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

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EFFECT OF WORM AND COLD PERIOD OF THE YEAR ON BOAR SEMEN QUALITY PARAMETERS*

BLAGOJE STANČIĆ, ALEKSANDAR BOŽIĆ, IVAN STANČIĆ, SAŠA DRAGIN, IVAN RADOVIĆ, MILICA PETROVIĆ [1]

SUMMARY: The effects of various seasons of the year on boars semen quality parameters were investigated on intensive swine production farms in the Autonomous Province of Vojvodina, Serbia. The total of 30 Large White AI boars were investigated, within one year. Two ejaculates per boar per month were investigated (total 720 ejaculates per year). The average ejaculate volume in the June-July-August and September-October-November season (213ml and 232ml), sperm concentration (220 and 210x10^6/ ml) and total sperm number per ejaculate (46,8 and 48,7x10^9), were significant (P<0,01) lower compared with December-January-February (293ml, 319x10^6/ml, 93,5x10^9) and March-April-May season (285ml, 284x10^6/ml, 80,9x10^9). The percentage of poor ejaculates significntly incerase in the wormer season (31,8% i 21,4%), compared with colder season (10% i 16,7%). Due to lower values *of ejaculate parameters,* fewer doses may be made from one ejaculate in the wormer season. *These facts should be taken into consideration when planning the intensity of boar reproductive exploitation in the wormer and colder season of the year.*

Key words: *season, semen, parameters, quolity, boar.*

INTRODUCTION

Number of doses per ejaculate is influenced by values of ejaculate parameters quality, such as volume, sperm concentration, total number of sperm in ejaculate and progressive motility (Tardif i sar., 1999; Stančić i sar., 2003; Knox, 2004). Season of

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Original scientific paper / *Originalni naučni rad*

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the year is one of the most important factor that influence semen parameters quality variations. It has been demonstrated that increasing of ambiental temperature in the wormer season is the most important factor that influence the decreasing of boar sperm production (Colenbrander i sar., 1990; Setchell, 1998; Corcuera i sar., 2002; Stančić i sar., 2003). The result is decreasing in boar reproduction exploatation on the one hand, and decreasing in sows fertility rate in the wormer season on the other hand (Stančić i sar., 2003; Okere, 2003).

The aim of this paper is to determine the effects of seasons on the main parameters of fertilization capacity in boar ejaculates, which are used for practical artificial insemination on swine farms in Vojvodina

MATERIALS AND METHODS

The researches were conducted on large swine farm in the Autonomous Province of Vojvodina, Serbia. During the period of one year (from January 2012 to December 2012), 2 ejaculates per boar were tested monthly, collected from 30 Large White boars, ranging from 2 to 2,5 years of age. Consequently, 720 ejaculates were tested in total.

Immediately after the collection on farm, the volume of each ejaculate was determined (ml) and the ejaculates were transported to the laboratory in air-conditioned boxes for boar semen (Minitüb) at +17^{o}C. The ejaculates were heated up to +37^{o}C in the laboratory.

The following parameters were determined for each ejaculate in laboratory: (1) the volume (ml), (2) the sperm concentration (x10^{6}/ml), (3) the total sperm count per ejaculate, and (4) the progressive sperm motility. The sperm concentration, the total sperm count, the number of insemination doses, and the level of required dissolution were determined by the photometer SDM5 (Minitüb, Germany). The progressive sperm motility was determined by a light microscope under the medium power magnification. The ejaculates with the progressive sperm motility < 65% were considered as poor for using in artificial insemination. The possible doses number per ejaculate were calculated with 4 x 10^{9} motile spermatozoa in 100ml diluted semen dose. The data were processed by *Statistica 10* software.

REULTS AND DISKUSSION

The average ejaculate parameters was: Volume = 253ml, spermatozoa concentration = 254x10^{6}/ml, total spermatozoa number = 65,7x10^{9} and progressive motility =78% (Table 1).

Table 1. Boars native sperm parameters

Tabela 1. Parametri nativne sperme nerastova

Ejaculate parameters Parametri ejakulata	Months of year / Meseci u godini				
	D-J-F (n=180)	M-A-M (n=180)	J-J-A (n=180)	S-O-N (n=180)	Ukupno (n=720)
Volume Volumen (ml)	293A	285A	213B	232B	253
Sperm concentration Konc. sptz. (x06/ml)	319A	284A	220B	210B	254
Total sperm No./ejaculat Ukupan br. sptz,/ejakulat (x109)	93,5A	80,9A	46,8B	48,7B	65,7
Prog. motility Progr. pokret. (%)	85A	85A	75A	70B	78
Bad ejaculates Loših ejakulata*	24/10%A	44/16,7%A	84/31,8%B	72/21,4%B	224/20,3%
Possible No. AI doses per ejaculate Moguć br. VO doza po ejakulatu	20A	17A	9B	8,5B	13
Required deluting degree Potreban stepen razređenja	1:7A	1:6A	1:4B	1:4B	1:5

n – Number of ejaculates / *broj ejakulata*;

* < 65% progressive motile spermatozoa per ejaculate / < *65% progresivno pokretnih spermatozoida u ejakulatu (n/%).*

Average ejaculate volume (213ml and 232ml), sperm concentration (220 and 210x10^6/ ml) and total sperm number per ejaculate (46,8 i 48,7x10^9) was significatly lower (P<0,01) i the period June-July-August and September-October-Novmber, compared with the period December-January-February (293ml, 319x10^6/ml, 93,5x10^9) and period March-April-May (285ml, 284x10^6/ml, 80,9x10^9). The percentage of poor ejaculates significntly incerase in the wormer season (31,8% i 21,4%), compared with colder season (10% i 16,7%). Therefore, in the wormer period of the year, it is possible to made significant lower number of doses per ejaculate (9 and 8.5), compared with in the colder period (20 i 17 doses) (Table 1).

