G1B	22.35±2.65	22.67±2.49	22.05±2.66	21.58±1.93
C2B	23.82±3.84	23.27±2.76	23.24±3.00	19.72±2.41
T2B	22.83±2.88	22.02±2.26	22.10±3.01	21.29±2.28
K2B	23.26±3.89	23.44^{A}±3.06	22.93±2.34	21.50±3.27
G2B	21.77±2.47	20.82±2.79	22.23±2.89	20.84±2.62

C – control group; T – trehalose; K – caffeine; G – glutathione; 1 – 1 mg/mL of the supplement; 2 – 2 mg/mL of the supplement;

$^{A}p<0.05$; $^{B}p<0.01$; $^{C}p<0.001$; X – mean; SD – standard deviation.

Generally, we observed a stimulation of all of the observed motility parameters, even though the stimulation was mostly non-significant ($p>0.05$). The few significant differences as well as occasional decreases in the motility parameters may have been caused by individual characteristics and viability parameters of the samples.

The effect of trehalose in our study was mainly stimulating. All of the motility parameters were increased when compared to the control, some of them significantly, especially during a long-time cultivation. Several investigators have found that the incorporation of trehalose in semen diluents can protect and stimulate the spermatozoa of many species. Woelders et al. (1997) demonstrated that an isotonic sugar medium containing sucrose and trehalose is significantly superior to a standard egg yolk medium in preserving the motility and acrosome integrity of bovine spermatozoa. These results were confirmed by Knazicka et al. (2010) who studied the effects of different energetic substrates used in culture media on bovine spermatozoa motility parameters during a 24-hour *in vitro* cultivation. The authors stated that the maximum viability was recorded while applying glucose (5%), sacharose (5%) and trehalose (1%), pointing out the beneficial effects of trehalose as a potential substance to prevent oxidative stress not only in freezing-thawing medias but also in culture medias used for a short-term cultivation and examination of spermatozoa. Moreover, in a comparison of raffinose and trehalose, Storey et al. (1998) showed that trehalose brings about a significantly better recovery rate in intact mouse spermatozoa. Additionally Aisen et al. (2002) observed that trehalose significantly improved the viability of ram spermatozoa assessed for motility and acrosome integrity.

Despite having evidence in favour of the beneficial aspects of trehalose, not all studies have found the same results. Chen et al. (1993) report that trehalose caused only minor improvement in bull spermatozoa survival, which is in the closest accordance with our data. Furthermore, it is now known that only adequate concentrations of trehalose are beneficial for spermatozoa survival as its high concentrations result to be toxic. Aisen et al. (2002) observed a favorable effect of reduced trehalose concentrations on spermatozoa motility and a deleterious effect of greater trehalose concentrations, which was consistent with observations by Hu et al. (2009) carried out in boar semen. In a later study by Hu et al. (2010), the greatest protective effects of trehalose were detected at the concentration of 100 mM, and a more reduced extent at 200 mM. The latter concentration resulted in increased osmolarity of the extender, which was in itself deleterious to the spermatozoa (Hu et al., 2009; 2010).

Regarding caffeine as a supplement for *in vitro* spermatozoa cultivation, we observed a stimulation of the motility parameters, especially over the first time periods,

from which we can conclude that caffeine could be an effective supplement for short-term preservation of spermatozoa. Moreover, various stimulating effects of caffeine on animal spermatozoa have been reported before. Positive effects of caffeine on the bovine semen at a concentration of 2.5 mmol/L were confirmed by Bird et al. (1989). Caffeine may also promote capacitation and/or acrosome reaction of boar spermatozoa and, when added to the fertilization medium, it accelerates spermatozoa penetration *in vitro* in pigs (Nagai et al., 1993) and mice (Fraser, 1979). At 10 mmol/L, caffeine increased rabbit spermatozoa motility after 24 h of semen refrigeration, whilst lower concentrations (2.5 or 5 mmol/L) did not affect spermatozoa motility (Lopez and Alvarino, 2000). According to Spalekova et al. (2011) the total and progressive motility of ram spermatozoa increased after 24h of incubation in presence of 1 or 4 mmol/L of caffeine.

However, along with stimulating effects, a number of negative effects of caffeine have been described as well. In humans caffeine concentrations greater than 2.5 mmol/L may adversely affect the spermatozoa fertilization and cleavage of embryos derived from such spermatozoa (Imoedemhe et al., 1992). Similarly, the results of Aitken et al. (1983) indicate that caffeine at concentrations of 5 mmol/L and above may have potential toxic effect on human spermatozoa. The fertilizing capacity of the normal human spermatozoa exposed to caffeine did not appear to be enhanced at low concentrations, whereas at higher concentrations (5 mmol/L) the capacity was adversely affected despite improving the motility (Imoedemhe et al., 1992). Furthermore, caffeine at higher concentration may cause a reduction of bovine spermatozoa motility (Bird et al., 1989), therefore, could be recommended to use caffeine-free fertilization media for insemination with capacitated spermatozoa in bovine IVF (Momoyawa and Fukuda, 2003). The effects of caffeine on spermatozoa characteristics may be species-specific. Caffeine at concentration of 5 mmol/L may cause decrease in fertilization capacity of human or bovine spermatozoa, however higher concentrations (10 mmol/L) may cause increased motility parameters in rabbits. Nevertheless, this motility increase did not lead to an improvement of the reproductive parameters.

Based on our spermatozoa motility analysis glutathione acts generally as a stimulating supplement as well. However we found controversial results at various time periods, which are in accordance with some previous studies. On one hand, Munsi et al. (2007) who examined the effects of several glutathione concentrations on bovine spermatozoa viability stored at 4-8°C during 5 days. The motility was significantly higher with the addition of 0.5 mM of glutathione. After 5 days, the optimal motility (over 50%) was recorded at concentrations from 0.5 to 2.0 mM, meanwhile at 3.0 mM the motility decreased significantly. These conclusions are proved by our results as well. Results of Triwulanningsih (2003) showed that the addition of 0.5 mM glutathione to the spermatozoa diluent medium was sufficiently effective in the production of liquid semen. Moreover Kim *et al.* (1999) reported that the addition of 1 mM glutathione into a fertilization medium during an *in vitro* fertilization improved its effectiveness.

On the other hand, Donnely et al. (2000) report that supplementation with glutathione to spermatozoa preparation media did not significantly improve spermatozoa motility. Additionally according to Whitaker (2008) a 5.0 mM glutathione supplemented treatment indicates that GSH may reduce the motility of the spermatozoa. Authors concluded that the decrease in motility could be related to the effect of glutathione on the function of the spermatozoa flagellum.

CONCLUSION

Our results show that trehalose, caffeine and glutathione supplementations have stimulating effects on spermatozoa motility parameters. We can conclude that the stimulating effects of trehalose were lasting over a longer time frame, which is why we would recommend trehalose supplementation for a long-time spermatozoa cultivation. Caffeine and glutathione had the most markedly stimulating effects especially over the first 24 hours of cultivation, which is why they could be used as additives for a short-term *in vitro* spermatozoa preservation. Nevertheless, very few of our results were significant, therefore further experiments should be done with a higher variability of trehalose, caffeine and glutathione concentrations and under different conditions to see a more significant positive or negative effect on spermatozoa motility characteristics.

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UTICAJ TREHOLAZE, KOFEINA I GLUTATIONA NA SPERMATOZOIDE BIKA: 1. POKRETLJIVOST *IN VITRO*

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Izvod

Rezultati ovog rada pokazuju da dodavanje treholaze, kofeina i glutationa u spermu bika, ima stimulativni efekt na parametre pokretljivosti spermatozoida. Efekt treholaze traje duže, pa se dodavanje treholaze preporučuje za dugotrajniju kultivaciju spermatozoida. Kofein i glutation ispoljavaju značajno delovanje samo tokom prvih 24h, pa se dodavanje ovih supstanci preporučuje za kratkotrajno čuvanje spermatozoida *in vitro*.

Ključne reči: trehalose, caffeine, glutathione, spermatozoid, bik, pokretljivost.

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ENTREPRENEURSHIP IN AGRICULTURE: NEW CHALLENGES OF INTERNATIONAL TRADE INTEGRATION

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SUMMARY: The international trade in agricultural production is still rather far from full liberalization despite the progress achieved after many negotiation rounds within the framework of the WTO. Some new rules in the international trade organizations and new obligations which were taken on by the participant countries of the WTO have opened new questions to be discussed and settled within the framework of the following negotiation rounds. The basic themes of such negotiations during Russia's introduction into the WTO should become the questions of the state trade regulation, the further perfection of sanitary control rules, the further decrease of the custom duties and administrative character of tariff quotas establishment on imported agricultural products.

Key words: *trade integration, agricultural development, entrepreneurship.*

INTRODUCTION

State trading companies continue to play important role on the global market. It is a serious problem since the sector of agricultural production exchange and character of activity in the given market of the state trading companies essentially differ by many parameters from other sectors of the global market. From the viewpoint of the import of agricultural production, the development of the state trade can result in the decrease of production on the market in comparison with what could be if on the global market there was a free competition with equivalent custom duties. Therefore, the state trading companies should operate by the precise rules which are not admitting discrimination of other participants of the global agrarian market. Basically, it should result in such situation at which the state trading companies cannot establish the internal price above the bottom level of the world price plus the custom duties. Hence, discrimination of consumers will be removed at a choice of sources of import production. The dealer, offering the bottom world price, will determine a price level on similar production on

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the domestic market. However, in practice there is no transparency in the state trading companies' activity, therefore frequently it is impossible to track, whether the given rules are carried out by them.

MATERIAL AND METHODS

It is difficult to limit the price discrimination. It is especially difficult on the foreign markets where the state trading agencies operate. It is also difficult when the government gives export grants to private trading agencies in the selective order, depending on a direction of their activity. Thus, it is much easier to limit the game on various price levels, since export subsidizing is under the positions of the WTO Agreement on Agriculture. The state trading agency realizes agricultural production on a foreign market under the higher prices than the internal prices for similar production while internal manufacturers receive the average price of a home market. Thus, export of agricultural production is subsidized. In some cases the given kind of indirect subsidizing is more effective for the government, than direct subsidizing of export deliveries. Therefore, the following rounds of trading negotiations should take into account such phenomenon, as game on a difference of the internal and external prices.

RESULTS AND DISCUSSION

The integral part of the WTO negotiations on the markets access is the negotiations on the agricultural issues. Besides the tariff aspect, they also include consideration of the Russian policy of the concerning state support of agrarian sector and export subsidizing.

The agricultural negotiations have been conducted from the end of 1998, when the initial offers on the Russian obligations in agriculture were submitted. The given document authorized by the Commission of the Government of the Russian Federation fixed the levels of internal support of agricultural producers (so-called Aggregated Measure of Support) both subsidizing the agricultural products export and foodstuffs in format established by WTO.

It is necessary to note, that AMS includes the measures that have the major influence on trade and manufacture (subsidies for animal and plant production, indemnification of the part of expenses on materials purchasing, preferential crediting, price support, privilege for transportation).

We created the model, which analyzes the matrix 6x6 (6 integrated regions on 6 integrated commodity groups).

6 regions are Russia, CIS countries, EU, East Asia, NAFTA countries, and others.

6 commodity groups are: sugar, meat (including poultry), grain, industrial goods, services, other agricultural products.

For the analysis of the received results the following parameters were used:

- Dynamics of import and export on each commodity group;
- Dynamics of manufacture on each commodity group;
- Dynamics of the investments and GDP of the country;
- Dynamics of the country wealth.

It is obvious, that at complete liberalization of the agricultural market the import of meat products in Russia will increase to the greatest degree, since the given commodity group is under the greatest quotas today. The import of meat at the Russian accession to WTO will increase more than twice. The Russian producers provide today not more than 50% of all meat consumption in the country. It is obvious that the cheap import will put appreciable impact on the Russian meat producers. The significant import growth will be observed in the case with sugar. Today the sugar consumption in Russia on 75-80% is provided by the import. By grain and other agricultural products we can predict the insignificant import growth.

On agricultural export we can observe the growth in all the commodity groups.

The significant reduction of trade barriers will cause the export growth. But if we pay attention to the absolute parameters, it will be possible to notice, that the export of agricultural products after the WTO accession will not cover import even by 10%.

The agricultural production will decrease, the GDP will decrease by 3%, the investments will be reduced by 0.8%. But, despite of such negative parameters, at complete liberalization of the agricultural market the general wealth of the country will increase on $1.3 bn. (because of the redistribution of the money resources to the more profitable manufactures).

However, it is necessary to note, that the Script 1 of complete trade liberalization is improbable. It is more theoretical model. In the greater degree it is possible to expect the realization of the Script 2, i.e. partial market liberalization.

The import of agricultural products in Russia is still increased, but already in a smaller degree. The import of meat products will increase by 29%, import of sugar – by 15%. As well as in the Script 1, the import of grain will not change. It is expected, that the import under the given script will grow gradually depending on the same gradual reduction of trade barriers by 36% during the five years' transitive period.

The situation with export is similar – the export grows, but at lower rates than in the case of theoretical complete market liberalization. It is connected, first of all, to the preservation of counter higher requirements to the domestic export from the importers.

Nevertheless, the volume of agricultural production is still decreasing.

The fact of recession of manufacture at partial and gradual market liberalization at the WTO accession tells that any attempt to free the access to the domestic market leads to the reduction of own manufacture. It has especially significant effect when the exporter is actively subsidized by the government. At the Script 2 the GDP will decrease on 1%, the investments will decrease on 0.2%. The general wealth of the country will increase on $661.2 mln. because of the redistribution of the financial resources to the more profitable manufactures.

Script 3 can be considered as the close to the Script 1, to theoretical model. But the modern stage of the agricultural negotiations shows that the Russian side had defended its trade positions in agriculture and the trade barriers for agricultural products will not change significantly.

The import of agricultural products is reduced under such circumstances (in particular, import of grain). With other commodity groups import grows insignificantly.

The export also grows (because of the back measures of the countries on protection of their producers)

The manufacture of agricultural products, as well as in the previous scripts, is de-

creased, but insignificant. The GDP and investments at the Script 3 will stay constant.

Even at the insufficient degree of reduction process regulation of the custom duties the further trading negotiations under the Russian accession into WTO should concentrate on the decision of two basic problems: on liquidation of a tariff dispersion and restriction of tariff escalation. It is natural, that the agreement on priority decrease of high custom duties that will help to solve a problem of a divergence in tariffing levels various agricultural products should be accepted. It will limit the countries in an opportunity of an establishment of higher protective measures on the priority goods for itself. The similar agreement on industrial products trade has been achieved during the Tokyo round of negotiations so it is obviously possible to make use of the given experience to regulation international trade by agricultural production.

In order to maintain the liberal and equal character of international trade in agricultural production it is still required the carrying out many trading negotiations rounds. But all the same the significant part of work on creation of the free global agrarian market is already made. Furthermore, it is necessary only to improve the main principles of the WTO and adopt them to constantly varying conditions of modern economic.

CONCLUSION

The WTO accession will be positive for Russian agriculture only if the conditions of the accession are favorable to Russia. Russia should be allowed by the WTO to provide the state support at the level of other countries, for example, USA or EU countries. The import tariffs should provide Russia with the real protection tool for internal agricultural producers from the expansion of import agricultural products. There is no doubt that Russia will enter the WTO because nobody is able to stop the global tendencies. The preparation to the WTO accession today should continue in a practical way – training of the staff, development of the appropriate normative documents.

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PREDUZETNIŠTVO U POLJOPRIVREDI: NOVI IZAZOVI U MEĐUNARODNOJ TRGOVINSKOJ INTEGRACIJI

VASILY EROKHIN, ANNA IVOLGA

Izvod

Međunarodna trgovina u poljoprivrednoj proizvodnji je, i dalje, prilično daleko od pune liberalizacije, uprkos napretku koji je postignut posle niza pregovora u okviru STO. Neka nova pravila u međunarodnim trgovinskim organizacijama, kao i nove obaveze koje su preuzele od strane zemalja učesnica u STO, su otvorila nova pitanja o kojima treba raspravljati i ustanoviti u okviru sledećih pregovora. Osnovna tema ovih pregovora, tokom uvođenja Rusije u STO, bi trebalo da postane pitanja regulacije trgovine, dalje usavršavanje u pogledu pravila o zdravstvenoj ispravnosti proizvoda, dalje smanjenje carina i uspostavljanje administrativnog karaktera tarifnih kvota za uvoz poljoprivrednih proizvoda.

Ključne reči: trgovinska integracija, razvoj poljoprivrede, preduzetništva.

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NUTRITION SPECIFICITY OF BROWN HARE (LEPUS EUROPAEUS) AS A CAUSE OF THE DECREASED NUMBER OF POPULATION

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SUMMARY: This paper reviews the specific choice of food and feed for brown hare (Lepus Europaeus) in Serbia and Europe, reducing the diversity of flora as a result of the intensification of agriculture, and the impact of these changes in population numbers. Examination of the composition of hare food is done gastric contents microscopy or fresh feces. Studies from Sweden, Austria and Hungary indicate that hare consume dozens species of plants. However, most authors agree that about 50% of meals are only 2-3 plant species. Based on the literature review can be concluded that the intensification of agriculture substantially reduced the diversity of wild plants in farming areas, most authors considered important to reduce the number of hares in Europe. In particular, it highlights the problem of food deficit in the summer, after harvest of winter crops. During that time comes to change the chemical composition of milk or falling energy value of 14.5 KJg-[1] (spring) to 11.03 KJg-[1] (autumn), which negatively affects the offspring. In contrast, the deficit in the winter diet in most areas is less pronounced, due to the wheat fields. As a solution to these problems according to the establishment of "green corridor" between the fields under the spruce monocultures, organic vegetable production, control the number of predators and possibly controlled production hare offspring in farm

Key words: *brown hare, food, feed selection, the size of the population.*

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INTRODUCTION

For Serbian hunting grounds brown hare is certainly the most interesting types of small furry hunting game. To the spatial distribution and abundance of brown hare in some hunting grounds primarily affect the natural factors, such as climate (Beukovic et al. 2009a; Popovic et al., 1996a,1997), diseases and predators (Beukovićet al. 2011b; Popovic et al. 2009; Ristisc et al. 2010a); and to some extent, and anthropogenic factors: agricultural activities, transportation, hunting ...(Beukovic et al., 1997, Popovic et al.,1996b).The density of population in Serbia is a brown hare from a few individuals to several dozen individuals per 100 ha, while the highest density was recorded in Poland fifties: 240 hares per 100 ha(Popovic and Djordjevic, 2010).

This kind of hunting game features extremely high degree increments, which makes up a large mortality of young (Gajic and Popovic, 2010). Although hares can live 10-15 years, in modern conditions only 3% of hares reach the age of 4 years. Young hares (up to one year of age) account for 50-75% of fall hares population of , and the rest of are hares mostly aged 1-2 years. According to Popovic et al. (2011) in the previous decade, the greatest number of rhares in Serbia has established the 2005th (629,639 animals), followed by a decline, with the exception of Vojvodina (Beukovićet al., 2002, 2007a,2009b). Same time, there was an increase in shooting percentage, with 14.26% to 18.02%. For obvious downward trend in the number of the most important are responsible changed living conditions, increased use of herbicides, as well as various infectious diseases(Popovic, 2006, Popovic et al., 2008) and it should add the direct influence of man. The biggest influence on the strength of the game population in the hunting grounds by the users level of utilization of game populations (Beukovic et al.,2011). Percentage of population must be consistent with the size of the parent fund and must be accompanied by its oscillations. In addition, one can contribute to maintaining and increasing the number of hares other measures, such as improving nutritional potential hunting grounds, winter nutrition, import brown hares from other hunting, predator number control, closed season, the establishment of protected areas and others (Beukovic et al., 2009c).