The decreasing value of boar ejaculate parameters in the wormer season of the year has not been entirely clarified. The variation of ejaculate parameters quality can be influenced by many factors, such as breed, individual boar, age of boar, semen collection frequency, ambiental temperature, daily photoperiod and some deseases (Colenbrander i sar., 1990; Stančić i sar., 2003; Wolf i Smital, 2009; Gerfen et al., 1994; Ciereszko et al., 2000; Jankevičiute and Žilinskas, 2002; Stančić et al., 2003; Smital et al., 2004; Chukwuemeka et al., 2005). However, based on the recently studies, this decreasing is mainly influenced by increased ambient temperature (Suriyasomboon et al., 2004) and prolonged daily photoperiod (Sancho et al., 2004) in warmer seasons, that reduce the process of spermatogenesis and testosterone synthesis. Furthermore, some researches indicate that this could be due to the genetic inheritance passed from wild progenitors to domesticated breeds. It is a well-known fact that wild boars demonstrate

extreme sexual activity seasonally and provide the best sperm quality during mating seasons, which last from late autumn to early winter (Kozdrowski and Dubiel, 2004; Macchi et al., 2010). The results of our researches indicate that the value parameters of sperm fertilisation capacity were significantly higher during cooler periods of the year in comparison with warmer seasons. Various authors have also obtained similar results which confirmed the influence of warm seasons on the reduction of the value parameters of sperm fertilisation capacity in boars (Liao et al., 1996; Kunavongkrit et al., 2005; Ciereszko et al., 2000; Jankevičiute and Žilinskas, 2002; Chukwuemeka et al., 2005).

The obtained results can enhance the reproductive efficiency in boar during cooler and warmer periods of the year on large farms in Vojvodina.

CONCLUSION

The results of ours investigations demonstrate that there are significant variations in boars ejaculate parameters value, within the seasons of the year. The ejaculate volume, the sperm concentration, the total sperm count and the progressive sperm motility are significantly lower during warmer seasons of the year, and hence the number of obtained insemination doses per ejaculate in almost twice as small as the number of obtained doses in cooler seasons.

These facts should be taken into consideration when planning the intensity of boar reproductive exploitation under production conditions. Therefore, it is possible to significantly reduce the negative effects of warmer seasons on sow fertility.

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UTICAJ TOPLOG I HLADNOG PERIODA GODINE NA PARAMETRE KVALITETA SPERME NERASTA

BLAGOJE STANČIĆ, ALEKSANDAR BOŽIĆ, IVAN STANČIĆ,
SAŠA DRAGIN, IVAN RADOVIĆ, MILICA PETROVIĆ

Izvod

Ispitivan je uticaj različitih godišnjih sezona na parametre kvaliteta sperme nerastova na farmama intenzivne proizvodnje svinja u PA Vojvodini (Srbija). Ispitano je ukupno 720 ejakulata (2 ejakulata po nerastu mesečno), od 30 nerastova rase Veliki jorkšir, koji se koriste za VO. Tokom perioda Jun-Jul-Avgust i Septembar-Okotobar-Novembar, prosečan volumen ejakulata (213ml and 232ml), koncentracija spermatozoida (220 and $210x10^6$/ ml) i ukupan broj spermatozoida u ejakulatu (46,8 and $48,7x10^9$), bili su značajno niži ($P<0,01$) u poređenju sa sezonom Decembar-Januar-Februar (293ml,

319x10^6/ml, 93,5x10^9) i Mart-April-Maj (285ml, 284x10^6/ml, 80,9x10^9). Procent loših ejakulata je bio znatno veći u topijem delu godine (31,8% i 21,4%), u poređenju sa hladnijim delom godine (10% i 16,7%). Zbog znatno nižih vrednosti parametara kvaliteta ejakulata, tokom toplijeg dela godine je moguće praviti manji broj inseminacionih doza po ejakulatu. Ovo treba uzeti u obzir prilikom planiranje reproduktivne eksploatacije nerastova u toplijem periodu godine.

Ključne reči : sezona, ejakulat, parametri, kvalitet, nerast.

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POSTCERVICAL ARTIFICIAL INSEMINATION OF SOWS IN COMBINATION WITH SYNTHETIC SEMINAL PLASMA (PREDIL MR-A®)

STANIMIR DIMITROV[1]

SUMMARY: The use of artificial insemination in pigs has been adapted worldwide as the reproductive method of election at farm level. The postcervical method is launch as a method to improve the reproductive efficiency and genetic progress. A strategy to enhance farrowing rate and litter size of artificially inseminated female pigs is to "stimulate" the uterus and oviduct by adding various compounds to extended boar semen or injecting a compound into the female before insemination. The aim of this study was to evaluate the effect of seminal synthetic plasma Predil MR-A® as a method to facilitate procedures with postcervical artificial insemination of sows. The postcervical insemination was performed with 1.5 x 10^9 spermatozoa in control group and 1.5 x 10^9 spermatozoa with 20 ml of chemical synthetic plasma Predil MR-A® in test group. The results show that after postcervical insemination with in combination of chemical synthetic plasma Predil MR-A® were obtained 84,27 % conception rate, 83,15 % farrowing rate, 10,79 total born piglets and 10.07 piglets born alive per liter.

Key words**:** *sows, artificial insemination, seminal plasma Predil MR-A®.*

INTRODUCTION

Artificial insemination of sows is effective method for intensive use of elite boars. Currently, three main pig artificial methods can be performed depending on the site of sperm dose deposition: cervical/conventional insemination, postcervical/intrauterine insemination and deep intrauterine insemination. Since 10 years ago, the postcervical method was launched as a method to improve the reproductive efficiency (increase in fertility and litter size) and genetic progress (due to the reduction in the use of elite boars) and several papers (Watson and Behan, 2002, Levis et al., 2002, Belstra, 2002, Dimitrov et al., 2007, Dimitrov et al., 2007) where published with diverse results obtained when comparing with the traditional cervical method for artificial insemination of sows. Other way to increase productivity rate of sows is by substances added

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Original scientific paper / *Originalni naučni rad*
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to semen with fertility-regulated function. Oxytocin, prostaglandins, estrogenes and synthetic seminal plasma (Predil MR-A®, Presus®) stimulate the uterine contractions, respectively the sperm transport and the metabolism of spermatozoa (Martin Rillo et al., 1996, Levis, 2002, Castaneda Morreno, 2002). The studies of some authors (Martin Rillo et al., 1996, Lyczynski, et al., 2000, Garcia Ruvalcaba et al.,2008, Garcia Ruvalcaba et al.,2009) showed, that the using of Predil MR-A® synthetic seminal plasma prior artificial insemination of sows (cervical or postcervical deposit of semen dose) have a positive effect on farrowing rate and litter size.

The aim of this study was to examine the effect of using of the synthetic seminal plasma Predil MR-A® in postcervical insemination of sows.

MATERIAL AND METHODS

The study was conducted in one pig commercial pig farm during June/August 2009 of Bulgaria. In the experiment were included total 173 females from Dunabe White breed. The lactation length was 25 days. After weaning the animals were housed in pens containing 10 to 13 individual animals. The sows were provided *ad libitum* access to feed and water.

Semen was collected no more than twice per week from boars housed at the same farm by using the gloved hand method. Obtained ejaculates were individually assessed for volume, number of sperm cells, and percent of motile spermatozoa. Volume (without gel fraction) was determined by using a graduated glass vessel with precision of 0.01 ml. Number of sperm cells was determined by using a Thoma counting chamber. Percentage of motile spermatozoa (0 to 100%) was subjectively evaluation with a light microscope at a magnification of 100X. Spermatozoa were diluted in DZNB extender and packaged in 100 ml plastic bottles. Diluted sperm was stored in a cooler unit at 15-17° C until used within 24 to 48 hours after collection.

Estrus detection of weaned sows was performed twice per day (09:30 to 10:30 and 15:00 to 16:00) starting on day 3 after weaning with a mature boar. Sows were randomly assigned to one of the following groups: control group (n=85) - postcervical insemination with 50 ml/1.5 x 10^9 spermatozoa; test group (n=88) - postcervical two-phase insemination with 50 ml/1.5 x 10^9 spermatozoa with in combination with 20 ml. chemical synthetic plasma Predil MR-A®. The postcervical insemination was conducted with postcervical catheter with supernatural long yellow trip including inner cannula. In postcervical two-phase insemination protocol, previously infused with 20 ml of chemical synthetic plasma Predil MR-A® warmed up to 37º C before beginning the introduction of the cannula. This procedure speed up the relaxation of the sow's cervix and makes easier the introduction of the cannula through the cervix rings into the uterus, followed by semen deposition and avoiding backflow. Catheters and chemical synthetic plasma Predil MR-A® were supplied from Kubus S.A, Madrid, Spain. The sows were inseminated 2 times per estrus. The technicians in the farm have good experience for using of post-cervical technique for artificial insemination. The gilts were not used in the experiment. The conception rate was determined by diagnosed at 28 to 30 days after insemination by ultrasonography (PREG-TONE®, Renco Corporation, USA). The farrowing rate was calculated as percent of inseminated females that farrowed. The total piglets born per litter and piglets born alive per litter were counted at farrowing. The farrowing index was calculated as piglets born alive per litter/100

inseminations (percentage of farrowing rate x piglets born alive per litter).

Prior to data analysis sows were categorized into two weaning-to-estrus interval of ≤ 6 days or > 6 days and the parities of females ≤ 5 parities or > 5 parities. A one way ANOVA with fixed effects statistical analysis were used. The post hoc comparisons were done by LSD test. All calculations were made with the statistical package, StatSoft® STATISTICA, Tulsa, OK).

RESULTS AND DISCUSSION

The mean conception rate and farrowing rate have a nonsignificant tendency for improving in the test group – 84,27% and 83,15% in comparison with control group – 79,07% and 76,74% respectively (Table 1).

Table 1. Reproductive performance in sows after postcervical insemination with Predil MR-A® (mean±SD)

Parameters	Control group	Test group
Number of sows	85	88
Conception rate, %	79.07	84.27
Farrowing rate, %	76.74	83.15
Total born piglets, nrs.	11.83±3,61	10.79±3,39
Live born piglets, nrs.	10.75±3,18	10.07±3,05
Farrowing index	824.95	837.32

Not significant differences between groups.

In contrast for the litter size parameters as total born piglets and live born piglets there have a nonsignificant tendency for the better results in control group – 11,83/10,75 nrs. and 10,79/10,07 nrs. piglets. In generally the farrowing index value is the more in test group with 12,37. Table 2 indicates the reproductive data according to the weaning-to-estrus interval. The using of synthetic seminal plasma Predil MR-A® did not significant effect on the reproductive parameters between groups depending weaning-to-estrus interval.