MATERIALS AND METHODS

Basic characteristics of brown hare nutrition and feed selection

The choice of food for wildlife dependent on the specifics of the hunting area, the degree of human activity, and most of the season. Feeding wild animals in hunting areas can be fully based on natural food, or a certain percentage involve different nutrients that a man entered the grounds in order to achieve certain goals such as increasing the number of animals in a territory, a better quality of trophies, a smaller loss of game. (Beukovic et al., 2006, 2007b; Popovic, 2007, Djordjevic et al.,2008a). Interventions in the diet of wild animals can give good results only if we have good knowlage with the specific use (digestion) of food, the affinity of certain species for certain nutrients, nutrition potential of hunting grounds, the possibility of increasing production of natural food in the hunting ground, opportunities for additional nutrition of game, et all.. Therefore, in this paper provides an overview of previous research in nutrition brown hare hunting in Central Europe, which is most similar to the many features of Vojvodina as a part of Serbia with a relatively stable population of brown hare (Beukovi et al.,2009c).

Brown hare belongs to a group of small herbivores with cecum fermentation of digest, which digest plant fibers with lower efficiency compared to larger herbivores. Therefore, small herbivores in the evolutionary developed two strategies for better utilization of food: a) selection digestible nutrients and b) faster passages non digestible parts meals and selective retention of digestible. That escaped of many predators, brown hare can develop a speed of 72 kmh^{-1}, compared with rabbits with a maximum speed of 52 kmh^{-1}. This feature requires fewer digestive system and the smallest ballast in the digestive organs. This is done by selecting digestive nutrient and faster flow of fiber. This is supported by the fact that brown hare are anatomically adapted to these requirements and has a smaller percentage of the digestive organs in relation to the brown hares (Table 1).

Table 1 Organ weight (g) of selected organs including Digest as a proportion of body wt %, ± SD, (n) (Stott,2008)

	Hare (% of gross carcass weight)	Rabbit (% of gross carcass weight)
Abdominal alimentary canal	13.77 ± 2.94 (79)	18:20 ± 3.44 (37)
Stomach	2:53 ± 0.72 (68)	5:09 ± 1:38 (46)
Small intestine	2.76 ± 0.45 (24)	3.23 ± 0.49 (25)
Caecum	4.97 ± 1:49(95)	6.79 ± 1.87 (38)
Liver	2.24 ± 0.39 (13)	2.65 ± 0.61 (43)
Heart	1:06 ± 0.12 (15)	0:28 ± 0.10 (37)

Determining the composition of the diet of wild animals is a very complicated method, all methods developed so far have certain disadvantages, such as monitoring - observation nutrition in the field (De-Wichatitsky Garin et al., 2005), the method with esophagus fistula (Vetetoet al.1972), microscopy and chemical analysis of gastric contents (Kamlerand Homolka, 2005) or microscopy and chemical analysis of feces(Katona and Altbäcker ,2002).

The fastest and easiest way to assess the composition and nutritional quality of wild animals is to examine the stomach contents shooted animals (Djordjevicet al.,2008b). Specifically, these samples can be taken from each animal, except in rare cases when a hunter shot a significant seriously damages the digestive system and caused extensive bleeding. However, analysis of rumen content requires the killing or capture animals (Watanabeand Takatsuki,1993).This can be a significant problem in hunting with a smaller number of animals in protected areas and parks, and selective culling can reduce the representativeness of samples.

Assessing the quality of food based on microscopy of feces its requirements collection for the area in places where the animals move up and retained. You should use only fresh feces that are immediately analyzed or frozen until analysis of botanical (Djordjevic et al.,2007). Size (number) of the sample is crucial for the accuracy of the data (Holecheket al.,1982). The great variety in the diet of some species and low similarity between individuals of one species require larger initial sample (Katonaand Altbäcker, 2002).

In previous studies, which did Frylestam (1986) in Sweden found that hares prefer a wild plant species and their eating significantly more diverse in areas of natural grass-

lands (37 species consumed) relative to the surface under the spruce monocultures (14 species consumed). Reichlin et al. (2006), stated that although the in diet of brown hare dominated cultivated plants, the animal prefers weeds and wild grass, if available to them. However, Jennings et al. (2006) have found from research in England and Wales to hares out of pastoral areas have less mass and less fat reserves, despite a similar quality food. The authors believe that the reason for the higher energy expenditure.

Table 2 Positiveli selected plant species (Reichlinet al.,2006)

Period	Scientific name	ε	N	is
February	*Malus domestica*	0.96	1	-
	Daucus carota	0.87	3	0.08
	Beta vulgaris	0.83	22	0.08
May	*Trifolium repens*	0.87	2	0.07
	Glycine max	0.61	8	0.25
	Trifolium pratense	0.39	4	0.35
	Papaver rhoeas	0.09	9	0.32
August	*Panicum miliaceum*	1.00	1	-
	Papaver rhoeas	0.93	1	-
	Trifolium incarnatus	0.27	2	0.68
November	*Beta vulgaris*	0.98	5	0.02
	Capsella bursa-pastoris	0.85	1	-
	Triticum aestivum	0.19	20	0.21

ε mean Electivity Index, N number of Hares that the plant used, the standard error of the mean.

Reichlin et al. (2006) are research nutrition of brown hare conducted in Austria, at an altitude of 140 m. Microscopy was used for the gastric contents shooted 110 animals and seven animals were found murdered on the road. The research was carried out in February (n = 37), May (n = 28), August (n = 32) and November (n = 20) to determine the composition of the winter, spring, summer and fall feeding. The authors found that in autumn and winter in hare food dominate crops, at the first place wheat (51%), and nutrients that are provided by hunters (sugar beets, carrots ...). In contrast, the share of weeds in these months is less than 5%. Nesvadba and Zaid (1989) in studies in the Czech Republic, also found that in the winter diet of brown hare, wheat has a dominant role. The analysis of stomach contents in February found that, beside to wheat, sugar beets and alfalfa account for about 91% of the total meal. During the spring feeding in the stomach contents of brown hare dominated arable crops (83%), primarily soybeans, while in August the three most consumed wild plant species, particularly after the cereal harvest (Table 2).

Katona et al. (2010) in five areas of Hungary and the three-year research investigated the composition of the autumn diet of brown hares (n = 350) microscopy of the stomach contents. The authors found that hares consuming a total of 24 plant species. Since the dominant species of cultivated plants in the diet of hares was wheat (*Triticum aestivum*), and a small scale was used, and alfalfa (*Medicago sp.*). In contrast, research Homolka (1983) indicate much higher share of alfalfa, as high as 21%. Of other crops Katona et al. (2010) have determined the presence of 0-3% of rape, and only in one

year and two of the area, while maize and sunflower in the investigated time already harvested. Frylestam (1986) considers that rabbits avoiding rape in late autumn due to the high content of glucosinolates and Chapuis (1990) argues that rape can be responsible for the mortality of wild animals. In addition to these plant species in the samples was established a significant presence Elder berry (Sambucus spp.) In the amount of 5-41%, although in previous studies in other parts of Europe it has not been established. Consuming large quantities of these plant species may explain the high protein content (36%). Significant participation in the meal had a browsing, which the authors believe that it is encouraged with the edges of forests and agricultural fields, and not from the nearby forests. Browsing has become an important part of the hare meal especially during the winter (Katonaet al.,2004). In contrast, consumption of seeds and herbaceous plants was small. The authors found that about 50% of the meal consisted of only 1-3 plant species. This observation agrees with previous studies Homolka (1983) who argues that only a few plant species seems most of the meals of brown hares. In conclusion, the authors note a high territorial and individual variability in the diet of hares.

DISCUSSION

Based on the literature review can be concluded that the intensification of agriculture greatly reduced the diversity of weeds in crop fields, which quoted the authors consider most important to reduce the number of brown hares in Europe. There are other interpretations of this great problem for the hunting industry. Smith et al. (2005) considered that the reduction in brown hare numbers drastically changed the most responsible environmental conditions, while the variety of problems in second-rate food.

In his research Vapa et al. (2006) believe that the disease of rabbits that appeared here with similar symptoms as in other countries, accompanied by significant mortality and at the same time of year (fall winter), and which were given different names, could be considered EBH syndrome.

The same authors state that it is necessary to take comprehensive measures to fully look into epizootic situation in regard to these and other diseases during the game and enable them to scientific institutions for the diagnosis EBHS.

Critical periods in the winter diet of brown hares and other half of the summer. From the literature review, it is obvious that brown hares during the winter months a significantly (or most) part of their needs satisfy, with thanks, at first place wheat. During this period, a problem may be a reduced availability of such foods because of deep snow and low temperatures growth maintace needs of hares (Popovic and Djordjevic, 2010). Except in winter, a big problem to feed the brown hares occurs in the period after harvest, when the arable land disappears until then the dominant food (and shelter), while the natural grasslands significantly reduced production of natural food due to summer drought. This phenomenon is known as “harvest stress” and can be extremely detrimental to the population of brpwn hares that live in large farming complexes under monocultures (Djordjevicet al.,2011). Another problem is the burning of stubble (and later mazefield), where the wild are directly threatened by fire and smoke, and indirectly due to complete mineralization of organic matter and destroying the natural feed. Since hares have a high fertility and their offspring make the world in the summer and autumn, harvest leaves a heavy stress effects on their offspring. Valenčak et al. (2009) stated that the European hares has large energy needs in reproduction, the very

fast growth offspring, as well as high energy value of milk. During the first four weeks of life, feeding the young is based solely on milk and its energy value depends on fat reserves accumulated in the previous autumn and winter, or directly from food. Given that the quality and quantity of food significantly reduced at the end of summer, it could be a significant problem in the rearing of young and reflected on the number. Valencek et al. (2009) have found that milk production does not change significantly during the reproductive season, but to significantly change its energy value of 14.5 KJg-1 (spring) to 11.03 KJg-1 (autumn), and due to a decrease in the percentage of milk fat (Table 3). (Liener, 1994).

Table 3 Energy budgets of lactating female European Hares (Valenceket al.,2009)

	Spring	Summer	Autumn
MEI[1]	918.1 ± 22.7	884.8 ± 15.4	961.9 ± 25.5
Milk total energy output[2]	356.8 ± 14.7	266.0 ± 8.2	272.6 ± 13.0
Milk energy reserves from[3]	88.9	0	0
Milk energy from food[4]	267.9 ± 14.7	266.0 ± 2.8	272.6 ± 13.0
Maintenance costs[5]	650.2 ± 16.8	618.8 ± 13.2	689.2 ± 16.1

[1]Metabolizable energy intake. [2]Milk energy content x milk mass. [3]Difference between milk energy content in spring and later in the year. [4]MEI-milk energy from food.

The solution to reducing the number of brown hare may be found in farm production and release in hunting ground again, much like pheasants. (Risrtic et al. 2010b) In this respect, the greatest progress was made in France and Italy, although the quarterly settlement "half-wild" hares in hunting grounds remains a problem(Gajic and Popovic, 2010). With this procedure brown hares are kept in special cages and feed pelleted feed, which is often the chemical composition and nutritive value corresponds to the needs of rabbits. In this regard it is necessary to further examine the needs of hares in nutrients and use of concentrate mixtures that are designed according to these needs. According to one female in the season so far can be obtained in this manner from 2.2 to 2.5 young (Mertinet al.,2010). That is, compared to production of pheasants, a small number, so this production is extremely expensive and unprofitable. However, intensive work on resolving outstanding problems of this procedure may be able to become significant in the foreseeable future to maintain the number of hares.

CONCLUSION

Based on the literature review can be concluded that the drastic reduction in brown hare numbers in Europe and Serbia, the most responsible intensification of agriculture and reducing the diversity of the natural diet. For the possibility of preserving or even increasing populations hare (Lepus Europaeus) in Serbia there are several solutions, some of which are currently most relevant:

a) the preservation of "green oasis" and the establishment of "green corridors" between areas with large monocultures that will provide food and protection, especially after the harvest of winter cereals,

b) organic vegetable production that will allow the survival and diversity of wild flora and
c) control the number of predators.
d) continuous monitoring of abundance and hunting rabbits for each area in accordance with management plans and test results the percentage of young rabbits
e) One should, perhaps, add controlled production of brown hares in the hares farm, which requires intensive work on resolving the many remaining problems in this technology.

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SPECIFIČNOSTI ISHRANE ZECA (LEPUS EUROPAEUS) KAO UZROK SMANJENJA BROJNOSTI POPULACIJA

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Izvod

U radu je dat pregled specifičnosti ishrane i izbor hraniva za zeca (*Lepus europaeus*) u Srbiji i Evropi, smanjenje raznovrsnosti flore kao posledica intenzifikacije poljoprivrede, i uticaj navedenih promena na brojnost populacija. Ispitivanje sastava ishrane zeca vrši se mikroskopiranjem želudačnog sadržaja ili svežeg fecesa. Istraživanja iz Švedske, Austrije i Mađarske ukazuju da zec konzumira nekoliko desetina vrsta biljaka. Međutim, većina autora se slaže da oko 50% sastava obroka čine samo 2-3 biljne vrste. Na osnovu pregleda literature može se zaključiti da je intenzifikacija poljoprivrede bitno smanjila raznovrsnost divljih biljnih vrsta u ratarskim područjima, što većina autora smatra značajnijim za smanjenje brojnosti zeca u Evropi. Naročito se ističe problem letnjeg deficita u hrani, nakon ubiranja ozimih kultura. U tom periodu dolazi i do promene hemijskog sastava mleka, odnosno pada energetske vrednosti od 14,5 kJg-1 (proleće) do 11,03 kJg-1 (jesen), što se negativno odražava na podmladak. Nasuprot tome, zimski deficit u ishrani u većini područja nije toliko izražen, zahvaljujući poljima sa pšenicom. Kao rešenje ovih problema navodi se zasnivanje «zelenih koridora» između polja pod monokulturama, organska biljna proizvodnja, kontrola brojnosti predatora i eventualno, kontrolisana proizvodnja zečića u odgajivalištima.

Ključne reči: zec, ishrana, izbor hraniva, brojnost populacija.

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ULTRASOUND DIAGNOSIS OF EARLY PREGNANCY IN SWINE*

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SUMMARY:Early pregnancy diagnosis significantly affects the efficiency of the intensive swine production. Furthermore, early pregnancy diagnosis of non-pregnant animals reduces the number of non-productive feeding days per sow, considerably increases the utilisation efficiency of housing space, and enables timely interventions during medical examinations of each animal. Traditional methods of pregnancy diagnosis, such as the detection of the lack of oestrous cycle in inseminated animals, the determination of progesterone and oestrone sulphate in blood and/or urine, the examination of vaginal and cervical discharge, and the rectal palpation of reproductive system, are either imprecise or impractical regarding production conditions. Ultrasound diagnosis is a sophisticated method of highly precise early pregnancy diagnosis deprived of negative effects on examined animals. Moreover, this method provides a real-time visualisation of conceptus, uterus, and ovaries. Ultrasound diagnosis enables pregnancy diagnosis as soon as 17 days after insemination. Well over 95% of accurate diagnoses are obtained during examinations conducted between 25 and 35 days after insemination. The only disadvantage of this method, which notably impedes mass application in production, is a fairly steep price of ultrasound equipment.

***Keywords**: ultrasound, early pregnancy, diagnosis, swine.*

Scientific Review Paper / *Pregledni naučni rad*

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INTRODUCTION

The reproductive efficiency of a sow herd is the result of the number of farrowed piglets per sow annually. One of the main parameters, which significantly influence the reproductive efficiency of a sow herd, is the farrowing index, i.e. the average number of farrowings per sow per year. This index is directly conditioned by the number (%) of sows and gilts, which conceive successfully after the first insemination. Thus, the number of non-productive feeding days per sow per year is reduced, and consequently the cost price of the total piglet production (Koketsu, 2005). Therefore, it is exceptionally important to use efficient methods of early pregnancy diagnosis in production within the period of 30 days after insemination. This procedure enables detection of non-pregnant sows and timely interventions. Moreover, the number of non-productive feeding days per sow per year is greatly reduced, and the reproductive efficiency of a sow herd is considerably increased from the technological, veterinary, and economic viewpoint (Stančić et al., 2003; Radović et al., 2006; Stančić et al., 2008; Stančić et al., 2008; Gagrčin et al., 2009; Stančić et al., 2009; Stančić et al., 2010).

The detection of pregnancy in sows and gilts on farms in our country is still conducted by the traditional method of detecting the lack of oestrus with a teaser boar 18 to 24 days after insemination. This method is not highly efficient due to numerous reasons which hinder the external exhibition of oestrus signs apart from pregnancy (Stančić et al., 2004). Pregnancy diagnosis methods such as the determination of progesterone and oestrone sulphate in blood and/or urine, the rectal palpation of reproductive tract, the histological and/or bio-electronic testing of vaginal discharge are not suitable for practical application in the intensive swine production (Flowers et al., 1999; Stefanakis et al., 2000; Stančić et al., 2001; Chadio et al., 2002; Stančić, 2002; Stančić et al., 2003; Stančić, 2004; Boma and Bilkei, 2008). The application of the ultrasound early pregnancy diagnosis in swine started in the late 1970s (Bosc et al., 1975). However, the practical application of ultrasonography is still very limited by a fairly steep price of necessary equipment. Nevertheless, the method of ultrasound pregnancy diagnosis is highly efficient because it enables the accurate diagnosis of early pregnancy as soon as 20 days after insemination. The method itself is very practical because it does not require any special preparations of animals, it does not cause great stress in animals, it does not have negative effects on animals, and the results are obtained in real time (Flowers et al., 1999).

Table 1. Efficiency of various techniques for pregnancy diagnosis in swine (Flowers and Knox, 2000)

Technique	Physiological Basis	Period of Efficiency	Accuracy
Detection of oestrus	Non-pregnant females exhibit oestrus	Any time during gestation	>98*
A-mode ultrasound	Identification of fluid in pregnant uterus via speed at which emitted sounds return to probe	Days 28 to 80 of gestation	>95
Doppler ultrasound	Identification of sound patterns of increased blood flow in uterine and umbilical arteries during pregnancy	After day 29 of gestation	>95

Real-time ultrasound (B-mode)	Visualization of fluid and fetal tissue in pregnant uterus	After day 21 of gestation	>95
Progesterone concentrations	Increased blood progesterone concentrations (>5.0ng/ml) in pregnant females	Days 17 to 20 of gestation	>85
Prostaglandin-$F_{2\alpha}$ concentrations	Increased blood prostaglandin concentrations in non-pregnant females (>200pg/ml)	Days 13 to 15 of gestation	>80
Oestrone sulphate concentrations	Increased oestrone sulphate concentrations in pregnant females (> 0.5 ng/ml)	Days 25 to 30 or after day 80 of gestation	>93

*Disadvantages: (1) pseudopregnant sows do not exhibit oestrus; (2) irregular exhibitions of oestrus prolong the period from insemination to the exhibition of oestrus; (3) the visualisation of reproductive tract is not possible; (4) repeated tests of pregnant sows are necessary to detect oestrus exhibition; (5) potential errors in detection of oestrus.

The aim of this paper is to show the basic technology and efficiency of the ultrasound early pregnancy diagnosis in swine, as well as the application of this diagnostic method in contemporary swine production.

DIAGNOSTIC ULTRASOUND TECHNOLOGY

The basic operation mode of an ultrasound apparatus consists of the following: (1) the apparatus transmits electric current to the wave-generating transducer (probe), (2) electric current stimulates piezoelectric crystals in the transducer to generate ultrasound waves (USW) of certain frequency, (3) USW are emitted towards organs and tissues, (4) some tissues reflect these waves (echogenic tissues), and some tissues or fluids (such as the allantoic fluid) absorb the waves (non-echogenic tissues) , (5) the obtained ultrasound echo of certain frequency is received by the transducer and transmitted to the apparatus where it is transformed in a black and white image displayed on the screen (Doppler real-time B-mode) or shown as a chart in a coordinate system (A-mode ultrasonograpgy).

Basically, the tissues and matters of higher density (e.g. fetal bones) generate stronger echo (so-called echogenic structures) so their ultrasound imaging is brighter, whereas the ultrasound imaging of tissues and matters of lower density is darker (e.g. the allantoic fluid, i.e. non-echogenic structures). The image is displayed in shades ranging from the absolute black to the absolute white colour. Fluids (liquids) are non-echogenic so they are shown black, whereas tissues, depending on their density, are shown in shades ranging from the absolute white to the bright grey colour (e.g. bones are very echogenic). Probes or transducers can generate USW ranging from 3.5 to 7.5 MHz. Probes with the total power of 3.5 MHz generate shortwave frequencies which can penetrate deeper into tissues, but the resolution of the obtained echo image is lower, whereas probes with the total power of 7.5 MHz generate high frequencies with weaker tissue penetration and higher resolution of the obtained image (Mannion, 2006).

Diagnoses can be obtained by means of transabdominal and transrectal probe. Transabdominal sonography is more common because it is less complicated and traumatic, but the visualisation of the organs and tissues is of lower quality in comparison

with the transrectal method. Transabdominal ultrasonography is performed by placing a probe on the borderline area between the ventral part of the abdomen and the final 3 mammary complexes, usually on the right side (Moeller, 2002). Easily detectable echo frequencies 15-20 cm deep imply pregnancy, whereas in non-pregnant animals this echo is 5 cm deep. Convex 3.5-5.0 MHz probes are widely used in practice for early pregnancy diagnosis in sows and gilts. Transrectal probes are inserted into the rectum of animals and this procedure shortens the distance between the probe and the examined organ, which enables images of higher resolution. Nevertheless, the procedure is complicated, it requires the sedation of animals, and creates substantial negative impact on the organs and tissues (Kyriazakis i Whittemore, 2006).

Nowadays, there are two types of ultrasound apparatus in use for early pregnancy diagnosis in sows and gilts: the Amplitude-depth sonography (A-mode or Pulse-Echo) and the Doppler ultrasonography (B-mode, real-time).

The A-mode is the simplest method. The reflected ultrasound echo is registered via graphic signals. The Y-axis of the chart shows the echo-amplitude, whereas the X-axis shows the depth, which is the distance from the organ. There is no visualisation of the organs or tissues. The physiological basis of this method is the determination of the fluid in the pregnant uterus, which reflects the ultrasound echo. The Doppler (B-mode, real-time, a black-and-white image) shows two-dimensional black-and-white anatomical image of organs, tissues, and various fluids in the pregnant uterus. It can detect fetal movements, heartbeats, and blood vessel pulsation in real time. This type of ultrasonography provides the information on the anatomy and function of the examined organs (Flowers and Knox, 2000).

Image 1. A-mode, Pulse-Echo (the apparatus and the graphic image on the screen)

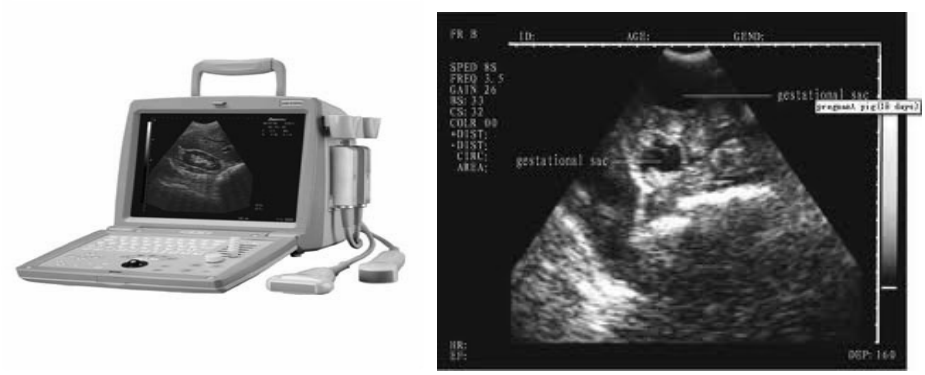

Image 2. Dopler B-mode real-time (the apparatus and the image on the screen)

THE APPLICATION OF ULTRASOUND DIAGNOSIS

The efficiency of the ultrasound pregnancy diagnosis entails the following: (1) it is possible to diagnose pregnancy very early (as soon as 17 days after insemination), (2) the results are obtained immediately and in real time, (3) the accuracy of the diagnosis is very high (over 90%), (4) the diagnostic procedure is simple and short, and (5) the procedure does not require any special preparations of animals, and it displays no negative effects on the health of animals. Therefore, it is very suitable for practical application in the intensive swine production. On farms in the USA, it is common practice to test the inseminated sows and gilts for the exhibition of oestrus with a teaser boar within the period of 17-24 days after insemination. Moreover, this traditional procedure is combined with the ultrasound pregnancy diagnosis obtained within the period of 28-45 days after insemination (Almond and Dial, 1987).

The accuracy of the diagnosis is increased with the length of pregnancy. Thus, on the 18th day after insemination positive pregnancy diagnoses were confirmed in 60% of sows, whereas on the 24th day after insemination they were confirmed in 96% of sows using the Doppler ultrasonography (Flowers et al, 1999). The studies conducted by Williams et al. (2008) show positive pregnancy diagnoses in over 90% of the observed sows within the period of 17-24 days after insemination using the Doppler ultrasound method. These authors came to a conclusion that there is no significant difference in diagnostic accuracy between the Doppler real-time and A-mode ultrasonography performed within the period of 17-24 days of gestation. In practice, it is very important to detect non-pregnant animals as soon and as accurate as possible. The results of the study indicate that the accuracy of the negative diagnosis is somewhat lower than the accuracy of the positive diagnosis. Furthermore, it is also demonstrated that the negative diagnosis is obtained more accurately using the Doppler real-time ultrasonography in comparison with the A-mode ultrasonography. Namely, the A-mode ultrasonography can hardly distinguish the bladder fluid from the allantoic fluid; whereas the Doppler real-time ultrasonography enables clear visualisation of the bladder. This is the main reason of the more accurate diagnosis by means of the Doppler real-time ultrasonography (Flowers et al., 1999). Therefore, it is highly recommended to repeat the diagnostic tests in sows with the negative diagnoses in 10 to 15 days (Maes et al., 2006). Repeated

tests are recommended even in the case of suspected early embryonic mortality because it is not possible to anticipate whether the pregnancy will be continued (Kauffold et sal., 1997; Millller et al., 2003; Knox and Flowers, 2004).

Basically, ultrasonography enables early pregnancy diagnosis via the sonogram image of a larger or a smaller black circle (the non-echogenic area of the allantoic fluid). The diameter of this area is increased with the length of pregnancy. The volume of the allantoic fluid per embryo is known to be increased from approximately 3ml on the 17th day of gestation to approximately 200ml on the 30th day of gestation. However, a substantial increase of the amniotic fluid is not detected prior to the 30th day of gestation. Nevertheless, it has been confirmed that the volumes of amniotic and allantoic fluids per embryo vary greatly within the first 25 days of gestation (Wildt et al., 1975; Knight et al., 1977). After the 21st day of gestation, there is a sufficient volume of the fluid in the allantoic sac to be clearly visualized by means of the Doppler real-time ultrasonography (Flowers et al., 1999).

According to the researches we have conducted (Stančić et al., 2011., unpublished data) by means of the Doppler real-time ultrasonography, it is possible to visualize the allantoic sac on the 20th day of gestation with the diameter of 9.54mm, and the embryo with the length of 0.8-1.6 cm at the depth of 24.8 cm (Image 3).

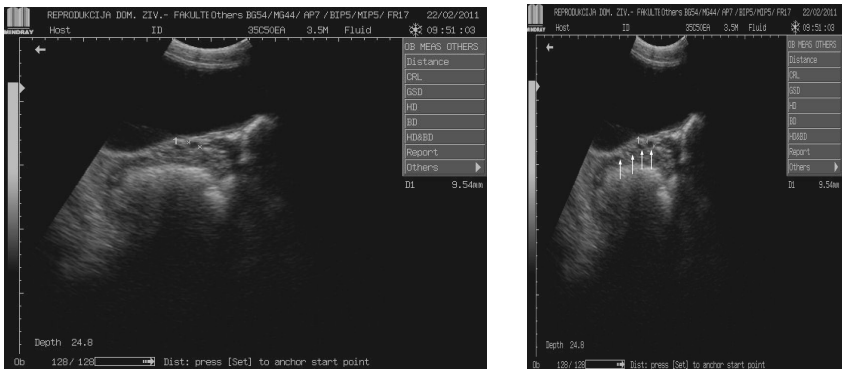

Image 3. The ultrasonogram on the 20th day of gestation *(Stančič I., 2011)*
The amniotic sacs are shown in the uterine horn (arrows).

Within the period of 23-26 days of gestation, the increase of the allantoic sac diameter is clearly visible ranging from 2.2 cm to 2.5 cm, as well as the embryo with the length ranging from 1.8 cm to 2.5 cm. The depth is decreasing from 10.80 cm to 7.55 cm due to the allantoic sac enlargement (Image 4 and 5).

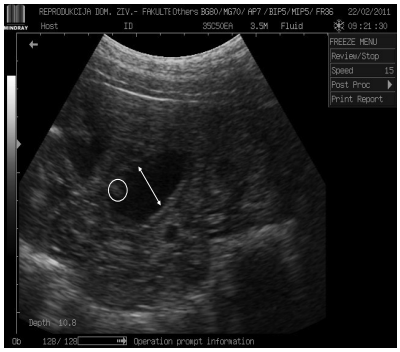

Image 4. The ultrasonogram on the 23rd day of gestation *(Stančič I., 2011)*
The amniotic sac (the arrow) and the embryo (in the circle) are shown.

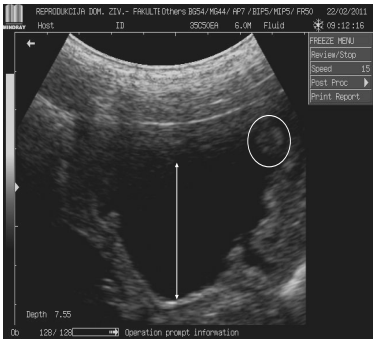

Image 5. The ultrasonogram on the 26th day of gestation *(Stančič I., 2011)*
The enlarged amniotic sac (the arrow) and the larger embryo (in the circle) are shown.

During the second half of the gestation period, the method of ultrasound pregnancy diagnosis is totally accurate. The fetal visualisation is completely clear, as well as the skeletal ossification. The foetuses are 5.7-6.0 cm long on the 56th day of gestation, and 8.0-9.0 cm on the 84th day of gestation (Image 6 and 7).

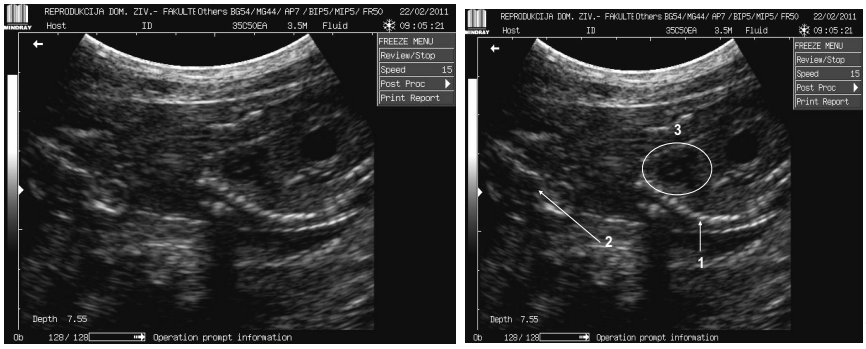

Image 6. The ultrasonogram on the 56th day of gestation *(Stančič I., 2011)*
The embryonic vertebral column (1), head (2) and heart (3, in the circle) are shown.

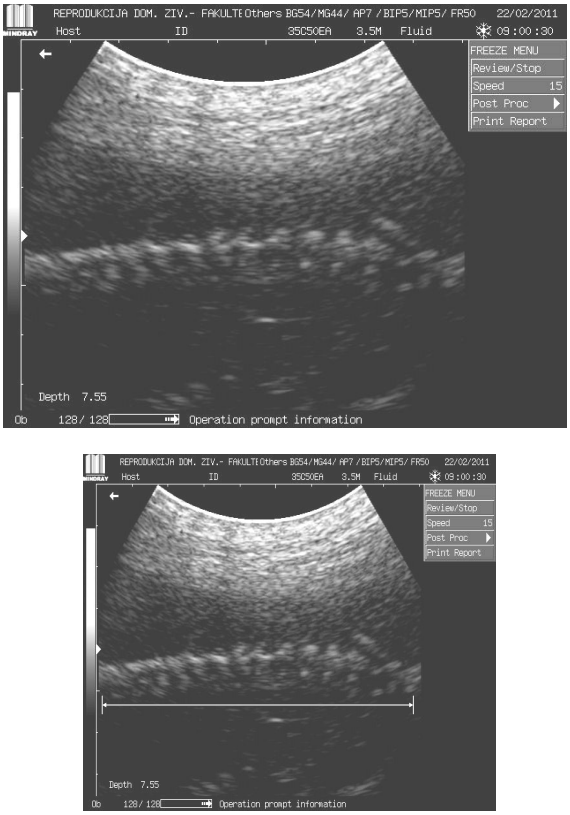

Image 7. The ultrasonogram on the 84th day of gestation (Stančič I., 2011)
Clearly defined vertebrae of the fetal vertebral column are shown (arrow)

The application of the real-time ultrasound methods (e.g. the Doppler or B-mode) enables pregnancy diagnosis approximately two weeks sooner than the A-mode ultrasonography. By means of the Doppler real-time visualisation it is possible to detect fetal mortality in sows previously diagnosed as pregnant. It is also possible to diagnose pseudopregnant sows (Flowers et al., 1999) due to the retention of the uterine luminal fluid after fetal mortality. Moreover, the fetal skeletal calcification does not commence prior to the 60th day of gestation and hence pseudopregnancy is extremely hard to detect prior to the 65th day of gestation. However, fetal heartbeats are not detected in pseudopregnant animals (Knight et al., 1977).

The Doppler ultrasound is used for the detection of the blood flow in the fetal heart and large umbilical cord blood vessels. The procedure enables the detection of the blood flow in these blood vessels, especially in the uterine artery, as soon as on the 21st day of gestation, while a significantly more accurate diagnosis can be obtained following the 30th day of gestation (Kyriazakis and Whittemore, 2006).

By means of the ultrasound technology it is possible to diagnose various pathological lesions of reproductive organs. Moreover, ultrasonographic examinations of the

function of ovarian structures (antral follicles and corpora lutea) enable the determination of the oestrous cycle phase in examined animals (Waberski and Weitze, 1998; Kauffold et al., 2004; Kauffold et al., 2007). This is very important for the practical production. It has been confirmed that in approximately 30% of breeding gilts on farms in Serbia, which are usually 8 months of age or more, the detection of oestrus exhibition is not possible with a teaser boar (Stančić et al., 2008; Stančić et al., 2008; Stančić et al., 2001). These gilts are not inseminated and they are excluded from the further reproduction as 'anoestrous'. However, the post-mortem examination of reproductive organs indicates that over 60% of these gilts exhibit the cyclic ovarian activity and their reproductive organs show no pathological lesions, which could cause them to be anoestrous. Therefore, it has been concluded that such gilts are not anoestrous and that they are unnecessarily excluded from reproduction due to the inefficient detection of oestrus exhibition with a teaser boar (Stančić et al., 2009; Stančić et al., 2011). Timely ultrasound examinations would detect the cyclic ovarian activity in these gilts and prevent the exclusion from reproduction based on the misdiagnosis 'prolonged pre-insemination anoestrus'. Consequently, the ultrasound procedure would be indubitably beneficial from the zoological, technological, and economic viewpoint.

CONCLUSION

The application of ultrasound technology enables highly accurate positive and/or negative early pregnancy diagnosis in sows as soon as 20 days after insemination. By means of the Doppler real-time ultrasonography it is possible to detect pseudopregnant sows after the 60th day of gestation. The method also ensures the detection of cyclic ovarian structures and pathological lesions in female reproductive system. This is greatly important for the intensive swine production due to the reduction of non-productive feeding days per sow per year.

The traditional method of oestrus detection with a teaser boar 18-24 days after insemination does not facilitate the detection of non-pregnant animals. Furthermore, according to numerous researches of various authors, it is not possible to detect oestrus within this period in 20-30% of sows which are not pregnant. On the other hand, a considerable number of sows exhibit oestrus following the 24th day after insemination (so-called irregular oestrus exhibition), as well as a considerable number of pseudopregnant sows (which are non-pregnant after insemination and do not exhibit oestrus almost until farrowing). Consequently, the average period from the first to the successful insemination is increased, as well as the average period from the first insemination to the exclusion from further reproduction (so-called non-reproductive period). The prolonging of this non-productive period causes significant reduction in efficiency of piglet production on farms: (a) the average annual farrowing index is decreased and thus the total number of produced piglets on a farm, (b) the average number of non-productive feeding days per sow per year is increased, and (c) the number of unnecessarily excluded sows from reproduction is increased. These factors substantially increase the cost price of piglet production and reduce the cost-effectiveness of such production on farms.

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ULTRAZVUČNA DIJAGNOSTIKA RANE GRAVIDNOSTI KOD SVINJA

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Izvod

Rana dijagnostika graviditeta značajno utiče na efiksanost intenzivne proizvodnje svinja. Naime, što ranijom dijagnozom negravidnih životinja, smanjuje se broj neproduktivnih hranidbenih dana po krmači, značajno se povećava efikasnost iskorištavanja smeštajnog prostora, a mouguća je i pravovremena adekvatna intervencija kod svake pregledane životinje. Klasične metode dijagnoze gravidnosti, kao što su evidencija izostanka estrusne cikličnosti, 18 i više dana od osemenjavanja, detekcija progesterona i estronsulfata u krvi i/ili urinu, ispitivanja vaginalne i cervikalne sluzi i rektalne palpacije reproduktivnih organa, ili nisu dovoljno precizne ili nisu praktične za primenu u proizvodnim uslovima. Ultrazvučna dijagnostika je sofisticiran metod vrlo preciznog dijagnostikovanja rane gravidinosti, bez negativnih posledica po ispitivanu životinju. Osim toga, ovom metodom se dobija dobra vizuelizacija konceptusa, materice i jajnika u realnom vremenu. Ultrazvučnom metodom se, kod svinja, može dijagnostikovati gravidnost već od 17. dana posle osemenjavanja. Preko 95% tačnih dijagnoza se dobija pregledom izvršenim između 25. i 35. dana od osemenjavanja. Značajniji nedostatak, koji znatno limitira masovniju primenu ove metode u prakstičnoj poizvodnje, je dosta visoka cena ultrazvučne opreme.

Ključne reči: *Ultrazvuk, rani graviditet, dijagnoza, svinje.*

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CURRENT DIAGNOSTIC PROCEDURES FOR CYSTICERCOSIS IN CATTLE AND PIGS*

VESNA LALOŠEVIĆ[1]

SUMMARY: Taeniosis/cysticercosis complex is a significant public health problem worldwide. Control of this zoonotic disease requires a good diagnostic test to identify animals harboring live metacestodes. Meat inspection, which is the only public health measure implemented to control this human infections, is a poorly sensitive method to detect such infected animals. The need for diagnostic tests superior to meat inspection have led to the development of serological tests. Antibody detection diagnostic tests indicate exposure to infection and not necessarily the presence of an established, viable infection. But from the public health point of view only living metacestodes are important. Contrary to meat inspection and antibody detection methods, the monoclonal antibody (MoAb)-based enzyme-linked immunosorbent assays (ELISAs) for the detection of excretory/secretory (ES) products indicate infection with live metacestodes The MoAb based antigen detection ELISA (Ag-ELISA) is much more sensitive to identify active infections of T. saginata and T. solium metacestodes.