Table 2. Reproductive performance in AI sows, in relation with weaning-to- estrus interval, WEI (mean±SD)

Parameters	WEI interval ≤6 days		WEI interval > 6 days	
	control group	test group	control group	test group
Number of sows	46	76	39	12
WEI, days	4.54±0.75	4.28±1.89	8.07±1.16	7.83±0.93
Conception rate, %	80.43	85.71	79,48	75.00
Farrowing rate, %	78.26	84.42	76.92	75.00
Total born piglets, nrs.	11.55±3.47	11.00±3.13	12.13±3.86	10.44±3.39
Live born piglets, nrs.	10.66±3.32	10.09±3.05	10.58±3.08	9.77±3.03
Farrowing index	834.25	85179	813/81	732.75

Not significant differences between groups.

Although not significant differences, sows cycling within up to 6 days after weaning and inseminated with synthetic seminal plasma had greater conception rate and farrowing rate (85,71% and 84,42%) when compared with control group – sows inseminated without synthetic seminal plasma up to ≤6 days and >6 days too. The lowest data

were obtained in animals inseminated with synthetic seminal plasma after 6 days of weaning. In generally, the better reproductive results are in the sows cycling up to 6 days (control and test group).

Table 3 indicates the reproductive performance of sows inseminated with synthetic seminal plasma Predil MR-A® depending of the parity of the animals.

Table 3. Reproductive performance in AI sows, in relation with farrowing parity (mean±SD)

Parameters	≤ 5 parity		> 5 parity	
	control group	test group	control group	test group
Number of sows	67	64	18	24
Parity, nrs.	3.25±1.07	3.40±1.10	6.26±0.45	6.84±0.85
Conception rate, %	79.10	82.81	77.78	87.50
Farrowing sows, %	77.61	82.81	77.78	83.33
Total born piglets, nrs.	11.63±3.95A	10.54±3.27 AB	12.53±2.03B	11.36±3.60
Live born piglets, nrs.	10.62±3.59 A	956±3.05 A	10.80±1.42	10.77±3.53
Farrowing index	824.21	791.66	840.02	897.46

[AB] Within a row, means with a common superscript differ ($p<0,01$).

The sows in test group inseminated with synthetic seminal plasma had a higher value of conception rate and farrowing rate – 82,81% and 82,81% (≤5 parity), and 87,50% and 83,33% (>5 parity) respectively. In contrast regarding piglets litter parameters (total born piglets and live born piglets) the sows in control group had a significant higher results. The intensive studies for application of postcervical insemination technique in sows show that the reduction of semen dose do not decrease the reproductive parameters. Depending of catheter model, semen dose and the experience of the technician were obtained various results – 88,70-92,60% conception rate and 10,80-10,90 nrs. live piglets (Watson and Behan, 2002), 83,30-95,16 % fecundity and 9,77-11,61 nrs. live born piglets (Levis, Burroughs and Williams, 2000), 87,80 farrowing rate and 10,20 nrs. alive born piglets (Roberts and Billkei, 2005). Normally this method is recommended in animals after first parity. In the gilts this manner of insemination is a problem of passing the inner cannula of the catheter trough the cervix and the low reproductive performance.

The idea of present study was to check how can to improve the reproductive efficiency of the postcervical insemination technique. The one of the main problems of artificial insemination of sows is that after dilution of boar semen to reduce some natural physiological stimulus in seminal plasma (Garcia Ruvalcaba et al., 1997; Srisuwan, 2001; Ramirez Ovalle, 2002; Rekiel and Sujka, 2007). The results of this experiment show that two-phase postcervical insemination with pre-treatment with synthetic seminal plasma Predil MR-A® have a positive effect of fecundity and farrowing rate. Simillar tendency was find in our previous study (Garcia Ruvalcaba et al., 2009). The effect of using of synthetic seminal plasma Predil MR-A® is by increment of sperm concentration in the oviduct isthmus brought by dilution effect in the cervix and due to uterine contractions improvement, on the other hand there is also an important effect in spermatozoa motility thus spermatozoa transport into uterus.

In present study the positive effect of postcervical insemination with in combination with synthetic seminal plasma was find in the weaning-to-oestrus interval. The

best conception rate and farrowing rate were find in animals inseminated within 6 days after weaning. The same positive effect of the combination of synthetic seminal plasma was find in the sow parity category. The better results (conception rate and farrowing rate) were obtained in test group. The significant better results in control group (postcervical insemination without synthetic seminal plasma) were obtained regarding piglets parameters.

CONCLUSION

The two-phase postcervical insemination with in combination of synthetic seminal plasma Predil MR-A® improved reproductive results in sows.

The usage of synthetic seminal plasma Predil MR-A® by mean two phase insemination method is a recommended technique to improve the reproductive results obtained trough postcervical insemination in pig farms.

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POSTCERVIKALNO VEŠTAČKO OSEMENJAVANJE KRMAČA U KOMBINACIJI SA SINTETIČKOM SEMENOM PLAZMOM (PREDIL MR-A®)

STANIMIR DIMITROV

Izvod

Veštačko osemenjavanje se koristi kao nezaobilazni metod reprodukcije na farmama svinja širom sveta. Tehnologija postcervikalne inseminacije se koristi kao metod daljeg unapređenja reproduktivne efikasnosti i genetskog unapređenja proizvodnje svinja. Strategija povećanja vrednosti prašenja i veličine legal, uključuje dodavanje supstanci za stimulaciju kontrakcija uterusa, u inseminacione doze ili injekciju ovih materija krmači pre osemenjavanja. Cilj ovog rada je da se ispita uticaj dodavanja sintetičke semene plazme Predil MR-A®, u inseminacione doze, kao metoda povećanja efikasnosti postcervikalne inseminacije. Postcervikalna inseminacija je izvedena inseminacionim dozama sa *1.5 x 10^{9} spermatozoida,pri čemu je dozama za osemenjavanje ogledne grupe krmača, dodato 20 ml* sintetičke semene plazme Predil MR-A®. Postcervikalno osemenjavanje dozama dopunjenim sa sintetičkom semenom plazmom, rezultiralo je sa 84.27% concepcije, 83.15% prašenja, 10.79 ukupno rođene I 10.07 živo rođene prasadi po čeglu.