Key words**:** *cysticercosis, meat inspection,antibody detection methods, ES Ag-ELISA.*

INTRODUCTION

Cysticercosis is a larval tapeworm infection acquired from ingestion of embryonated *Taenia saginata* (cattle) or *Taenia solium* (pigs and humans) eggs excreted with faeces from human carriers who harbour the adult tapeworm in the intestines. The hatched embryos migrate throughout the body and develop into cysticerci (Minozzo *et al.,* 2002; Boa *et al.,* 2002). In humans, the invasion of the central nervous system with *T. solium* cysticerci known as neurocysticercosis (NCC) is one of the emerging diseases worldwide, and the major cause of epileptic seizures in areas of endemicity (Cruz *et al.,*

Review scientific paper / *Pregledni naučni rad*

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1999; Doder et all. 2004). Moreover, cysticercosis is an important cause of economic losses in pig and cattle production due to downgrading and/or condemnation of carcasses as untreated infected meat is an important source of this zoonosis (Carabin *et al.,* 2006;). Meat inspection, which is the only public health measure implemented to control human infections, is a poorly sensitive method and detects mainly heavily infected animals (Wanzala *et al.,* 2003).Detection of cysticerci depends on the expertise of the meat inspector as well as on the stage of development of the cysticerci (Minozzo *et al.,* 2002; Wanzala *et al.,* 2003). The method is more sensitive to detect dead, degenerated, or calcified cysticerci than viable cysts, as the latter they have the same pinkish-red color as the meat (Onyango-Abuje *et al.,* 1996; Wanzala *et al.,* 2003). Moreover, the method detects infection only after slaughter when it is too late to make decision on treatment of animals.

The need for diagnostic tests superior to meat inspection have led to the development of serological tests. Antibody detection diagnostic tests indicate exposure to infection and not necessarily the presence of an established, viable infection. But from the public health point of view only living metacestodes are important. Contrary to meat inspection and antibody detection methods, the monoclonal antibody (MoAb)-based enzyme-linked immunosorbent assays (ELISAs) for the detection of excretory/secretory (ES) products indicate infection with live metacestodes (Harrison *et al.,* 1989; Brandt *et al.,* 1992). The 158C11 and 60H8 MoAb based antigen detection ELISA (Ag-ELISA) is much more sensitive to identify active infections of *T. saginata* and *T. solium* metacestodes (Brandt *et al*., 1992; Van Kerckhoven *et al*., 1998; Dorny *et al*., 2000). The assay is an important tool to detect individual cases or to screen populations for the presence of viable cysticerci. This information is required by public health services in order to make decisions on treatment or control programs (Wanzala *et al.,* 2002; Sikasunge *et al.,* 2008). The assay requires relatively expensive laboratory equipment. The dipstick methods are more applicable in the resource deprived field laboratories of developing countries (Birku *et al.,* 1999). Studies that have used the polystyrene surfaced Nunc-immunoTM dipsticks for the detection of pathogens in food samples demonstrated the usefulness of the sticks for immunological tests (Aldus *et al.,* 2003; Blazkova *et al.,* 2006).

Immunodiagnostic tools

Immunodiagnostic techniques include methods for specific antibodies and for circulating parasite antigen in serum or cerebrospinal fluid. The antigens used in immunoblot and enzyme-linked immunosorbent assay (ELISA) for antibody detection have evolved from crude extracts to highly purified specific fractions and recombinant antigens of the glycoprotein family, increasing both the sensitivity and the specificity of the tests. The application of ELISA for the detection of circulating parasite antigens may present some diagnostic advantages since it demonstrates not only exposure but also active infections. Until now only a few of the current techniques have been standardised and fully validated, making comparisons between studies difficult. In surveys on cysticercosis, antibody detection systems have been useful in identifying the risk factors associated with transmission of *Taenia spp.* High seroprevalence in a community indicates a situation where preventive and control measures should be applied.

Diagnosis of cysticercosis in cattle

Carcass inspection

Meat inspection of cattle is the only public health measure implemented to prevent *T. saginata* transmission to people and is based on the partial incision and inspection of target organs/muscle groups: heart, masseters, and if necassary tongue, *triceps brachii*, and diaphragm; and careful visual search of *T. saginata* cysts. Studies on naturally infected calves have demonstrated that only 14.1% of the total carcass cysts were found in inspection sites indicated above and that only 5.8% of the total carcass cysts appear on cut surfaces created by meat inspection technique (Wanzala *et al.,* 2003). A higher proportion (34%) of cysts was found in the target organs/muscle groups in experimentally infected calves (Kyvsgaard *et al.,* 1990). Another study found a sensitivity of 38% in carcass dissection positive naturally infected calves. They demonstrated that 27% of animals with one to 10 cysts, 42.9% of animals with 11 to 20 cysts, and 77.8% with more than 20 cysts were revealed by the routine procedure (Walther et Koske, 1980). The lack of precision of the visual identification of specific cysts might overestimate the prevalence through misdiagnosis of other morphological alterations in affected muscles. It is difficult to differentiate between old lesions caused by cysticerci and other lesions. For example, of meat inspection identified *T. saginata* cysticercus lesions only 76.7% of them gave positive PCR result: 91% of 78 viable cysts and 70.7% of 239 dead (degenerating and calcified) cysts (Geysen *et al.,* 2007). In another study PCR confirmed only 52.4% of the cysticercus lesions recovered during routine meat inspection: 80% of 25 viable cysts and 49.6% of 242 dead cysts (Abuseir *et al.,* 2006).

Enzyme-Linked Immunosorbent Assay

Antibody detection ELISA

The antibody detection ELISA (Ab-ELISA) for the diagnosis of *T. saginata* cysticercosis detects specific antibodies in serum of infected cattle from three weeks post-infection onwards (Kamanga-Sollo *et al.,* 1987). The diagnostic antigens are either from crude homogenates of *T. saginata* cysticerci, or from cyst fluids and/or crude homogenates of related parasites *T. hydatigena, T. crassiceps* and *T. solium* (Craig et Rickard, 1980; Geerts *et al.,* 1981; Kamanga-Sollo *et al.,* 1987; Monteiro *et al.,* 2006). Such crude antigens give cross-reactions with serum from cattle infected with heterologous helminths, such as *Fasciolla hepatica*, and *T. hydatigena* (Craig & Rickard, 1980). Since *T. hydatigena* metacestode is not commonly found in cattle, cross reaction may not present a major drawback in practice (Geerts *et al.,* 1981).Such assays are deficient to diagnose lightly infected animals at slaughterhouses (Monteiro *et al.,* 2006). In natural conditions light grade infections are common. For example, an ELISA using hydrosoluble antigens of *T. crassiceps* demonstrated a sensitivity and specificity of 37.5% and 95.7% respectively (Geerts *et al.,* 1981). The ELISA using a recombinant antigen of *T. saginata* oncosphere adhesion protein (Hp6-Tsag) showed 100% sensitivity and a specificity of 93.2% in experimentally infected cattle (Ferrer *et al.,* 2007).

Enzyme-Linked Immunosorbent Assay

Antigen detection ELISA

The level of antibody titer did not correspond with live cysticerci burden in naturally infected animals (Onyango-Abuje *et al.,* 1996). In slaughterhouse cattle antigen assay was found three times as sensitive as meat inspection. Furthermore, the assay detects live cysticerci which are most likely missed, as they have the same pinkish-red color as the meat (Onyango-Abuje *et al.,* 1996; Wanzala *et al.,* 2003). Two MoAb-based ELISA systems, HP10 and (158C11 and 60H8), were developed for the detection of *T. saginata* cysticercosis (Harrison *et al.,* 1989; Brandt *et al.,* 1992; Van Kerckhoven *et al.,* 1998). Both assays recognized the circulating glycoprotein antigens secreted by the viable metacestodes from four weeks after infection onwards (Harrison *et al.,* 1989; Onyango-Abuje *et al.,* 1996). A mouse monoclonal antibody IgM coded HP10, developed against glycoproteins from surface enriched extract of *T. saginata* cysticerci, was used for the development of a diagnostic ELISA to detect these glycoproteins in the serum of *T. saginata* infected cattle. However, only cattle harboring 200 live 8 to 16 weeks-old cysticerci were consistently detected (Harrison *et al.,* 1989). In a recent study conducted in Kenya the assay detected 75% (n=20) of naturally infected cattle harboring one or more live cysts at carcass dissection. All animals with five and more cysts were Ag-ELISA positive (Wanzala *et al.,* 2007). Another study found a sensitivity of 83% (n=6) for animals with ≥30 live cysticerci, which dropped to 22% (n=23) for animals with 1-29 live cysts (Onyango-Abuje *et al.,* 1996).

The other MoAb-based antigen detection ELISA system was developed by Brandt *et al.* (1992) and modified by Van Kerckhoven (Van Kerckhoven *et al.* 1998) and Dorny (Dorny *et al.* 2000). The assay yielded a sensitivity of 92% and a specificity of 98.7% in heat treated sera from cattle harbouring more than 50 viable cysts.

Electroimmunotransfer Blot

There is limited information on the use of the electroimmunotransfer blot (EITB) for the diagnosis *T. saginata* cysticercosis. A hydrophobic fraction, 10 to 18 kDa, isolated from cyst fluid of *T. hydatigena* metacestodes, collected from naturally infected goats, was evaluated in immunoblot and dot blot procedures and detected *T. saginata* infectionin calves (Bogh *et al.,* 1995).

Dipstick-immunoassay

Hayunga (Hayunga *et al.* 1991a) developed a dipstick antibody detection ELISA for the diagnosis of *T. saginata* and *T. solium* cysticercosis using ammonium sulphate-soluble fractions of *T. hydatigena* cyst fluid antigen adsorbed on Immobilon P membrane dipsticks. The assay detected 6 out of 7 (85.7%) cysticercotic cattle three weeks after experimental infection (Hayunga *et al.,* 1991).

Dot-ELISA

Although considerable progress has been made to develop simple dot-immunoassays for *T. saginata* cysticercosis, the techniques are not standardized (Jiang *et al.,* 1990; Draelants *et al.,* 1995b; Biswas *et al.,* 2004; Agudelo *et al.,* 2005). Draelants

(Draelants *et al.* 1995a) developed an antigen detection dot-ELISA using MoAbs (2H8 and 12G5) of IgM isotype and nitrocellulose membrane as described by Brandt (Brandt *et al*. 1992). The assay gave 87.5% and 93.5% sensitivity and specificity, respectively in cattle with more than 100 viable cysts.

Diagnosis of cysticercosis in pigs

Carcass inspection

Meat inspection is the only diagnostic method carried out on large scale in slaughterhouses for the post-mortem detection of pig cysticercosis. The method is more sensitive to detect dead, degenerated, or calcified cysticerci; but is most likely to miss quite a number of viable cysticerci, as they have the same pinkish-red color as the meat (Wanzala *et al.,* 2003). The procedure is based on the partial incision and careful observation in the "predilection" sites such are: heart (obligatory), masseters, tongue, and *triceps brachii*(optionaly) (Boa *et al.,* 2002). The technique has demonstrated low sensitivity. Dorny (Dorny *et al*. 2004) estimated a sensitivity of 22.1% and specificity of 100%. Boa (*Boa et al.* 2002) showed that routine meat inspection involving visual inspection of incised and intact surfaces of heart, tongue, external and internal masseter muscles, and *triceps brachii* muscles can only reveal 10.6% of the total carcass cysts. In other words, the inspection can only detect 10.6% of infected animals.

Tongue palpation

Tongue examination for detection of *T. solium* cysts in live pigs by palpation and visual inspection is a low cost ante-mortem diagnostic method of porcine cysticercosis. The technique has a high specificity (100%), but generally low sensitivity (Dorny *et al.,* 2004; Phiri *et al.,* 2006). The sensitivity of the method depends on the level of infection. Approximately, 76 and 78% of positive tongue palpation pigs were found seropositive in ELISA and EITB, respectively (Sato *et al.,* 2003). The diagnostic antigens were isoelectric-focusing purified glycoproteins according to Ito (Ito *et al.* 1998). In an endemic area, the tongue palpation detected up to 70.8% of meat inspection positive pigs (Gonzalez *et al.,* 1990). In comparison to total carcass dissection as low as 16% sensitivity was also recorded (Phiri *et al.,* 2006). Despite the low sensitivity the method is used in epidemiological studies of porcine cysticercosis (Sarti *et al.,* 1992; Mutua *et al.,* 2007; Sikasunge *et al.,* 2008). In a pig population tongue palpation showed prevalence of 10.8% while this was 23.3% by Ag-ELISA (Sikasunge *et al.,* 2008).

Enzyme-Linked Immunosorbent Assay

Antibody detection ELISA

The assay detects IgG. Antigens used for coating of most ELISAs for the detection of antibodies against *T. solium* cysticercosis in pig serum are from cyst fluid or crude homogenates of the *T. solium* cysticerci, or from the related parasite *T. crassiceps* (Nunes *et al.,* 2000). These crude antigens have shown cross reactions with sera from pigs infected with *T. hydatigena, E. granulosus*, *Ascaris suum, Fasiolopsis buski, Hymenolepis diminuta, and Diplydium caninum* (Kumar et Gaur, 1987; Cheng et Ko, 1991;

Ko et Ng, 1998; Pinto *et al.,* 2000). Fractionation and/or purification of the antigens have improved the specificity of the assay (Ito *et al.,* 1998; Assana *et al.,* 2007). A fraction of 14 kDa antigen purified using an ion exchange column on high performance liquid chromatography from crude cyst fluid of *T. solium* was found specific (Assana *et al.,* 2007). The isoelectric-focusing purified glycoprotein antigens from cyst fluid (Ito *et al.,* 1998) have shown a specificity and sensitivity of 100% in detecting antibodies against *T. solium* metacestodes in pig serum (Ito *et al.,* 1999).

Antigen detection ELISA

The presence of antibodies does not constitute direct evidence of a living parasite within the host (Garcia *et al.,* 1997; Fleury *et al.,* 2007). It may indicate transient antibodies from exposure to infection (Garcia *et al.,* 2001) and/or persisting antibodies of previously established infection after elimination due to immune mechanism and/or drug therapy (Harrison *et al.,* 1989; Garcia *et al.,* 1997). A mouse monoclonal antibody IgM coded HP10, developed against glycoproteins from surface enriched extract of *T. saginata* cysticerci (Harrison *et al.,* 1989), is used for the detection of these glycoproteins in the serum of *T. solium* infected people. The assay displayed 84.8% (n=46) sensitivity and 94% specificity in serum from patients with active infection (Fleury *et al.,* 2007). In another study a similar sensitivity (85%) and specificity (92%) was found (Garcia *et al.,* 2000). The assay is used for serodiagnosis and follow-up of NCC (Garcia *et al.,* 2000; Garcia, 2007).

Another MoAb-based antigen detection ELISA system was developed by Brandt *et al.* (1992) and modified by Van Kerckhoven *et al.* (1998) and Dorny *et al.* (2000). The monoclonal antibody-based ELISA is being used for clinical management and epidemiological surveys of human cysticercosis (Erhart *et al.,* 2002; Prado-Jean *et al.,* 2007). The sharing of antigens between the metacestode and adult tapeworm might influence seropositivity in endemic areas (Draelants *et al.,* 1995a; Correa *et al.,* 1999). In the hamster model of taeniosis, adult antigens have been demonstrated to cross the intestinal epithelium and enter the circulation (Correa *et al.,* 2002).

The detection of viable cysts is achieved through capturing circulating antigens by MoAbs. The two MoAb-based ELISA systems (Harrison *et al.,* 1989; Brandt *et al.,* 1992; Van Kerckhoven *et al.,* 1998) are used for detection of circulating antigens of viable metacestodes in pig serum. The assays could detect antigens in serum of pigs harbouring live cysticerci from four weeks after infection onwards, in contrast no antigen is detected in those containing only dead cysticerci (Nguekam *et al.,* 2003).

Electroimmunotransfer Blot

The EITBs are the most sensitive and specific assays for the detection of antibodies specific to *T. solium* cysticercosis in pigs. The EITB (Tsang *et al.,* 1989) was evaluated on serum samples from naturally infected pigs with *T. solium* and other heterologous infections including echinococcosis. The assay was determined to be 100% sensitive and specific. It detected antibodies in experimentally infected pigs between 5 and 8 weeks post infection (Tsang *et al.,* 1991). This assay was very sensitive (98%) and specific (100%). No sera from *Echinococcus granulosus*/*E. multilocularis* and other heterologous infections recognized the GP bands (Tsang *et al.,* 1989). The assay has been used for epidemiological surveys of human cysticercosis (Garcia *et al.,* 1991).

Dot ELISA

The performance of antibody detection dot-ELISAs depends on antigens and/or reference tests used. The complete homogenate of *T. solium* cyst antigen dotted on nitrocellulose membrane detected antibodies in 56.5% sera from patients with CT/MRI confirmed NCC. The assay was 92% specific (Biswas *et al.,* 2004). In another studies the assay detected 58.3% of LLGP EITB positive individuals during immunological screening of endemic population (Agudelo *et al.,* 2005).

CONCLUSIONS

Cysticercosisis a significant public health problem worldwide. Control of this zoonosis requires a good diagnostic test to identify animals harboring live metacestodes. Visual meat inspection, which is the only public health measure implemented to control human infections, is a poorly sensitive method to detect such animals. It may underestimate the prevalence of the disease by a factor 3 to 10.

The need for diagnostic tests superior to meat inspection have led to the development of serological tests. Antibody detection diagnostic tests indicate exposure to infection and not necessarily the presence of an established, viable infection. But from public health point of view only living metacestodes are important. Contrary to meat inspection and antibody detection methods, the monoclonal antibody based enzyme-linked immunosorbent assays (ELISAs) for the detection of excretory/secretory (ES) products indicate infection with live metacestodes. The assay is an important tool to detect individual cases or to screen populations for the presence of viable cysticerci. The assay requires relatively expensive laboratory equipment. The dipstick methods are more applicable in the resource deprived field laboratories of developing countries.

There is limited information on the prevalence of *T. saginata* and *T. solium* cysticercosis in Serbia. Recent studies of neurocysticercosis (Doder et all. 2004) have shown that the prevalence is very low, but cysticercosis is present in pigs and humans.

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SAVREMENE DIJAGNOSTIČKE PROCEDURE U DIJAGNOCTICI CISTICERKOZE KOD GOVEDA I SVINJA

VESNA LALOŠEVIĆ

Izvod

Tenijaza i cisticerkoza predstavljaju značajan javno-zdravstveni problem širom sveta. Kontrola ove zoonotske bolesti zahteva korišćenje zadovoljavajućih laboratorijskih testova koji identifikuju zaražene životinje kao rezervoare infekcije ljudi. Inspekcija mesa je jedina važeća mera prevencije i kontrole humanih infekcija, ali je slabo osetljiv metod za detekciju živih metacestoda kod životinja. Postoji potreba za razvojem savremenih i osetljivijih dijagnostičkih testova. Testovi zasnovani na detekciji antitela ne mogu da utvrde da li su cicticerkusi u muskulaturi vijabilni, što je sa aspekta javnog zdravlja najvažnije. Imunološki testovi zasnovani na detekciji sekretorno/ekskretornih antigena (Ag-ELISA) mogu da dokažu žive metacestode. U radu jed dat prikaz svih metoda u dijagnostici cisticerkoze kod goveda i svinja, iako za sada, nema standardizovanih, komercijalnih testova.

Ključne reči: cistcerkoza, inspekcija mesa, imunoenzimski testovi, ES Ag-ELISA.

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KEEPING AND NUTRITION OF DAIRY COWS IN ORGANIC MILK PRODUCTION*

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SUMMARY:Organic dairy farms are closed agroekosystem within which is implement a balanced cultivation of plants and animals according to strict principles and regulations of organic agriculture. Organic production of plants for animal nutrition, organic nutrition, natural fertilization of soil are processes that define organic production of milk to the greatest extent and contribute to the natural fertility of land, clean air and water, and biological differences, avoiding possibilities of soil contamination. Organic milk production in developed countries takes up an increasing share of total agricultural production. In Europe, organic agriculture is led by members of the European Union. Market for organic food and beverages in the countries of Central and Eastern Europe is still underdeveloped, but the demand for organic products is increasing. Despite undoubtedly a natural prerequisite for the development of organic agriculture, and especially clearly expressed agroecological diversity of national agricultural area, Serbia enters the group of countries where this form of agricultural production is still underdeveloped. Although the organic production has been written about almost every day, the fact is that at this time a few number of Serbian products may bear the sign »Serbia Organica.« However, optimism is awakening by the fact that the number of registered Serbian organic producers increases. It is estimated that Serbia currently has about 15,000 hectares under organic crops, and potentially, organic food could be produced on 600,000 acres.

Key words: *organic production, organic food, animal nutrition.*

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INTRODUCTION

Stables for cattle - a factor for success in organic livestock production

The basic strategy when building the stables for dairy cows must below cost to build. Prior to construction it should verify preliminary and main location projects and construction permits, municipal approvals and contributions. When creating a project for the construction or reconstruction of facilities for keeping cows in organic milk production should be ensured that stables must be constructed and equipped so that the animals are kept in the most natural way. It should create conditions in which animals are not exposed to stress. It should be kept in mind that the stable structures, in which animals live and produce and people works in them for several hours a day. They must therefore meet the needs of man and animals to the greatest extent, must be built according to ethological, ecological, zoohygienic and ethical principles with the aim of producing high-quality organic milk.

The internal arrangement of the stables must ensure the logical flow of daily operations, without disturbing the normal behavior of animals.

Ensuring quality by selecting animals, maintenance of fertility and the application of proper nutrition is most important, but not enough for an economical and efficient production of organic milk. Also important is the choice of location for the construction of appropriate buildings: barns, storehouses for food, water tanks, manure pits, etc.

The choice of sites is primarily affected by: fitness of location, road connectivity, electricity connections, the possibilities of water supply, macro-ventilation, sunlight, etc. Furthermore, it is also important technological and technical equipment and the mutual arrangement of objects (barns, warehouses and ancillary premises which have a large impact on the functionality of the facility and performance)

Transport pathways for roughage, litter, manure equipment is usually not permitted to cross-over. Areas where the animals are moving, should not end up in a “blind street”.

The barn should have suitable climatic and microclimatic conditions (Krajinović, 2006) Animals in organic production must have enough space for feeding, drinking, lying down (resting) and movement (Tosić, 2001). Access to fresh water and food must be free. Animals must not be kept tied and individually. Minimal inner surface of the breeding dairy cows is 6 m2/cow (surface area available for animals) and external surface (to move, not including pasture) to 4.5 m2/cow (Popovic Vranjes, 2010). Free housing system is more convenient because it is a natural way of keeping the cows of which is ensured freedom of movement, where the cows have access to a number of places for lying. All other functions of the free ways of keeping are separated, Figure 1

This means that feedinig, drinking, lying, and milking are separated. Freedom of movement and separation of functions that are forcing the cows often waking up and moving is very important, because it positively affects the general state of health, physical condition of the cows, the length of poduction life and production results. Free system of holding is almost without exception used for any larger herds ranging from 10-20 cows, and so forth. Automation of work processes in the free system of keeping cows led to the proportion of human labor was reduced to 40 hours per cow per year. The advantage is also that the job is easier, because it is fully automated the most

complicated operations like milking. There are very significant advantages in terms of microclimate because this system are supplied with optimal zootechnical and zoohygienic conditions. In fact, there are secured sufficient quantities of fresh air, vent, and the optimum temperature.

It can be freely recommend to all farmers who wish to engage in organic milk production adaptation of old buildings in the stalls with free grazing.

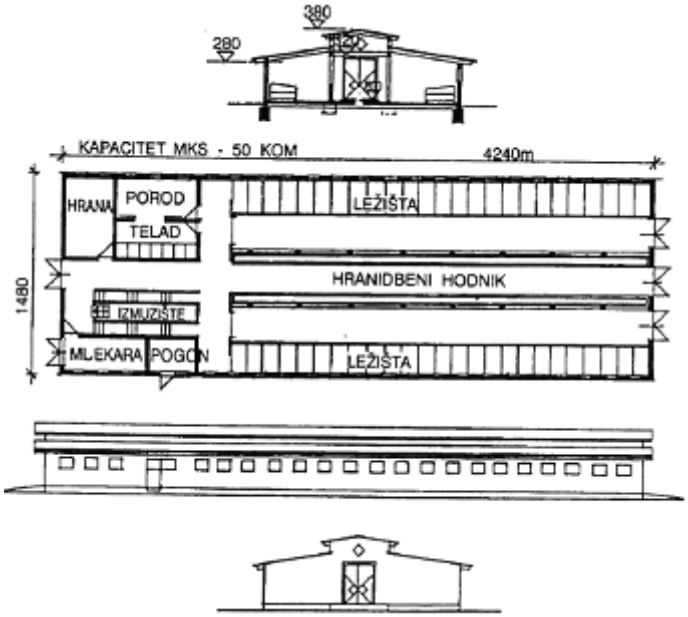

Figure 1. Stable for dairy cows, free grazing system with capacity of 50 head

Slika 1. Štala za mlečne krave, slobodan sistem držanja sa kapacitetom od 50 grla

The main characteristics of these farms are specializations for the production of milk, with modern equipment, wherever this is possible and profitable, then the free way of keeping; functional parlor and a system of holding on deep litter cages or league.

The traditional way of keeping the cattle must be fundamentally changed. Small farms with inadequate facilities (very bad microclimate), inadequate nutrition and poor management have to amend and adapt in every way to succeeded in organic livestock production. The best farms for milky cows are stables with external environment. Organic livestock production can be based on production units whose members are composed of pastures or other land on which the products are fodder, or provides fodder for which the certificate is issued (the Law on Organic Production, Official Gazette of RS, No. 30/10)

Inclusion of livestock production in organic farming can begin at least one year from the date of inclusion of land plots in organic agriculture to ensure that when organically produced feed for livestock from these plots.

Organic livestock production is a new type of food for humans but also a new challenge for the entire zootechnics. Its precondition is organically produced food for the animals which practically means a return to ekstenzivnost to some extent.

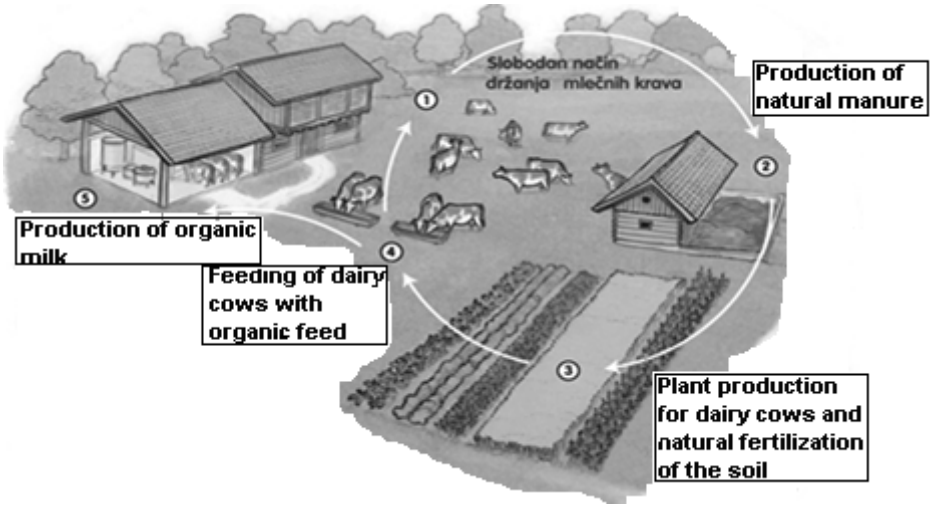

Figure 2. Interconnectivity of organic livestock and crop production in the organic milk production (http://www.dukat.hr)
Slika 2. Međusobna povezanost organske stočarstve i ratarske proizvodnje u organskoj proizvodnji mleka

DAIRY COW'S FEEDING IN ORGANIC PRODUCTION OF MILK

Animal feeding main principle applied in organic breeding is that animal feeding has to be adjusted to their physiological requests with maximum using of available food in rounded production process. The food has to be prepared in the form that allows animals to show their natural feeding habit and satisfy their needs. Animals, especially cuds, are the part of ecological agriculture. Thans to their ability to use feed rich with raw fiber, the cuds take active role in the closed process of matter and energy circulation in ecosystem.

Main principles of milk cow feeding are:

- sufficient and complete feeding in order to use genetic milk potential maximally
- even feeding that meets the requests of each phase in cow life during the whole year, which is especially important in the time of pregnancy and lactation.
- balanced meals concerning nutrient content
- healthy food without harmful impurity
- economical meals

Cow feeding has great influence to profitability of one organic farm. (Jovanovic, 2009). Knowing of the main principles in milk cow feeding is necessary for making good results in milk production and in keeping animals in good health as well. In order to make as optimal as possible meals for milk cow feeding it is necessary to know the kind of food and its content.

Pasture is the most simple and the healthiest but also the cheapest way of cow feeding in organic production, but this is not enough to make high milk production so it is necessary to introduce concentrated feed as well.

Pasture may be free or controlled. Controlled pasture is more recommended because of lesser wastage (grass trampling) and at the same time it allows better utilization and maintenance of the pasture.

Green feed made on farm in accordance with organic production principles is the sort of bulky food used in organic production. Among those feed, utilization of green grass pasture mass, green mass from plow, dry feed like hay, straw, corn straw, barley straw, oats straw, wheat straw, chaff, peelings and so on, is the most practical.

Silage of whole corn is one of the most important energy sources in DAIRY cow feeding because this plant gives a lot of green mass, has relatively high content of energy in dry matter and represent infallible component for preparing of completely mixed meal. (Forouzmand et al., 2005)

Winter feeding of cows is usually based on quality hay that can be given during the whole year as well. Hay received from different kind of clover (lucern, "leptirnjaca", red clover…) is the best because of high content of nutrient (proteins) (Dewhurst, 2001). Meadow hay, like hay of several years grass, is good as well. Feeding of milk cow with quality hay only is not enough to provide optimal milk production so it is necessary to introduce concentrated and juicy feed as well. Concentrated feed also have to be produced in accordance with organic production principles or bought on the organic farm that deals with husbandry and processing of grain produced in organic way.

The feeding of cuds is based on maximally using of pasture in accordance with pasture availability during the year. (Popovic-Vranjes, 2009). At least 60% of dry matter in daily meal is consisting of fodder, fresh or dry bulky feed or silage. Organic production unit can provide the most 10% of conventional feed for cuds that is 20% of feed for other animal when another organic food can not be provided. Given percents are calculated on the yearly base as the percent of meal dry content.

Participation of conventional feed in daily meal must not be more than 25% of meal dry content. In the case of extreme climate condition, weather disaster or men's harmful influence when it is not possible to produce green, bulky feed, federal inspector may allow higher participation of conventional feed during limited time period, in specific region. For meal preparation and conservation it can be used preservatives like bacteria, fungus and enzyme. For silAGE preparation, like preservatives it can be used formic, acetic, LACTIC or propionic acid.

Animal origin feed can be used in organic production if they are produced and prepared in accordance with regulations concerning organic production and preparation (milk, milk powder, skim milk, skim milk powder, whole milk, whole milk powder, whey, whey powder, whey powder with little sugar content, whey powder protein and so on). Following mineral elements can be used for animal feeding in organic production: sodium, calcium, phosphorus, magnesium, sulfur as well as following elements in small quantities: iron, iodine, cobalt, copper, manganese, zinc, molybdenum, selenium in the form that is in accordance with the Regulations. Vitamins, provitamins and chemical substances that have similar effect used for animal feeding must be FROM natural origin. Antibiotics, coccidiostatics, medical preparation, growth stimulators or any matter tha stimulate growth or production can not be used for animal feeding.

Animal feed must not contain growth stimulator, remedy for appetite, artificial colors, urea, amino acid, clinical waste and droppings.

CONCLUSION

Organic agriculture development should contribute to optimal use of natural resources, local production increase and comprehensive prosperity of rural region habitants. In organic production animal WELFARE are of high priority. First of all it should be provided conditions for animal growth and develop in accordance with natural genetic potential. That means respect of animal physiological and ecological needs and making the conditions them to satisfy their natural function and behavior. The number of animal at organic farm is in relation with farm area in order to avoid industry farm and too much excretion of nitrates to the ground and underground water.

The animals should be from organic system breeding. Building for animal keeping should be in accordance with animal sort (enough space, light and possibility to be out). The animals should be feeded with organic food made on own farm or made on the farm at the same region.

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Zakon o organskoj proizvodnji, Sl. glasnik RS, broj 30/10.

DRŽANJE I ISHRANA MUZNIH KRAVA U ORGANSKOJ PROIZVODNJI MLEKA

KRAJINOVIĆ MILAN, POPOVIĆ VRANJEŠ ANKA, CVETANOVIĆ DAVID, JEŽ GORAN, JEFTIĆ MARIJA, NOVAKOVIĆ IVANA

Izvod

Organske mlečne farme predstavljaju zatvoren agroekosistem unutar kojeg se provodi uravnotežen uzgoj biljaka i životinja prema strogim načelima i propisima organske poljoprivredne proizvodnje. Organska proizvodnja biljaka za ishranu životinja, celokupna , organska ishrana te prirodno đubrenje zemljišta su procesi koji u najvećoj meri definišu organsku proizvodnju mleka i doprinose prirodnoj plodnosti zemljište, čistoći vazduha i vode te biološkoj razlici, izbjegavajući mogućnost zagađenja zemljišta.

Organska proizvodnja mleka u razvijenim zemljama zauzima sve veći udeo u ukupnoj poljoprivrednoj proizvodnji. U Evropi organsku poljoprivredu predvode zemlje članice Evropske unije.Tržište organske hrane i pića u državama srednje i istočne Evrope i dalje je nerazvijeno, ali potražnja za organskim proizvodima stalno raste. Uprkos nesumnjivim prirodnim preduslovima za razvoj organske poljoprivrede, a posebno jasno izraženoj agroekološkim razlikama nacionalnog poljoprivrednog prostora, Srbija ulazi u grupu zemalja u kojima je ovaj oblik poljoprivredne proizvodnje i dalje nedovoljno razvijen. Iako se o organskoj proizvodnji gotovo svakodnevno piše i govori, činjenica je da u ovom trenutku malen broj srpskih proizvoda može nositi znak „Serbia organica“. Ipak, optimizam budi činjenica da broj registrovanih srpskih organskih proizvođača neprestano raste. Procenjuje se da u Srbiji trenutno ima oko 15.000 hektara pod organskim usevima, a potencijalno bi organska hrana mogla da se proizvodi i na 600000 hektara.

Ključne reči: organska proizvodnja, organska hrana, ishrana životinja.

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APPLICATION OF PROPER MECHANICAL MILKING IN ORGANIC MILK PRODUCTION*

POPOVIĆ VRANJEŠ ANKA, KRAJINOVIĆ MILAN, CVETANOVIĆ DAVID, JURAKIĆ ŽELJKA, JEŽ GORAN[1]

SUMMARY: Milking is the most important process in organic milk production, which greatly affects both the quality of milk and the number of somatic cells and the occurrence of mastitis. One of the consequences of the introduction of mechanical milking at the farm was the occurrence of mastitis in the herd. Inflammation of the udder or mastitis is still one of the biggest problems on the farms for milk production, which is always accompanied by high costs. It is therefore very important that the correct procedures before, during and after milking are used in order to reduce mastitis to a minimum. Mastitis causes reduced ability of milk synthesis by the mammary gland and prevents the complete synthesis of certain ingredients. For treatment of mastitis in organic milk production in addition to treatment for sanitation and disinfection allowed in organic production, homeopathic remedies made based on plant and mineral salts are largely used.

Keywords: *milking, organic milk, mastitis*

INTRODUCTION

Organic agriculture is a management system that requires the integration of cultural, biological and mechanical practices that promote better utilization of existing resources, improve and maintain the biological balance of biodiversity and biological diversity (AMS National Organic Program 2008).

With regard to organic livestock production in Serbia, in all branches of animal husbandry there is a significant potential for the development of organic livestock pro-

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Rewiev paper / *Pregledni rad*

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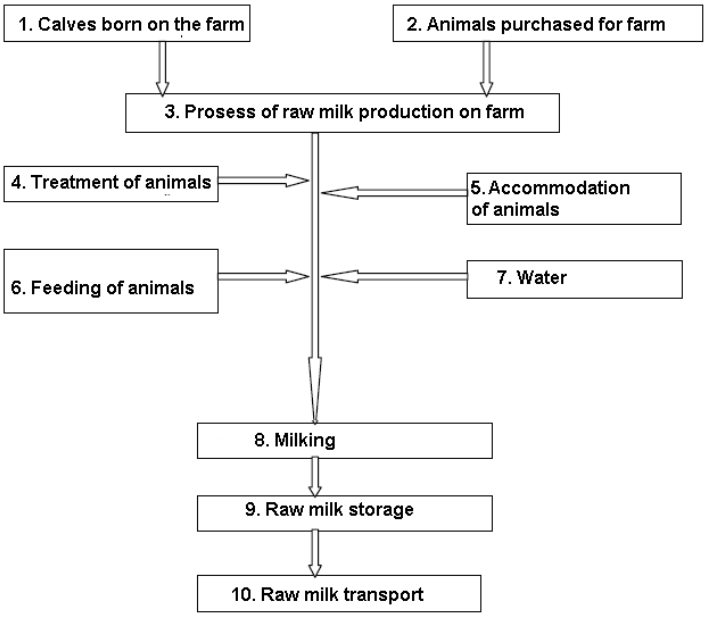
duction, especially in mountainous regions. In cattle and sheep the predominant racial composition is in favor of traditional livestock and traditional production systems on pastures in mountainous regions that preserves the traditional production of indigenous species of dairy products (cheese and cream). Milk production based on organic principles is showing the increase around the world, especially in EU countries (Popović-Vranješ, 2011). In order to preserve the nutritional value of milk and suitability for processing, as well as to achieve the best price, it is needed to supply the milk with preserved quality, in the condition it was during the healthy milking of well fed and properly nurture cows. Producers effort to maintain good udder health after milking, to delivery and maintain the quality of milk, helps to reduce risk and has better marketing and profitability of production in organic livestock production (Jovanovic, 2008). Mechanical milking in organic production systems provides: significant reduce of the physical effort of workers – the milker, increases productivity and reduces the human labor required for these jobs on the farm, and secures bacteriological and chemical quality of milk (Popovic-Vranjes, 2009).

Program for the safety of milk at the farm level

Program for the safety of the milk at farm level must be designed so that all process steps are defined, hazards identified and sources of relevant criteria for risk analysis, monitoring and control measures must be defined and corrective actions and records proposed. After defining the requirements for prerequisite program a flow chart for raw milk and process-related activities in accordance with the principles of HACCP must be designed (Scheme 1).



Scheme 1: Flow diagram of raw organic milk-primary production

Šema 1: Dijagram toka sirovog organskog mleka- primarna proizvdnja

Milking procedure, whether it's a manual or mechanical, is managed after the preparation process for milking, which consists of washing the udder, drying the udder, and udder massage for the first jets of milk.
These procedures provide:

- Stimulation of receptors in the udder, which is necessary for secretion of the hormone oxytocin and formation of the reflex for milk release;
- obtaining a good hygienic quality of milk;
- fast and efficient milking from the udder;
- shorter duration of milking;
- obtaining the maximum amount of milk and
- Healthy udder that is capable of further production of milk.