Ključne reči: veštačko osemenjavanje, sintetička semena plazma Predil MR-A, krmača.

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AESTHETIC VALUE OF SMEDEREVO FORTRESS EXPRESSED THROUGHOUT PARAMETERS OF BIOECOLOGICAL ANALYSES

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SUMMARY: Smederevo fortress is a fortified medieval town, an area of 10,5 ha. As a city park, belongs to a category of green space of general purpose. Bioecological analysis had the aim to highlight the important parameters for assessing the aesthetic values of the area. Cluster analysis was applied to determine the relatively homogenous groups of tree species. The results showed that the current state of greenery composition is not at a high level. Further improvement of the space should be directed towards highlighting a monumentality of vegetation, in order to justify the space and its history.

Key words: *Smederevo fortress, aesthetic values of the greenery, bioecological parametres.*

INTRODUCTION

Green spaces (gardens, parks and other landscape formations) are important elements of each city. As a part of the city image and its surroundings, they expand ecological diversity and essentially make cities and urban areas more suitable for living. The urban forests are green areas, made up of trees and other vegetation within the urban environment (Peschardt et al., 2012; McPherson et al., 2005). They have an important place in the biodiversity of the cities, as a segment of urban greenery, but their vegetation is affected by a number of anthropogenic stressors (LaPaix and Freedman, 2010). With the aim of increasing the functionality of urban greenery, it is necessary to assess their state, which is an initial step towards the rehabilitation and improvement of plant health and visual appearances (Stavretović et al., 2010). Urban green spaces, and urban forests, as the areas of multiple sociological functions (Ranković and Keča, 2007), are directly used for active or passive recreation of the population, and their presence, indirectly affect the

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quality of the urban environment awareness (Hadžidervišagić, 2011). In the city, plants and "green space" are considered to be psychologically and socially, as well as environmentally beneficial. They give people a "contact with nature" considered both calming and restorative; they encourage people to spend time in public spaces, thus enhancing community safety, and they produce various well-known positive environmental effects (Despard, 2012). Many existing urban green spaces in the city of Smederevo slowly losing its previous sociological value. Smederevo fortress, as the central city park as well as cultural-historical resource of great value, is one of the few green areas in the city, which is still active in this field. However, the degradation of its landscape composition, as well as aesthetic and decorative values of dendroflora, will significantly reduce a sociological value of the space.

Smederevo fortress lies on the right bank of the river Danube The Danube region is on of the most interesting regions in Europe in geographical and historical viewpoint (Kovács, 2011). The numerous sites of Roman and medieval periods on its shores are just witnesses of this state. The largest Serbian medieval town, Smederevo, was built in the third decade of the 15th century. With a triangular shape, 25 towers, walls with height over 10 m and width around 4.5 meters, the fortress of Smederevo covers an area of nearly 10,5 hectares. The fortress is surrounded by water on two sides while the third side is faced towards land. It is constituted from the Big and the Small Town. The entire town was built of stones of various composure (Stojsavljević et al., 2011).

A Special importance Smederevo got early in the fifteenth century, at the time of despot Đurađ Branković, when the city became the last capital of the Serbian medieval time and the seat of ecclesiastical and economic life. At that time the importance of Smederevo exceeded local and regional dimension and Smederevo became the center of the crossroads of civilizations, the center of overlapping influences of the East and the West (Petronijević, 2006).

MATERIAL AND METHODS

The results obtained in this study were analyzed by using several methods. First of all concerned research had the approach with qualitative type and included the study of accessible literature sources, namely the available documentation, which served as a source of general information about the historical development of the park.

In a further phase of this research bioecological analysis of vegetation was applied, actually bioecological analysis of dendrofond of the researched area of Smederevo fortress. As an indicators of biological and ecological values according to Anastasijević (2000) the following characteristics of trees species were measured: tree height (m), tree height to first branch (cm), trunk diameter (cm) and width of a canopy (cm). Height of trees was measured with Vertex laser altimeter. Standard diameter was used for determination of trunk diameter at a height of 1,30 m. At the end with the aim of overall estimation of dendrofond of Smederevo fortress, the values of vitality and decorativeness were given from 1-5 (1 as the lowest and 5 as the best grade).

In order to organize obtained data, their interpretation and the relevant evaluation, data were analyzed by using the statistical software Statistica10 (StatSoft , OK, USA).

RESULTS AND DISCUSSION

Within 27 plant species and among 27 genotypes, located in Smederevo fortress there are 123 individual adult trees. Considering its surface spreadness, the composition of space and vegetation is very monotonous and modesty. Within total number of adult trees, deciduous have a dominance, while there are only two individuals of coniferous species *Juglans regia* L. and *Picea pungens* Engelm. The most numerous individuals are: *Platanus acerifolia* (Ait.) Wild. witht 32 individuals (26%), *Populus nigra* L.*'Italica'*/ Mnch./Du Roi with 20 individuals (16%), then *Fraxinus excelsior* L. with 11 individuals (9%), followed by *Acer saccharinum* L. with 10 individuals (8%), *Fraxinus angustifolia* Vahl with 7 individuals (6%), *Morus alba* L. *'Pendula'*/Dipp. with 4 individuals (4%) and *Celtis occidentalis* L. also with 4 individuals (4%). Other plant species are represented mostly in identical percentage rating with less than 4%.