Washing of udder

Before milking thoroughly cleaning of the udder should be done to eradicate all visible dirt, which contains a large amount of microorganisms, harmful and dangerous for the milk. Washing is carried out with hot water, with temperatures of 35 ° C to 45 ° C from the milking pail in the stall or with a shower (strong jet of hot water) when the milking is performed in the parlor.

When washing out from bins for each cow water should be changed. Very dirty udders can be washed with neutral pH means allowed in organic production, and then rinse with clean water.

Drying the udder

The water left behind after washing the udder is often contaminated with bacteria. When placing the liner sets there is a possibility that such water is inlet in the udder cups and thereby contaminate the milk. To prevent this, it is important to wipe udders after washing or to dry them. To wipe the udder dry paper towels are used, in the absence can clean cloths or towels can be used, in a way that one cloth that is used to wipe only the udder of one cow. This prevents transmission of pathogens between cows.

Milking of first jets of milk – trial milking

Milking of first jets of milk (two or three jets from each teats) is performed in a container for sampling of milk with dark bottom, which allows the milker to easily spot any changes to it (curd flakes, blood, pus). The goal is to, based on visual inspection of the milk, to make control of udder health status, or secretion. In this way very early mastitis can be detected and potentially contaminated milk can be removed.

Milking

The space for milking must be located and constructed in a manner that ensures satisfactory hygienic conditions during milking and it must be kept clean. During milking a sufficient quantity of clean water must be available to clean dirty teats and udders, equipment, arms, floors, walls (Havranek et al. 2003). When choosing a device for milking, one should pay special attention to: that the device does not damage the cow's udder in physiological and biological terms, that the flow control of milking is secured (initial phase of milking, active milking, the end of milking with the possibility of full milking so-called "blind milking"), that it is simple to operate and maintain (http://

poljoprivreda.info/?oid=10&id=213).

Right moment to start milking is when the udder is swelling, getting a slight pink color, and teats becomes full and firm to the touch. Milking should be carried out always at the same time and in the same way over time to maximize the cows developed a conditional reflex milking. It is important to use the time after preparation of the udder (3-5 minutes), when it is most effective time for giving milk.

Various types of parlors are used around the world; according to the layout of boxes for cows and capacity. The main types of parlors, which are most used in practice: a tandem, parallel parlor, herringbone and rotolactor. Tandem type milking parlors, herringbone and parallel are stationary and rotolactor milking parlor is rotary. By the degree of automation, parlors are divided into standard, semi-automatic and automatic. With standard milking all operations are carried out by hand except the act of milking which is done with machine. In semi-automatic milking parlor next to the device, there is an automatic mechanism for exclusion and removal of the liner sets, control of the end of milking, full milking, exclusion and removal of the liner sets automatically. Out of the newer systems the most efficient is robot milking for the full automation of the milking process, figure 1,2.

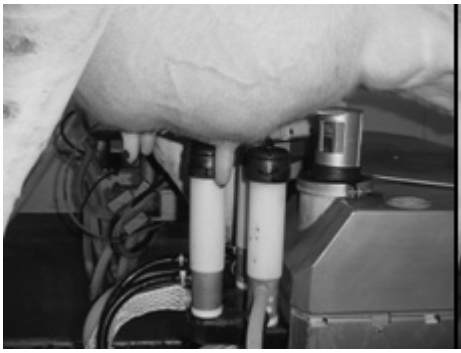


Figure 1. Detection of teats
Slika 1. Detekcija sisa

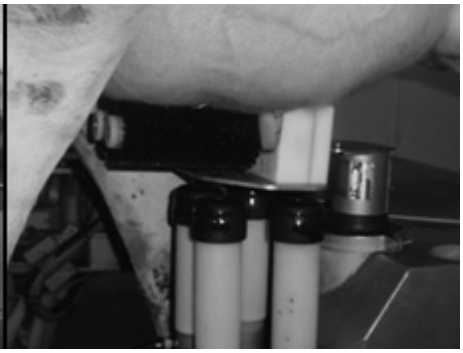


Figure 2. Cleaning of teats
Slika 2. Čišćenje sisa

Benefits of robot milking are reduced physical work on the farm to the extent that the worker does not even attend the milking; better animal health (which is controlled by the robot); achieved a greater degree of hygiene of cows and teats; amount of milk is higher than the previously described milking systems (Ostojić, 2007). Automatic milking systems are routinely equipped with automatic feeders. Complete milking robot system consists of: a box for milking, a system for detection of teats, teats cleaning system, the robotic arms that attach the teat cups, the control system including sensors and devices for milking.

Liners should provide: tightness of the joint at each end of the teat cups, tightness of the joint of the teat liners, no air leaks, to reach the basic cleaning and hygiene requirements, excluding the entire milk from the teats, adjusting the shape and size of teats in the determination of categories of dairy cows and a longer life (6-12 months) (http://poljoprivreda.info/?oid=10&id=213).

Under certain conditions milking equipment may cause significant vacuum fluctuations in the teat glass shell. This results in milk turbulence, which normally flows from the teat, but in this case returns to the teat (i.e. reverse flow). In this way it is pos-

sible to transmit pathogens to the milk in the teat and to penetrate to teat canal ant to cause infection. Infections caused this way can be avoided if you use milking equipment that can not slip during milking (Cergolj and Tomašković, 2003). For stable milking system turning off vacuum and liner removal sets is done by automatic remover. They are connected to the flow meter, and when the milk flow drops to 0.2 l/min, automatic removers partially or completely exclude the vacuum, the liner sets off and milking is interrupted. They are of great importance, because their use prevents the blind milking, which otherwise can lead to internal and external damage which causes the appearance of mastitis. Mastitis is inflammatory disease of the mammary gland and milk channels and is of great economic importance in dairy cows, since it can lead a change in quality and reduced volume of milk production. As agents of mastitis occur following microorganisms: *Staphilococcus aureus, Streptococcus agalactia, Str. dysgalactia, Str. uberis; koagulaza neg. stafilokoki, E. coli, Str. pyogenes, Micobacterium tuberculosis, Salmonella, Listeria monocytogenes, Bacillus cereus, Clostridium perfigens and Corinebacterium bovis.*

Efficient milking is very important for maintaining the health of the teats, damaged teats tops are the door to bacteria that enter from the environment in the mammary gland.

Subsequent milking

Milk, which is left over in the teats after milking, does not stand out on its own from channels into the dairy milk cistern and teat canal. For this reason, in the end of milking each of four teats should be massaged in order to milk final (residual) milk. Subsequent milking begins when there is a sudden drop in milk flow (0.2 l/min). Efficient subsequent milking increases milk yield and helps to prevent mastitis.

Disinfection of the teats and equipment after milking

At the end of milking, the teats must be disinfected immediately, to prevent the entry, growth and colonization of pathogenic microorganisms in the teat canal, and especially *S.aureus, Str.agalactiae* and *Str.dysagalactiae*, which causes mastitis. Disinfection encourages healing of minor wounds on teats, which are suitable for the propagation of microorganisms. Teats can be disinfected by dipping in a disinfectant solution or spray disinfectants permitted for use in organic production. These are products which form a physical barrier at the bottom of the channel of mammary gland and thus reduce the risk of new infections that enter through the teat (Popović Vranješ, 2009), figure 3.

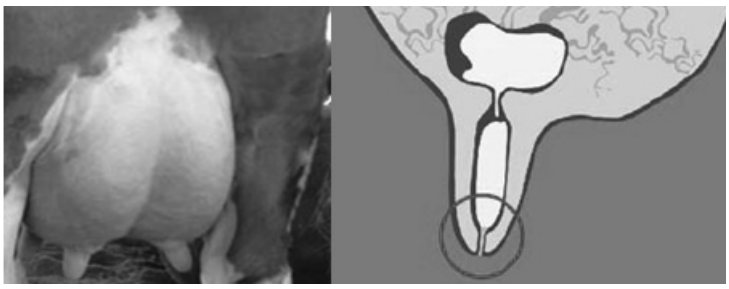


Figure 3. Use of the preparation for the closing of channels in the prevention of mastitis
Slika 3. Primena preparata za zatvaranje kanala mlečne žlezde u prevenciji mastitisa

From herbal preparations, the most efficient are those on the basis of aloe Vera, seaweed, whey, essential oils and healing herbal oils for teats massage, tinctures based on Echinacea, garlic, fennel and chamomile. Some of these products are in the form of pills and should be placed under the tongue or in the vulva of cows with mastitis. Aloe Vera and garlic tinctures are used in an increased number of somatic cells, and a tinctures of Echinacea plants have success with summer mastitis and the incidence of teats gangrene. The success of these products depends on the immune system of cows and proper nutrition and hygiene for farmers, milking equipment and facilities for accommodation and milking (Dettloff, 2009).

Milking parlor and equipment must be properly washed, cleaned and disinfected to prevent infection and propagation of carriers of infection. Proper milking technique and selection of adequate milking equipment reduce risks and protect the production process of raw milk. Funds are granted for cleaning and disinfection of buildings, installations, equipment and accessories in organic livestock production (potassium and natrium soap, water and water vapor, limestone, lime, sodium hypochlorite (liquid bleach), caustic soda, baking stone, hydrogen peroxide, natural plant extracts, citric, acetic, formic, lactic, oxalic and acetic acids, alcohol, nitric acid (for equipment in the dairy industry), phosphoric acid (for equipment in the dairy industry), formaldehyde, cleaning and disinfection of the nipple and the milking equipment and sodium carbonate.

The use of antibiotics in organic milk production in the prophylaxis is prohibited, although these can be used in exceptional cases. Antibiotics are only allowed in cases of emergency. Milk from such animals should not be used, but they can keep their organic status. Many European countries require all antibiotics treatments to be recorded in a national database, and recording the use of alternative botanical and homeopathic treatments is no required (Popović Vranješ, 2010).

Organic milk is a potentially profitable alternative to conventional milk producers. Depending on the price that is available and possibility to control costs it is realistic to expect a decent profit. Many states subsidize this production in many ways. Premiums are generally higher for organic than conventional milk. It is conceivable that organic milk production is more expensive, requires more work, more control and is still struggling in the market. Consumers have to understand that for the higher quality need to pay a higher price, which is up 30% higher compared to the conventional. For consumers such a milk is pure and natural product (Ostojić and Cvijanović, 2004).

CONCLUSION

It is known that with the introduction of mechanical milking on the farm mastitis has appeared as a problem. The most important factor affecting the number of somatic cells in milk is the beginning and development of inflammation of the teats. NSC increases over the number of 200.000 in ml of milk is generally considered abnormal and indicates teats infection. Since mastitis leads to disturbances in the secretion of milk, consequently appears a large number of microorganisms that secretes with milk which is further reflected in the hygienic quality of milk. It has consequences in creating large costs as in the primary production of organic milk as in milk processing. Correct application of milking machine has multiple significance in ensuring the health of the teats and reduce production costs in organic livestock production.

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PRIMENA PRAVILNIH POSTUPAKA MAŠINSKE MUŽE U ORGANSKOJ PROIZVODNJI MLEKA

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CVETANOVIĆ DAVID, JURAKIĆ ŽELJKA, JEŽ GORAN

Izvod

Muža predstavlja najvažniji postupak u organskoj proizvodnji mleka, koji u velikoj meri utiče kako na kvalitet mleka tako i na broj somatskih ćelija, odnosno pojavu mastitisa. Jedna od posledica uvođenja mašinske muže na farme bila je pojava mastitisa u stadu. Upala vimena tj. mastitis danas predstavlja jedan od najvećih problema na farmama za proizvodnju mleka, koji je uvek praćen velikim troškovima. Zbog toga je veoma bitno da se ispravnim postupcima pre, tokom i nakon muže mogućnost pojave mastitisa svede na najmanju meru. Mastitis uzrokuje smanjenje mogućnosti sinteze mleka od strane mlečne žlezde i onemogućava potpunu sintezu pojedinih sastojaka. Za lečenje mastitisa u organskoj proizvodnji mleka pored sredstava za dezinfekciju i sanitaciju dozvoljenih u organskoj proizvodnji u velikoj meri se koriste i homeopatska sredstva napravljenu na bazi biljaka i mineralnih soli.

Ključne reči: muža, organsko mleko, mastitis.

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SEROPREVALENCE OF *NEOSPORA CANINUM* IN DOGS

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SUMMARY: Neospora caninum is an apicomplexan parasitic protozoa that seriously impacts economic performance of diary and beef industries by causing abortion in cattle. Likewise, it is considered as a cause of severe neuromuscular disease in dogs around the world. Since canine neosporosis has not yet been properly investigated in Serbia,the aim of our work was to determine seroprevalence of N. caninum antibodies in a group of dogs from one region of Vojvodina (Serbia) and to evaluate the importance of the age as a possible risk factor for higher seropositivity to N.caninum in dogs. For this purpose, sera from 31 dog from territory of Krčedin and Vršac were examined using indirect fluorescent antibody test. All sera were screened at 1:50 dilution and positive samples were then titrated in two-fold dilution series to the respective endpoint (1:100). Our findings showed that 12,9% of tested dogs was positive to N. caninum antibodies, but no statistically important association between seroprevalence and the age of the dogs was discovered.

Key words: *Neospora caninum, dogs, seroprevalence, Vojvodina, age, risk factor.*

INTRODUCTION

Neospora caninum is an intracellular protozoan parasite of domestic and wild animals with worldwide distribution (Lyon, 2010). Until 1988 it was misdiagnosed as *Toxoplasma gondii*. Since then, *N. caninum* is considered as a major cause of reproductive failures in cattle, as well as the most common cause of infectious inflammatory myopathies and neurological diseases in dogs (Dubey et al., 2007). Beside woolfs and coyoties, dogs are definitive hosts of *N. caninum.* In dogs, ingestion of infected tissues of intermediate host (e.g. cattle) as well as transplacental transmission are dominant routs of acquiring the disease, although lactogenic infection has also been reported (Dubey et

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Short communication / *Kratko saopštenje*

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al.,2011, Dubey and Schares, 2011, Cavalcante et al., 2011). In infected puppies, disease tends to be more severe than in adult dogs. Clinical signs are usually noticed between 3 and 9 weeks of age and include ascending, often progressive and potentially lethal paralysis of the limbs and their rigid hyperextension (Lion, 2010, Reichel et al., 2007). In adult dogs, polymiositis and multifocal CNS signs are the most common clinical presentations, while ulcerative dermatitis is common in older and immunosuppresed dogs (La Perle et al., 2001, Reichel et al., 2007, Lion, 2010). Other possible manifestations of canine neosporosis are: myocarditis, hepatitis, pneumonia, dysphagia and abortion (Lion, 2010, Moura et al., 2011). Although the exact role of the dogs in the epidemiology of neosporosis has not yet been completely revealed, several epidemiological studies have indicated that the presence of dogs on farms may be an important risk factor for bovine neosporosis (Cedilo et al., 2008). This assumption has further led to recommendations for dog control on the farms as one of the preventive measures that should be implemented in farm management procedures in order to avoid infection in cattle (Reichel and Ellis, 2009, Dubey et al. 2007, Innes et al.,2002). Therefore, although the circulation routes of *N. caninum* between dogs and cattle require further investigation, presence of *N. caninum* antibodies in dogs should impose a reasonable doubt for the presence of bovine neosporosis in local herds. The aim of our study was a quick evaluation of seroprevalence of *N. caninum* in dogs from two regions of Vojvodina and investigation of possible connection between the seropositivity of the dogs and their age, since age is (together with gender, breed, diet, envirement and contact with other animals), according to some authors (Moura et al., 2011, Regidor-Cerillo et al., 2010, Collantes-Fernández et al. 2008, Paradies et al., 2007, Haddadzadeh et al., 2007), considered to be a risk factor for presence of *N. caninum* antibodies in dogs. To our knowledge, there have been no previous data concerning canine neosporosis in Serbia.

MATERIAL AND METHODS

A total of 31 dogs were included in this study, 10 of which were kennel dogs from Krčedin and 21 were the ambulatory patients from Vršac. All dogs were of a different breed, mostly hounds, of both gender, with age of dogs varying from 8 months to 11 years. Acquired anamnestic data and thorough clinical examination confirmed that there were no clinical signs of neosporosis in dogs at the moment of serum sampling.

A total of 31 serum samples were transported to the Laboratory of Parasitology of the Department of Veterinary Medicine, Faculty of Agriculture, Novi Sad, where they were tested for the presence of IgG antibodies against *N. caninum* using a standard method of indirect fluorescent antibody test (IFAT). For this purpose comerciall IFAT reagents (VMRD, USA) were utilised, using a 1:50 dilution as a cut-off value (screening test). All positive sera were further examined in a two-fold dilution series (1:100).

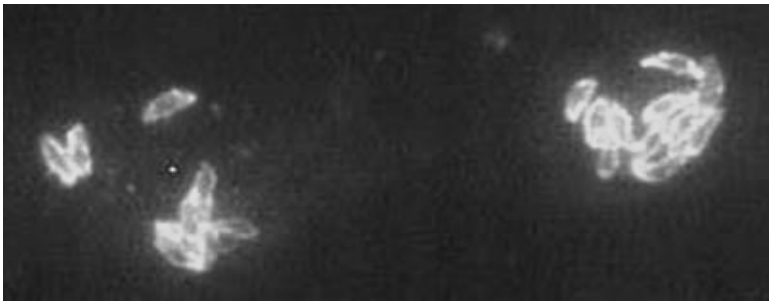
„Nikon“ fluorescence microscope and „Leica“ digital camera were used for visual examination of the asseyd sera and recordance of the positive ones, respectively.

RESULTS AND DISCUSSION

N. caninum antibodies were detected in 12,90% (4/31, 95% CI: 3,6% - 29,8%) of samples, which matches the results of other authors (Reichel et al., 2007, Lyon, 2010

) who reported seroprevalences ranging from 0% to 20% in clinically healthy dogs. Moreover, our findings appear quite similar to those from municipalities of Lages and Balneário Camboriú (Santa Catarina State, Brasil) where Moura et al. (2011) determined seroprevalence of 12,3%. On the other hand, in their study titers of positive sera ranged from 1:50 to 1:800, while ours were on a low, cut off level, of 1:50.

An example of positive IFAT, recorded during our research, is given in a picture below (Picture 1).



Picture 1. Indirect fluorescent antibody test (VMRD, USA). *Neospora caninum,* fluorescence of the tachizoites in reaction with positive dog sera
Slika 1. Indirektni imunofluorescentni test (VMRD, USA). Neospora caninum, fluorescencija tahizoita sa pozitivnim serumom psa

Regarding the age of the dogs, no statistical differences were observed between the occurrence of anti-*N.caninum* antibodies (p = 1.000, 2-sided Fisher's exact test) in dogs from the two studied age groups (Table 1).

Table 1. Seroprevalences according to the age of the dogs (N=31)
Tabela 1. Seroprevalenca u odnosu na starost pasa (N=31)

Dog age groups *Starosne grupe pasa*	No. of dogs in the group *Br. pasa u grupi*	No. of seropositive dogs Br. seropozitivnih pasa	Prevalence *Prevalenca*
Dogs ≤ 2 years old	16	2	12,5 %
Dogs > 2 years old	15	2	13,3 %

This finding is consistent with findings of researchers from Turkey (Çoŝkun et al., 2000), Brasil (Romanelli et al., 2007) and Poland (Goździk et al., 2011), despite differences between sample sizes and formulation of the age groups. For example, we have decided to divide our dogs in two groups, one consisting of dogs ≤ 2 years old and another containing dogs > 2 years old, in order to get uniform distribution within the groups which seemed necessary for statistical processing of such small sample. In Poland, however, large size of the sample (257 dogs) allowed Goździk et al. (2011) to compare seroprevalences between five different age groups (under 1year old, 1 to 5, 5 to 10 and dogs over 10 years old).