In the area of Smederevo fortress there are eight plant species that are present only with one individual. Some of those species are: *Morus alba* L., *Betula pendula* Ehrh., *Broussonetia papyrifera* L'Her ex Vent., *Picea pungens* Engelm, *Acer negundo* L., *Tilia parvifolia* Ehrh, *Junglas regia* L., *Populus tremula* L. It is a fact that their participation in total vegetation composition can't make a significant contribution to the overall value of aesthetic quality of the space.

Vegetation composition of the entire area is dominantly created with tree lined planting, also partly in groups, and what is more, there are a lot of solitary species spread through whole area. This makes whole space composition very chaotic and without clear idea. Table 1 shows the studied parameters of the bioecological analysis. The species emphasized by the highest mean values for height are: *Populus x euroamericana* (Dode) Guinier (22,50 m), *Populus alba* L.(21,50m), *Quercus robur* L.(21m), *Populus nigra* L.*'Italica'*/ Mnch./Du Roi (19,35m), *Platanus occidentalis* L. (18,50m). Plant with the lowest height is *Picea pungens* Engelm. with mean value of 2 m. Comparing to other plant species *Picea pungens* Engelm. has the narrowest canopy as well (1 m), which is in accordance to it hight value. None of the present plant species has not yet reached its full height, even the existing environmental conditions are positive for them. This can be explained by not reaching their full age, also by negative impact that comes from a poor maintaining. Considering the fact that individuals of the following plant species *Platanus occidentalis* L., *Catalpa bignonioides* Walt., *Acer pseudoplatanus* L., *Acer platanoides* L., *Quercus robur* L., *Populus alba* L., *Populus x euroamericana* (Dode) Guinier, *Fraxinus ornus* L., are found in the space in a small groups (2-3 individuals), they can be consider almost as a solitary species. This is probably a reason why they were highlighted as a species with the high mean values of trunk height (3,50m, 3,10m, 4m, 4m, 4,75m, 3m), *Populus x euroamericana* (Dode) Guinier and *Fraxinus ornus* L. are out of this group considering the values (2,25m, 2,75m). In contrast to them are folowing plant species: *Populus nigra* L.*'Italica'*/ Mnch./Du Roi (1,29m), *Morus alba* L. *'Pendula'*/Dipp. (1,56m) and Celtis occidentalis L. (1,97m). This low value of *Populus nigra* L.*'Italica'*/ Mnch./Du Roi can be explained by its age and with the growth of adventitious branches on the tree.

The widest canopy (which again may be explained by their position in small groups) have the following plant species: *Populus alba* L. (19,50m), *Quercus robur* L. (18m), *Morus alba* L. (17m), *Populus x euroamericana* (Dode) Guinier (16,50m), while the species with the lowest canopy are: *Picea pungens* Engelm. (1 m), *Morus alba* L.

'Pendula'/Dipp. (2,46m), *Juglans regia* L. (2,50m) and *Populus nigra* L.*'Italica'*/ Mnch./ Du Roi (4,39m). Since this species do not reach a big width, it can be concluded that they were adapted to existing environmental conditions.

Table 1. Pattern of testified parameters of bioecological analyses of dendrofond of Smederevo fortress

Tree species	N	Height (cm)			Trunk height (cm)			Trunk Diameter (cm)			Width of the canopy (cm)			Value of the vitality (VIT)			Value of the decorativeness (DEK)		
		Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Morus alba 'Pendula'	5	2.30	0.27	11.91	1.56	0.13	8.60	22.20	2.59	11.66	2.46	0.09	3.64	2.80	0.45	15.97	3.00	0.00	0.00
Platanus occidentalis	2	18.50	3.54	19.11	3.50	2.12	60.61	56.50	23.33	41.30	15.50	6.36	41.06	2.50	2.12	84.85	3.50	2.12	60.61
Fraxinus angustifoila	7	15.57	3.60	23.11	2.29	0.39	17.21	47.71	6.10	12.79	12.93	4.15	32.08	3.29	0.49	14.85	3.29	0.49	14.85
Catalpa bignonioides	3	13.00	3.50	26.92	3.10	0.53	17.07	29.33	4.04	13.78	8.03	2.52	30.20	2.33	0.58	24.74	3.00	0.00	0.00
Celtis occidentalis	5	10.25	1.67	16.25	1.93	1.11	57.38	42.17	10.94	25.95	8.83	2.58	29.23	3.00	0.00	0.00	3.17	0.41	12.89
Populus nigra 'Italica'	20	19.35	1.57	8.13	1.29	0.75	58.65	46.20	9.13	19.77	4.39	3.24	73.89	2.40	0.50	20.94	2.95	0.39	13.36
Acer pseudoplatanus	2	13.00	0.00	0.00	4.00	0.00	0.00	39.00	4.24	10.88	11.50	2.12	18.45	2.50	0.70	1.41	3.00	28.28	47.14
Acer platanoides	3	12.50	0.86	6.93	4.00	0.00	0.00	36.67	12.01	32.77	10.67	2.08	19.51	3.00	0.00	0.00	3.33	0.58	17.32
Fraxinus lanceolata	4	14.13	1.75	12.39	3.27	0.74	22.49	31.25	9.47	30.29	8.50	1.73	20.38	2.50	0.58	23.09	2.75	0.56	18.18
Fraxinus excelsior	11	14.77	1.13	7.62	3.14	0.46	14.70	33.82	6.66	19.70	9.00	1.95	21.66	2.27	0.47	20.55	2.64	0.50	19.14
Platanus acerifolia	32	17.89	3.39	18.94	2.66	1.14	42.80	44.84	17.28	38.54	11.94	2.22	18.60	2.87	0.42	14.89	2.90	0.54	18.56
Quercus robur	2	21.00	1.41	6.73	4.75	2.47	52.10	52.50	10.61	20.20	18.00	9.90	55.00	3.50	0.71	20.20	3.50	0.71	20.20
Populus alba	2	21.50	2.12	9.87	3.00	1.41	47.14	80.00	28.28	35.36	19.50	0.70	3.63	4.00	0.00	0.00	5.00	0.00	0.00
Acer saccharinum	10	16.20	1.23	7.59	2.20	0.95	43.12	54.50	12.79	23.45	9.75	1.99	20.41	2.60	0.70	26.89	2.70	0.82	30.49
Populus x euroamericana	2	22.50	0.70	3.14	2.25	0.35	15.71	50.00	7.07	14.14	16.50	2.12	12.86	2.00	0.00	0.00	3.00	0.00	0.00
Fraxinus ornus	2	15.00	4.24	28.28	2.75	0.35	12.86	42.50	24.75	58.23	12.00	4.24	35.36	2.50	0.71	28.28	2.50	0.71	28.28
Morus alba	1	18.00	–	–	2.50	–	–	50.00	–	–	17.00	–	–	3.00	–	–	4.00	–	–
Betula pendula	1	14.00	–	–	1.50	–	–	30.00	–	–	9.00	–	–	4.00	–	–	4.00	–	–
Broussonetia papyrifera	1	13.00	–	–	2.00	–	–	49.00	–	–	13.00	–	–	4.00	–	–	5.00	–	–
Picea pungens	1	2.00	–	–	0.80	–	–	5.00	–	–	1.00	–	–	2.00	–	–	2.00	–	–
Acer negundo	1	13.00	–	–	1.50	–	–	40.00	–	–	12.00	–	–	2.00	–	–	2.00	–	–
Salyx babylonica	1	13.50	–	–	2.50	–	–	50.00	–	–	12.00	–	–	4.00	–	–	4.00	–	–
Salyx alba 'pendula'	1	13.00	–	–	2.00	–	–	60.00	–	–	12.00	–	–	3.00	–	–	3.00	–	–
Junglas regia	1	16.00	–	–	3.00	–	–	50.00	–	–	14.00	–	–	3.00	–	–	3.00	–	–
Populus tremula	1	4.00	–	–	1.50	–	–	10.00	–	–	2.50	–	–	5.00	–	–	3.00	–	–
Tilia parvifolia	1	15.00	–	–	2.25	–	–	25.00	–	–	10.00	–	–	3.00	–	–	3.00	–	–
Acer obtusatum	1	13.00	–	–	5.00	–	–	30.00	–	–	13.00	–	–	3.00	–	–	4.00	–	–

Over 50% of total analyzed existing plant species, in Smederevo fortress, have received a low grade of vitality and decorativeness (up to 3). As the most prominent vital and decorative species were found: *Populus alba* L. (VIT= 4, DEK=5), *Salyx babilonica* L. (VIT=4, DEK=4), *Betula pendula* Ehrh. (VIT=4, DEK=4), *Quercus robur* L. (VIT= 3,5, DEK=3,5). The lowest grades for the vitality received *Populus x euroamericana* (Dode) Guinier (2), *Picea pungens* Engelm. (2), *Acer negundo* L. (2), while the lowest grade for the value of decorativeness was given to *Fraxinus ornus* L.
(2,50), *Picea pungens* Engelm. (2) and *Acer negundo* L. (2). Such low values can be explained with the species unadaptible to the natural environmental conditions.

Cluster analysis grouped the researched species into subclusters (Figure 1). Hierarchical classification of 25 tree species was divided into 10 groups of subclusters. Groups are composed of the following species:

1) Populus alba L.
2) Celtis occidentalis L.
3) Salyx babilonica L., Acer saccharinum L., Populus nigra L. 'Italica'/ Mnch./Du Roi
4) Acer obtusatum Wldst. et Kit, Tilia parvifolia L., Betula pendula Ehrh.
5) Fraxinus lanceolata Borkh., Catalpa bignonioides Walt.
6) Populus x euroamericana (Dode) Guinier, Quercus robur L.
7) Morus alba L.
8) Fraxinus excelsior L., Platanus acerifolia (Ait.) Willd., Fraxinus ornus L., Acer negundo L., Acer platanoides L., Acer pseudoplatanus L., Broussonetia papyfera L'Her. ex Vent., Juglans regia L., Fraxinus angustifolia Vahl
9) Platanus occidentalis L.
10) Picea pungens Engelm., Populus tremula L., Morus alba L. 'Pendula'/ Dipp.