On the other hand, results of our study directly oppose to those of Moura et al. (2011), Regidor-Cerillo et al. (2010), Collantes-Fernández et al. (2008), Paradies et al. (2007) and Haddadzadeh et al. (2007) who all detected an increase in seroprevalence in dogs > 1 year old, which they explained by domination of postnatal over congenital infection of dogs with *N. caninum*, suggesting a higher exposure of adult animals to the sources of infection.

The small size of the sample was, once again, the main determinant of our choice of statistical test (Fisher's instead of $\chi 2$ test) used in this study.

CONCLUSION

This study demonstrated that *N. caninum* is present in dog population from the territory of Krčedin and Vršac, with prevalence of at least 12,90% (4/31, 95% CI: 3,6% - 29,8%). Furthermore, we find that age of the dog should not be of importance as a risk factor for the presence of *N. caninum* antibodies.

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SEROPREVALENCA *NEOSPORA CANINUM* KOD PASA

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Izvod

Neospora caninum je parazitska protozoa iz kola *Apicomplexa* koja pričinjava ozbiljne ekonomske gubitke mlečnoj industriji i proizvodnji tovnih grla izazivajući abortuse kod goveda. Takođe, ovaj parazit se smatra uzročnikom ozbiljne neuromuskularne bolesti kod pasa širom sveta. Pošto neosporoza pasa u Srbiji još uvek nije adekvatno ispitana, cilj našeg rada je bilo utvrđivanje prevalence antitela na *N. caninum* unutar grupe pasa iz jednog regiona Vojvodine (Srbija) i ocena uticaja starosti pasa, kao faktora rizika, na visinu seroprevalence. U ovu svrhu, upotrebom testa indirektne imunofluorescencije ispitani su serumi 31-og psa sa teritorije Krčedina i Vršca. Svi serumi su pregledani pri razređenju 1:50 ("screening test"), a pozitivni uzorci su zatim titrirani ponovo, u dvostrukom razređenju (do 1:100). Antitela na *N. caninum* utvrđena su kod 12,9% ispitanih pasa, ali statistički značajna povezanost između starosti pasa i visine seroprevalence nije pronađena.

Ključne reči: *Neospora caninum*, psi, seroprevalenca, Vojvodina, starost, faktor rizika.

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PASTURE CONTAMINATION WITH STRONGYLE EGGS ESTIMATED WITH COMPOSITE FAECAL EGG COUNTS IN GRAZING SHEEP: REPORT FROM A SMALL FLOCK*

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SUMMARY: Production limits in grazing sheep and increase of antihelmintic resistance are caused by strongyle parasites found on pasture. The aim of this study was to estimate spring and autumn pasture contamination using composite faecal egg counts (FEC). Sheep from one small flock from Srbobran (South Bačka province) were sampled in spring and autumn 2011. Estimation of contamination intensity was expressed as average spring and autumn FEC. Our results showed high and moderate level of pasture egg contamination in the spring and autumn, respectively, found in that flock and that composite FEC can be used in this estimation. Hence the presence of high egg numbers alter the number of infective larvae on pasture, obtained results may help development of successful and sustainable parasite control programs in our region, both biological and commercial.

Key words: *pasture contamination, composite faecal egg counts, sheep, control programs*

INTRODUCTION

Domestic ruminants, especially sheep, are very important protein source for people worldwide (Nwosu et al., 2007). In sheep production, grazing is very important practice for economic sustainability (Simin, 2009), but there are certain production limits due to parasitic gastroenteritis (PGE) caused by range of gastrointestinal nematodes

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Short communication / *Kratko saopštenje*

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(GIN, also known as strongyle parasites or strongyles) found on pasture (comprehensive checklist of sheep parasites see in Lalošević et al., 2005; Taylor et al., 2007). PGE results in various kinds of losses with significant cost for sheep industry (Charon, 2004; Pavlović et al., 2003), especially in subclinical infected sheep (West et al., 2009).

Beside ill thrift, the importance of GIN is much higher nowadays because of increase of resistance to antihelmintics routinely used for helminth control (Papadopoulos et al., 2003). Thus, carefully targeted prophylaxis and metaphylaxis can improve the efficiency of parasite control and slow the rate of development of resistance by identifying groups of livestock that are in increasing need for treatment (Morgan et al., 2005; Abbott et al., 2009).

There are several approaches for diagnosing PGE in sheep (Taylor, 2010), but among these, faecal egg counts (FEC) are often favored because they are simple, non-invasive, relatively cheap and provide direct measure of rates of pasture contamination with nematode eggs (Morgan et al., 2005). However, since conducting multiple FEC tests on large number of animals can be uneconomical and time consuming, composite faecal egg count methods have been developed as useful tool for estimating level of parasite infection of a group of sheep (Morgan et al., 2005; Abbott, 2009).

Šibalić and Cvetković (1996) emphasized that there is no universal pattern for parasite control, but basic principle for deworming practice in sheep flock was recommended: pre-turn out dehelmintization in the spring to minimize pasture contamination high due to spring rise phenomena, and in the autumn, after housing, to decrease worm burden in winter months when nutrition is poor (common in some sheep flocks in Serbia) and possibility of infective larvae (L3) to overwinter and become available in the spring next year.

One of alternative methods to minimize number of L3 on pasture is the use of nematophagous fungi (Gómez-Rincón et al., 2006) which trap nematode larvae with three-dimensional sticky nets and chlamidospores (Larsen et al., 1997). Out of several species of fungi, *Duddingtonia flagrans* was one of most promising candidates for control of L3 in several animal species, including sheep, because of it's ability to pass unharmed through animal's digestive tract and trap larvae in faeces (Larsen, 1999). This leads to decrease of larval population on herbage resulting in lower availability for sheep which prevents both clinical and subclinical infection.

The aim of this study was to determine spring and autumn pasture contamination in small sheep flock infected with known GIN genera during one grazing season, following basic principles of control given by authors from Serbia (Šibalić and Cvetković, 1996). Estimation of egg contamination intensity via composite FEC will provide useful data as aid in planning fungus administration program.

MATERIAL AND METHODS

Sampled sheep originated from one small flock of about 120 Merinolandschaf ewes near the town of Srbobran (45.34° N; 19.48°E) in South Bačka region, Vojvodina province. All adult ewes graze for minimum eight months during the year at natural pasture beside Krivaja river, from early spring (end of March, beginning of April) to late autumn (end of November, start of December), depending of weather conditions.

For each coprological examination and determination of FEC, ten fresh faecal

samples were randomly collected from the pasture as described by Abbott et al. (2009). Since average prepatent period for majority of species is about three weeks (Taylor et al., 2007), we have created sampling schedule for two sampling occasions in the spring and two in the autumn approximately 21 days apart, which is presented in Table 1.

Table 1. Sampling schedule for estimation of average composite FEC in sheep
Tabela 1. Raspored uzorkovanja fecesa za utvrđivanje broja jaja prosečnog zbirnog uzorka u ovaca

	Time of sampling / *Vreme uzorkovanja*	
	Spring /*Proleće*	Autumn /*Jesen*
First sampling / *Prvo uzorkovanje*	Middle of April / *Sredina Aprila*	End of October / *Kraj Oktobra*
Second sampling / *Drugo uzorkovanje*	End of May /*Kraj Maja*	End of November /*Kraj Novembra*

The samples have been packed separately in small plastic cups, sealed and shipped to the laboratory until the end of the day, and refrigerated until examination. Following procedure described by Morgan et al., 2005, composite sample was made by mixing of 3 g of faeces from each sheep, and two McMaster slides were examined with modified McMaster technique (Taylor et al., 2007) to obtain average composite FEC, where number of counted eggs was expressed as egg per gram (epg) of faeces. Sensitivity used in counting method was 50 epg – each egg counted under the grid is equivalent to 50 eggs in one gram of faeces.

Average values of two composite FEC in the spring and autumn are calculated, and expressed as spring and autumn FEC used for indication of pasture contamination in that particular time of year.

After two sampling in the spring, all sheep in the flock have been treated twice with antihelmintics one month apart. First treatment was carried out with ivermectin (Alfamec®, Alfasan) while the second time the ewes received a treatment with doramectin (Dectomax®, Pfizer).

RESULTS AND DISCUSSION

Average FEC (obtained at two separate occasions in spring and autumn) are showed in Table 2. Parasite genera *Trichostrongylus spp., Ostertagia spp., Chabertia spp., Bunostomum spp., Trichuris spp., Nematodirus spp.* and *Strongyloides papillosus* species in monoinfection or mixed infection have already been determined earlier in the same flock by Lalošević et al. (2008) and Simin (2009).

According to Taylor (2010), FEC determined in our study in the spring can be classified as high since FEC over 1000 epg of faeces are generally considered as heavy infections, while autumn findings suggest that there is moderate infection since number of epg exceeds treshhold limit of 500 epg. Similar interpretation of FEC found in grazing lamb flocks in southern Western Australia is given by Sweeny et al. (2011).

Table 2. Average composite FEC in sheep in spring and autumn
Tabela 2. Prosečan broj jaja u prolećnom i jesenjem zbirnom uzorku fecesa ovaca

	Composite FEC values / *Vrednosti prosečnog zbirnog uzorka*	
	Spring sampling *Prolećno uzorkovanje*	Autumn sampling *Jesenje uzorkovanje*
First sampling / *Prvo uzorkovanje*	1525 epg (April / *April*)	950 epg (October / *Oktobar*)
Second sampling / *Drugo uzorkovanje*	725 epg (May / *Maj*)	500 epg (November / *Novembar*)
Average FEC	1125 epg	725 epg

However, FEC have some limitations and should be viewed as additional diagnostic information to be considered with history and clinical signs (Abbott et al., 2009). It is impossible to calculate actual worm population of the host from epg of faeces, since many factors influence egg production of worms which may vary between parasite genera due to different female fecundity. As sheep grow older, they develop an immunity that reduces worm fecundity, so egg count becomes a less reliable indicator of the size of worm burden (Taylor, 2010). Despite these limitations, Taylor points that FEC can be used to help decide if treatment is necessary, while Morgan et al., (2005) suggest that a composite sample from 10 individual sheep is likely to give good results for routine examination of mixed trichostrongyle egg counts in grazing sheep. Papadopoulos et al. (2003) reported the highest FEC in sheep in the spring (FEC=1200 epg and 1100 epg; March and April; two regions of Greece) suggesting that this might be associated with the post-partum or spring egg rise. The second but smaller peak was observed in October (around 400 epg) due to reinfection of animals when increased rainfall provided a more suitable environment for the survival of infective larvae, as well in the spring.

These findings were similar with our data showing high degree of infection in spring in both studies, but less in the autumn. Findings of these authors support our decision to investigate pasture contamination level on this time of year, simultaneously validating basic parasite control recommended by Šibalić and Cvetković (1996).

In Sweden, Waller et al. (2004) found that high number *Haemonchus contortus* abomasal nematodes altered their life cycle adapting to cooler climate. They have developed very successful strategies to survive harsh winter conditions, together with other nematode genera, surviving in tissues of animal host as early L4 larvae (EL4), in stage known as hypobiosis or arrest (Waller et al., 2004; Lalošević et al., 2005). When the well known peri-parturient relaxation of immunity occurs, they resume their development and are primarily response for elevated egg counts of the ewes 4-6 weeks following lambing. So, if early contamination on pasture allow rapid development of L3, they could, together with other species capable to overwinter outside animal host (*Teladorsgia circumcinta*, for instance), trigger clinical episodes of PGE in young lambs and possibly in the ewes by auto-infection (Waller et al., 2004).

Same authors had to deworm their experimental group of lambs, because of high worm burden. Similarly, after finding high level of spring FEC in our research, we have treated sheep flock, as described earlier. One month after first treatment with ivermectin (Alfamec®, Alfasan), the owner requested a treatment with doramectin (Dectomax®, Pfizer) since we have proved it's efficacy in this flock (Lalošević i Simin, 2008).

Discussed data also clearly show the importance of autumn contamination, because high number of eggs on the pasture will develop to L3 under favorable conditions (Papadopoulos et al., 2003), increasing its possibility to be ingested and survive winter as EL4 or to be ingested in the spring after successful outside survival. On the other hand, during the hot and dry summer there is less infection risk since animals are faced to shortage of the green vegetation, and larvae do not easily survive such climatic conditions (Papadopoulos et al, 2003).

Innovative ways of controlling subclinical parasitism on pastoral farming systems need to be considered (West et al., 2009), and already mentioned capability of *D.flagrans* in reducing number of infective larvae on sheep pasture will may be useful innovative tool for biological control, regarding increasing resistance of parasites to antihelmintics and growing public demand for food without drug residues (Lalošević et al., 2009).

CONCLUSION

Our study showed that composite FEC can be used for estimation of pasture contamination and that there is high and moderate level of contamination in spring and autumn, respectively. These are important facts regarding higher availability of infective larvae on pasture and associated risks for sheep infection. The authors underline that these results are from one small flock only, so no generalizations can be made in estimation of pasture contamination level in this region of Serbia. Further studies are needed including more farms from region where grazing practices are common, so detailed information can be obtained. Our findings may help in developing successful and sustainable parasite control programs in our region, both biological and commercial, to minimize impact of GIN on production parameters in grazing sheep.

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PROCENA KONTAMINACIJE PAŠNJAKA BROJEM JAJA STRONGILIDA U ZBIRNOM UZORKU FECESA OVACA: NALAZI U MALOM ZAPATU

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Izvod

Ograničenja u uzgoju pašnih ovaca i povećanje rezistencije na antihelmintike izazvani su strongilidnim parazitima prisutnim na pašnjaku. Cilj ovog rada jeste procena prolećne i jesenje kontaminacije pašnjaka metodom brojanja jaja u zbirnom uzorku fecesa ovaca. Uzorci su sakupljeni u proleće i jesen 2011-te od ovaca iz jednog malog stada iz Srbobrana (grad na jugu Bačke). Utvrđeni intenzitet kontaminacije izražen je kao prolećni i jesenji prosek broja jaja u fecesu. Naši rezultati su pokazali da su na pašnjaku ispitivanog stada prisutni visoki i umereni stepen kontaminacije jajima u proleće i jesen (tim redom), kao i da brojanje jaja u zbirnom uzorku fecesa predstavlja odgovarajuću metodu za vršenje ovakve procene. Kako prisustvo velikog broja jaja na pašnjaku utiče na porast broja infektivnih larvi, dobijeni rezultati mogu doprineti razvoju efikasnih i primenljivih, kako bioloških tako i komercijalnih, programa kontrole parazita u našem regionu.

Ključne reči: kontaminacija pašnjaka, broj jaja u zbirnom uzorku fecesa, ovce, programi kontrole.

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PROMEMORIA

REMINISCENCE ON NORMAN BORLAUG (1914-2009) NOBEL PRIZE WINNER AND GREAT WHEAT BREEDER

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SUMMARY: In this paper we describe our memories of Norman Borlaug, American wheat breeder and Nobel Prize winner, and the relationship between him and Slavko Borojević, wheat breeder and geneticist from Novi Sad, Serbia (part of the former Yugoslavia).

Key words: *Wheat, Borlaug, Green Revolution, Borojević.*

INTRODUCTION

Norman Borlaug, or Norm as we used to call him, died at the age of ninety-five on September 12th, 2009—ten years after his colleague Slavko Borojević, a wheat breeder and geneticist passed away in Novi Sad, Vojvodina, Serbia (part of the former Yugoslavia). On Norm's ninety-fifth birthday, Katarina promised to visit him in Dallas where she planned to visit her grandson Miloš. Norm, already weak, was eager to welcome her and planned to discuss a project to improve wheat in Africa. Unfortunately, Norm did not survive to see Katarina. The plans on how to improve agriculture in Africa are left, as his legacy to the future generations of scientists and politicians worldwide.

BIOGRAPHY OF NORMAN BORLAUG

Comprehensive biographies of Norman Borlaug may be found in many encyclopedias, journals, and on the Internet (Borlaug 2006, Lumpkin and Berdegué 2009, Quinn 2007, Swaminathan 2009). Moreover, Vietmeyer wrote a fascinating book, describing life and work of Borlaug, "The Mild-Mannered Maverick who Fed a Billion People"

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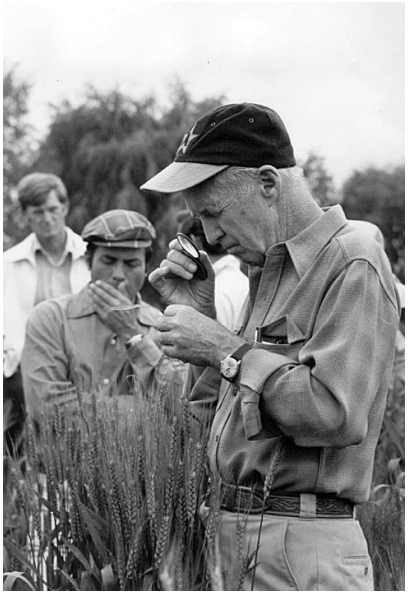
(2008), and we will present a short biography of him incorporating some of the information from the book.

Norman E. Borlaug was born on March 25, 1914, in the small Iowa community of Cresco to a farmer's family originating from Norway. He began his education in an Iowa one-room grammar school. In his life Borlaug had several facilitators who helped him shape his brilliant future. The first of them was his cousin Sina, a local school teacher, who encouraged him to enter a high school in Cresco.



Dr. Norman Borlaug, (1914-2009) at a field day in Mexico.
Dr. Norman Borlaug (1914-2009) na danu polja u Meksiku.

(Source: CIMMYT; by permission from Dr. Thomas Paine, March 19, 2010.)

The second facilitator was David Bartelma, Cresco's high school principal. The third facilitator was the All-American football star George Champlin, who enticed Borlaug into going to the University of Minnesota even though he had no money, no job, and nowhere to live (Vietmeyer 2008). Thanks to the roles of these three, Borlaug found himself the first of his family to attend college. He enrolled at the University of Minnesota in St. Paul in 1933, supported by his family, especially by his grandfather.

At the University of Minnesota, Borlaug was admitted into forestry although his first choice was agriculture. Borlaug earned his all three degrees at the University of Minnesota: Bachelor of Science in 1937, Masters Degree in 1940, and Doctorate Degree in 1942 (with Professor Stakman). Borlaug's Ph.D. dissertation was in plant pathology on the resistance to disease in flax. Throughout his years at the University, Borlaug diligently studied and participated in many different athletic clubs while working hard as a waiter. During the summers, thanks to his major in forestry, Borlaug often worked as a forest ranger in some of the most remote parts of the United States. At the university café, Borlaug met his future wife Margaret Gipson—a student at the College of Education. Borlaug married Margaret in 1937; "without her unwavering support, Borlaug might not have accomplished nearly so much in his long and demanding career

(Swaminathan 2009)." Borlaug had two children with Margaret—a son, William, and a daughter, Jeanie.

After graduation, Borlaug worked as a microbiologist at DuPont Corporation. In 1944 he joined the Rockefeller Foundation research project on wheat in Mexico. Later, in 1964 he joined the CIMMYT, the International Maize and Wheat Improvement Center located near Mexico City, where he served as the director of wheat research from 1960 until his retirement in 1982. Even after his retirement from CIMMYT he continued to serve as a consultant. From 1984 to 2007 he held the position of distinguished professor of International Agriculture at Texas A&M. In 1985, Borlaug joined the Sasakawa Africa Association whose mission is to end starvation and malnutrition in Africa.

MEXICO AND THE GREEN REVOLUTION

Working for the Rockefeller Foundation, Borlaug arrived in Mexico in 1944. At that time, the country's farmers were growing less than half of the wheat necessary to meet the demands of the large population. Mexico's wheat varieties were particularly susceptible to rust and lodging. Borlaug worked for 13 years before he and his team of agricultural scientists developed a disease resistant wheat variety; however, it was not resistant to lodging. At the CIMMYT, Borlaug started a new project, breeding wheat varieties with short straw that would be resistant to lodging. He crossed Japanese dwarf varieties, specifically Norin 10 with Mexican varieties. The history of the arrival and incorporation of the short straw traits are particularly revealing (Borojević and Borojević 2005a and 2005b). The wheat variety Norin 10 was brought to the United States by an agronomist returning from his military service in Japan. In United States, Norin 10 was first crossed with Brevor variety by the American wheat breeder Vogel (who cooperated with Borlaug). Vogel created the new variety Gains. From variety Gains, Borlaug transferred the "Japanese genes" for short straw and daylight insensitive genes (discovered later and named Rht 1, Rht 2, and PpD genes) to many Mexican varieties (Koenig 2009). To accelerate the breeding procedures of new wheat varieties, Borlaug applied the "shuttle breeding method" (Borlaug 1968). Transferring genetic material from one part of Mexico to the other, because of climate differences, he was able to produce two generations of wheat in one year. Aided with fertilizer and irrigation, Borlaug's new wheat varieties were very high yielding and enabled state of Mexico to achieve self-sufficiency in wheat production by 1956. These results were considered an agricultural miracle (Quinn 2007).

After increasing Mexican wheat production several times, Borlaug turned to countries in South Asia, where India and Pakistan were facing starvation after the Second World War. Borlaug convinced the political leaders of these two countries to accept his new concept of greater investment in agriculture by using new high-yielding wheat varieties and improving farming practices. His new strategies in agriculture increased yields by four times in India and Pakistan, making them self sufficient in wheat production and contributing to saving hundreds of millions of people from starvation. Later, the wheat varieties created at the CIMMYT spread to Turkey, Morocco, and across the world, wherever spring wheat varieties could grow. Today, high-yielding, disease-resistant wheat varieties based on Dr. Borlaug's pioneering work are grown on 80 million hectares (200 million acres) throughout the world. Later this movement became known as the "Green Revolution," and Borlaug's work gave rise to science-based agriculture in

developing countries (Lumpkin and Berdegué 2009).

CRITICS OF GREEN REVOLUTION

The Green Revolution was often criticized by environmentalists for promoting practices that used fertilizer and pesticides and focusing on a few high-yield crops that benefited large landowners without considering pollution, environment effects and the needs of small landowners. Borlaug was indignant: "Using manure would require a massive expansion of the lands required for grazing the cattle and consume much of the extra grain that would be produced. At best, he said, such efforts could support no more than 4 billion people worldwide, well under the nearly 7 billion now inhabiting the planet..." (Larkin 2009). The Green Revolution, despite the critiques that it received, demonstrated how humanity can resolve the most fundamental needs, such as food production. It is to be hoped that future generations will explore the experience and the achievements of the Green Revolution in the other fields of human needs, for example energy, in a similarly innovative and sustainable way (Boroyevich Dushan, personal communication, 2009).

RECOGNITION

In 1970, Borlaug won the Nobel Peace Prize. It was estimated that more than a billion lives were saved by his breakthrough contributions in agriculture. It is said that Borlaug did more for humanity than any politician or monarch in history. Borlaug believed that it was possible to attain a balance between the human population and food production, without starvation (Lumpkin and Berdegué 2009). During his life, Borlaug received more than 150 international honors, including the United States Congressional Gold Medal and the Presidential Medal of Freedom (Quinn 2007).

BORLAUG HUMANIST

Borlaug was not only an agronomist, but he was a humanist. Thomas A. Lumpkin, CIMMYT Director General, cited Borlaug on the occasion of his farewell: "Norm once said: 'I personally cannot live comfortably in the midst of abject hunger and poverty and human misery.'" Today millions of small-scale farmers in developing countries still practice low-input, subsistence agriculture and live in poverty. They typically spend at least 70% of their income on food and most are at risk of being malnourished. The World cannot be at peace until these people are helped to feed themselves and escape poverty (Lumpkin and Berdegué 2009). Norm was also involved in defeating malnutrition and poverty in Africa. Since 1985, he actively participated in the Sasakawa Africa Association, whose activities focus on bringing science-based crop production methods to the small farms of subSaharan Africa (Quinn 2007). Borlaug founded the World Food Prize in 1986, now considered the "Nobel Prize" for food and agriculture. It is given for outstanding contributions in increasing the quantity, quality, and availability of world food supplies. (Slavko Borojević was nominated twice for the Prize, in 1991 and 1996 but, due to the international sanctions imposed on the former Yugoslavia at the time, he was not eligible.)

RELATIONSHIP WITH SLAVKO BOROJEVIĆ

Slavko Borojević met Borlaug at several international conferences during the 1960s. They became colleagues and friends, sharing similar ideas on how to create high-yielding varieties of wheat (Borojević and Borojević personal correspondence 1960-2009). Both had extraordinary and very similar life trajectories. They were both wheat breeders, passionate about solving hunger Borlaug in the World, Borojević in his country and in East-Central Europe.

In 1958, Slavko Borojević started the project of breeding high-yielding wheat varieties in Novi Sad, with the purpose of increasing wheat production in his country (the former Yugoslavia). After the Second World War, the country was importing wheat for human consumption. Under Borojević's program, the semi-dwarf daylight insensitive winter wheat varieties were developed with a yield capacity over 10 t/ha. This was achieved by transferring the Rht8 gene for dwarf straw and PpD genes for daylight insensitivity from the Italian varieties to continental wheat varieties. The genes Rht8 and PpD in Italian varieties originate from the Japanese variety Akakomugi, which was brought to the Italian breeder Strampelli by a visitor returning from Japan before the Second World War. The new varieties significantly increased the national average yields and the total volume of wheat production. Some of the countries in the region turned from importer to exporter of wheat (Bošković 2002). The results achieved in wheat breeding made Borojević the most successful wheat breeder in the former Yugoslavia and he is considered the founder of the "Yugoslav school of plant breeding" (Mihaljev and Petrović 2005). Particularly impressive is the coincidence that both Borojević and Borlaug, working independently in different parts of the world, were using the "Japanese genes" in their wheat projects but from different Japanese varieties. Because the genes (Rht and PpD) were not discovered at the time (Borojević and Borojević 2005a and 2005b), they both worked only on phenotypic expression of the genes in the varieties that they crossed. Such work required extensive and tedious biometrical, genetic research but also scientific intuition in the combination of genes for the creation of a new variety (the short straw, resistance to diseases and extreme temperatures, increase in grain quality, and high yield). Their vision and accomplishments in creating the high-yielding wheat varieties were a great success at the time and remain so today.

Borojević Slavko had a great respected for Borlaug and his work. He was talking about Borlaug's projects everywhere: at home, in the field, at meetings, and at lectures at the University (Borojević 1970). He visited Borlaug in Mexico several times during the 1970s. In 1979, Borojević was invited to the Trustees Board meeting of the CIMMYT by Borlaug and late Glen Anderson, another famous wheat breeder. On this trip he was accompanied by his wife Katarina, a plant geneticist, and daughter Ksenija, then a student of archeology/palaeoethnobotany (the authors of this paper).

Every summer scientists from the CIMMYT visited the experimental fields of the Institute for Crops and Vegetables in Rimski Šančevi (Novi Sad), where Borojević Slavko and Katarina were working. Borojević also sent his younger coworkers to the CIMMYT in Mexico for training and to attend conferences. When Borojević Slavko died, Borlaug has located Ksenija at Washington University and had expressed his condolences in a letter reprinted below (P.S.1). After Slavko's death, collaboration and correspondence with Borlaug and the CIMMYT has continued by Katarina and Slavko's coworkers. Breeders from the CIMMYT and from Novi Sad exchanged genetic mate-

rials, transferring genes from Mexican varieties to Novi Sad varieties and vice-versa, thanks to the cooperation established by Borlaug and Borojević.\

-----Original Message-----
From: Viesca, Eva (Secretary to Dr. Norman E. Borlaug)
Sent: Monday, August 06, 2001 11:34 AM
To: 'Kborojev@artsci.wustl.edu'
Subject: FW: Passing away of Dr. Slavko Borojevic

Mrs. Borojevic
7563 Buckingham Drive Apartment 2s
St. Louis, MO 63105

Dear Mrs. Borojevic:

I assume you are the daughter of Drs. Slavko and Katarina Borojevic; am I correct?
I knew both of them well, since I was also a wheat scientist.
I have heard that Dr. Slavko Borojevic passed away in 1999; is that correct?
What is your mother's address? I would like to write to her.
Starting September 5th, I will be stationed at Texas A&M University where I am a Distinguished Professor of International Agriculture. If you have any news before that date, please send me an e-mail: eviesca@cgiar.org, otherwise, please contact me through my secretary at Texas A&M, Mrs. Glenda Kurten as follows: g-kurten@tamu.edu

Sincerely,
Norman E. Borlaug
PO Box 6-641
CIMMYT
CP 06600
Mexico, D. F.
MEXICO

LITERATURE

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SEĆANJE NA NORMANA BORLAUGA (1914-2009) DOBITNIKA NOBELOVE NAGRADE I VELIKOG OPLEMENJIVAČA PŠENICE

KATARINA BOROJEVIĆ I KSENIJA BOROJEVIĆ

Izvod

Norman Borlaug ili Norm kako smo ga zvali, umro je u 95-oj godini života, 10 godina nakom smrti svog kolege i saborca za visoke prinose pšenice Slavka Borojevića u Novom Sadu. Prilikom čestitanja 95. rođendana, Katarina Borojević obećala je Borlaugu, da će ga posetiti u Dallasu na jesen. Norm, već jako oslabljen bolešćcu rekao je: "dodjite moramo diskutovati o Africi, tamo još nije završen posao unapređenja proizvodnje pšenice". Na žalost, Norm nije dočekao Katarinu, a unapređenje pšenice u Africi je ostavio buducim generacijama u amanet.

Norman E. Borulaug se rodio 25. marta 1914. godine u malom mestu Cresco, u Iowi u Sjedinjenim američkim državama, u porodici farmera poreklom iz Norveške. Osnovnu školu završio je u školi sa jednom učionicom u rodnom mestu. Na preporuku svoje rođake učiteljice, nastavlja srednju školu radeći i baveći se fiskulturom. Posle završene srednje škole, odlazi na studije, u St. Paul u Minnesoti, kao prvi iz svoje porodice. Porodica ga u tome podržava, naročito deda. Na univerzitetu u St. Paulu, Borlaug se upisao na šumarstvo, jer nije bio primljen na poljoprivredu, koju je želeo da upiše. Za vrema studija izdržava se radeći, najčešće u studenskim restoranima, ali i radeći kao šumarski rendžer, često u najzabačenijim krajevima Američkih država Idaho i Massachusetts. U studentskom kafeu, na Universiteta u Minnesoti, sreće Margaret Gipson studenta (College of Education) sa kojom se oženio 1937. godine. Margaret ga prati kroz život i podržava njegove projekte, tako da ju smatraju heroinom Zelene revolucije. Umrla je 2007. godine. Borlaugovi imaju kćer Norma Jean (Laube) i sina Willam-a. Borlaug je završio je šumarski fakultet 1937., magistrirao 1940., doktorirao 1942. godine iz fitopatologije kod čuvenog profesora Stakman-a na Universitetu Minnesota u St. Paulu, na otpornosti bolesti lana. Nakon doktoriranja, opredeljuje se za naučni projekat Rockefellerove Fondacije, unapređenje proizvodnje pšenice u Meksiku, a zatim za CIMMYT (Centar za unapredjenje proizvodnje kukuruza i psenice) nedaleko od Meksiko Citi-a. Tada, 1944. godine, Meksiko nije uspevao da proizvede ni polovinu svojih potreba u pšenici. U CIMMYT-u, Borlaug je počeo unošenjem gena za nisku stabljiku, i gena neutralnih za fotoperiodizam iz japanske sorte Norin 10. (Japansku sortu Norin 10, doneo je jedan agronom u SAD vraćajući se sa svoje ratne misije posle drugog svetskog rata iz Japana.) Norin 10 prvo je ukrstio sa sortom Brevor, američki selekcioner pšenice Vogel i stvorio sortu Gains. Ova nova sorte je imala gene za nisku stabljiku, kao i gene nautralne za fotoperiodizam, koji su kasnije nazvani Rht i PpD geni. Te gene je Borlaug preneo u mnoge meksikčke sorte za pšenicom. Uz primenu đubrenja i navodnjavanja, što su nove sorte zahtevale, prinosi su se toliko povećali da je Meksiko 1956. godine, podmirivao svoje potrebe. Nakon Meksika, Borlaugov zadatak u CIMMYT-u bio je povećanje proizvodnje pšenice na Srednjem Istoku i Južnoj Aziji. Nakon drugog svetskog rata u nekim delovima Indije i Pakistana zavladala je strašna glad, kada je oko 160 miliona ljudi umrlo od gladi. Borlaug je uveo CIMMYT-ove pšenice niske stabljike u te

zemlje, uprkos velikom instiktivnom otporu seljaka. Sa uvođanjem sorata niske stabljike u proizvodnju, Borlaug je uveo i novi način proizvodnje pšenice, primenom fertilizacije i navodnjavanje zemljišta. Ovaj novi pristup u poljoprivredi sa novim sortama, doveo je do četverostrukog povećanja prinosa u tim zemljama. Taj pokret nazvan je kasnije Zelena revolucija. Nakon primene Zelene revolucije na velikim površinama, te zemlje počele su da proizvode dovoljno pšenice za svoje potrebe. Borlaug je uveo CIMMYT-ove pšenice i na Srednji Istok, Severnu Afriku, sve do Maroka i Turske. Za svoj uspeh na povećenju proizvodnje pšenice, Borlaug je 1970. godine dobio Nobelovu nagradu za mir "jer je učinio više nego i jedan čovek svoga vremena da osigura hleb za gladni narod", više nego i jedan političar ili monarh u istoriji. Ali mnogo važnije od toga je, što je Borlaug promenio shvatanje o mogućnosti postizanja balansa između broja ljudi i mogućnosti ljudi da proizvedu hranu. To je njegova najveća zasluga. Pokraj Nobelove nagrade za svoj rad i dostignuća, Borlaug je primio i velik broj drugih priznanja. Bio je član 11 nacionalnih akademija poljoprivrednih nauka, dobio je 60 počasnih doktorata nauka universiteta iz raznih zemalja, kao i veliki broj priznanja poljoprivrednih, farmerskih i drugih društvenih organizacija, ukupno preko 150. Na državnom federalnom nivou primio je Nacionalnu medalju za nauku u SAD, kao i Kongresnu zlatnu medalju, najviše civilno odlikovanje u SAD.

Saradnja sa Slavkom Borojevićem

Razvijajući svoje projekte stvaranja sorata, Slavko Borojević i Borlaug se često sastaju 1960-tih godina na internacionalnim konferencijama, izmenjuju iskustva, postaju kolege i prijatelji. Vezivala ih je ista želja da povećaju prinose pšenice, Borlauga u svetu, a Slavka u svojoj zemlji (bivšoj Jugoslaviji) koja je posle drugog svetkog rata veliki deo svojih potreba u pšenici obezbeđivala iz uvoza.

Radeći u Meksiku, Borlaug početkom šesdesetih unosi u jare (prolećne) sorte pšenice gene za nisku stabljiku (Rht1 i Rht2) i za neutralni fotoperiodizan PpD, iz japanske sorte Norin 10. Slavko Borojević u Novom Sadu u ozime sorte pšenice unosi gen za nisku stabljiku Rht8 i PpD gene iz Japanske sorte Akakomugi, koju je pred početak drugog svetskog rata doneo jedan Japanac, selekcioneru pšenice Strampelli-u u Italiju. Sa uvozom talijanskih sorata, gen Rht8 i PpD stižu u Jugoslaviju, krajem pedesetih godina. Borlaug u Meksiku, a Slavko u Novom Sadu, oba koriste Rht gene za nisku stabljiku i PpD gene iz Japanskih sorata. Ovi geni, su mnogo kasnije otkriveni i dokazani u njihovim sortama. U vreme kada su oni razvijali svoje projekte malo se znalo o ovim genima. Tada se mogao uočiti i izmeniti samo efekat pojedinih gena na fenotipskom nivou. To je zahtevalo posebna biometrijska istraživanja i genetička proučavanja, ali i naučnu intuiciju u kombinovanju gena (za nisku stabljiku, otpornost prema bolestima, kvalitet i drugo) pa su zato njihova dostignuća u to a i sadašnje vreme velika. Slavko je Borlauga je veoma cenio i voleo. Uvek je o njemu pričao, u kući, na predavnjima, na sastancima i pisao o njegovom uspehu. Davao je diplomske i magistarske radove o Meksičkim sortama pšenice. Posetio je nekoliko puta CIMMYT u Meksiku, gde je Borlaug radio, a 1978/79. godine sa Katarinom i Ksenijom, tada studentom arheologije na Kalifornijskom universitetu u Berkliju (autorima ovog rada).

Svako leto, su Borlaugovi saradnici iz CIMMYT-a dolazili u Novi Sad i posećivali Ogledno polje Zavoda za pšenicu, Instituta za ratarstvo i povrtarstvo u Rimskim Šančevima, gde su Slavko i Katarina radili. Slavko je takođe slao svoje saradnike u Meksiko na naučne seminare i posete. Izmenjivali su genetski material godinama, unosili gene iz meksičkih sorata u novosadske sorte i obrnuto.

INSTRUCTIONS TO AUTHORS FOR WRITING PAPER

The journal "Contemporary Agriculture" publishes original scientific papers, review papers and short communications.

A paper is written in English. It should comprise a short summary in Serbian. The whole script of the paper, including tables, graphs, schemes, drawings and photographs, can have maximum 6 typed pages, Portrait, in single spacing. Margins: Top 2.5 cm, Left 2.5 cm, Bottom 2.5 cm, Right 2.0 cm. For typing the paper the Times New Roman font, 10 pt , should be used. Justify with the indent of the first line 0.6 cm. (Portrait - Paragraph – Indents and Spacing – Special – First Line 0.6. No pagination.

THE PAPER TITLE is written in bold letters, Font Size 11 , centred. The title should be lowered below the upper margin clicking enter 4 times and writing should be commenced in the fifth line.

THE NAME AND SURNAME of the authors are written in normal letters, Font Size 10, centred, with a single space below the paper title. With mark 1, in superscript, (click Insert Footnote) above the name of the last author, the Footnote is marked, stating the title, the name and surname, the rank and the institution in which the respective authors are employed.

SUMMARY: (italic), Font Size 10 (Justify) with a single space below the name and surname of the author of the paper. The summary presents the basic objective, the material and method of the study, the significant results and the conclusion (500 characters maximum).

Key words: minimum 3 and maximum 6 words. Below the summary, Font Size 10.

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STANČIĆ, B., BOŽIĆ, A., RADOVIĆ, I., GRAFENAU, P. sen., PIVKO, J., HRENEK, P., STANČIĆ, I.: Veštačko osemenjavanje svinja dozama sa samanjenim brojem spermatozoida (pregled). Savremena poljop., 56(1-2)1-11, 2007.

PhD Thesis:

TAST, A.: Endocrinological basis of seasonal infertility in pigs. PhD Thesis, Faculty of Veterinary Medicine, Finland, Helsinki,2002.

In Scientific Books:

TOMES, J.G., NIELSEN, H.E.: Factors affecting reproductive efficiency of the breeding herd. In: Control of Pig Reproduction (D.J.A Cole and G.R. Foxcroft, eds.). Butterworths, London, pp.527-540,1982.

At Scientific Meetings:

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We are grateful to all the authors for their cooperation.

Editor –in-chief
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STANČIĆ, B., BOŽIĆ, A., RADOVIĆ, I., GRAFENAU, P. sen., PIVKO, J., HRENEK, P., STANČIĆ, I.: Veštačko osemenjavanje svinja dozama sa samanjenim brojem spermatozoida (pregled). Savremena poljop., 56(1-2)1-11, 2007.

Doktorska disertacija:

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