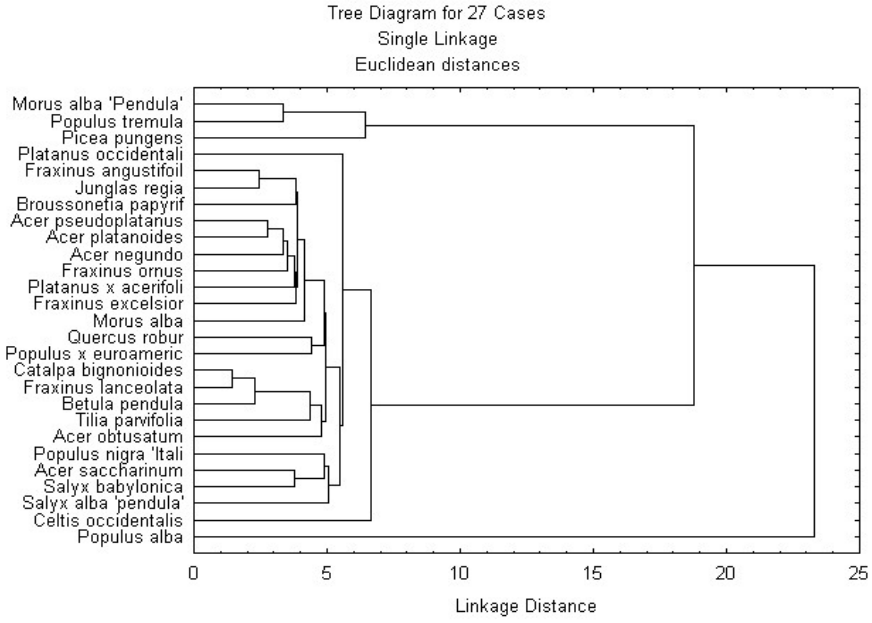

Fig. 1. Cluster analysis of 27 adult tree species

White poplar (*Populus alba* L.) is a very effective tree for monumental compositions, for big parks and park forests on alluvial terrain. It can be planted in groups, small forests mixed with red oak, black poplar, black alder or other types with darker foliage (Vukićević, 2006). What is more white poplar is suitable as a soliter because of its broad and bright canopy (Ocokoljić and Ninić-Todorović, 2003). In the area of Smederevo fortress, trees of this kind are extremely well adapted, and the reason for that are certainly appropriate and natural conditions. White poplar has good grades for all parameters of bioecological analysis and as such is highlighted in a separate subcluster.

In the second group of sublusters is *Celtis occidentalis* L. as a tree species with very high value of trunk diameter (42,17) and very low value of trunk height (1,93).

The third group consists of Weeping Willow (*Salyx babilonica* L.), Silverleaf Maple (*Acer saccharinum* L.) and Lombardy Black Poplar (*Populus nigra* L.*'Italica'*/Mnch./Du Roi); the species that were emphasized to its values of vitality and decorativness. Not so far in the past, the individuals of Weeping Willow were much more numerous in Smederevo fortress, and with its decorativness had a lot of influence to the aesthetic value of the space. Since, they were directly exposed to gust of wind, branches were broken and many individuals had to be removed. Considering a Black Poplar species, due to their strong root system, this type is very suitable for binding a river banks, also very some of its clones showed a good results (*Populus x euramericana* Dode (Guinier) and *Populus deltoides W.Bartram* ex Humphry Marshall) (Blagojević et al., 2011). This data can be used in order to avoid a formation of monoculture in the field. So, besides individuals of Lombardy Black Poplar trees (*Populus nigra* L.*'Italica'*/Mnch./Du Roi), as a variety of Black Poplar, also in the vegetative composition it can be introduced some of its clones, in order to avoid the danger of monoculture.

Bosnian maple (*Acer obtusatum* Wldst. et Kit), Small-leaved Lime (*Tilia parvifolia* L.) and Birch (*Betula pendula* Ehrh.) were distinguished in the fourth group on the basis of average values of parametres of the bioecological analysis. What is more these three species are emphasized in the researched area as the individuals.

Green ash *(Fraxinus lanceolata* Borkh.) and Southern Catalpa (*Catalpa bignonioides* Walt.) are species separated in the fifth group of the subclusters. Southern Catalpa is a species that belongs to a tropical family, but has been introduced in many countries as ornamental. Although this plant is consumed by indigenous cultures of South America for medical uses (Muñoz-Mingarro et al., 2003). The individuals of Green ash also possess the aesthetic characteristics desired of urban trees, such as autumn color, attractive bark and flowers (Pericval et al., 2006). All the above facts are the reason of their frequent occurrence in the green areas.

As part of the sixth group, Euroamarican Poplar (*Populus x euroamericana* (Dode) Guinier) and English oak (*Quercus robur* L.) were distinguished by their height and values of decorativnesses. The species are grouped by big number and poor aesthetic values, what indicates a low values of decorativnesses. White mulberry (*Morus alba* L.), as the species that stands as the individual in the space has a high values of all parameters and as such distinguishes from the other individuals in the seventh subcluster.

In the eighth group of subclusters there were found the species that can be found in the space in a group planting and as individuals. The species that stand in the groups (*Fraxinus excelsior* L., *Platanus acerifolia* (Ait.) Willd., *Fraxinus ornus* L., *Acer platanoides* L., *Acer pseudoplatanus* L., *Fraxinus angustifolia* Vahl) have proven to be very weak and with poor values of decorativness and vitality. Although the natural en-

vironment conditions are good for them, weak measures of maintaining and bad spatial distribution caused the lower values of researched parameters of bioecological analysis. The species that are planted in groups and they are very suitable for greenery. On the other hand, Norway Maple, as the most numerous species in Smederevo fortress is located separately within subcluster of the sixth group. The species is highly resistant to dust and fumes in the air and as Vukićević (1996) considered this species is one of the most adaptable species to urban conditions. The species that stand as the individuals (*Broussonetia papyfera* L'Her. ex Vent., *Juglans regia* L.) have high value of of decorativness and vitality. What is common for all species in this subcluster is the high values of trunk diameter, ranking above 30 to 50.

In the ninth group is American Sycamore (*Platanus occidentalis* L.), which distinguish itself by its height and good assessment of vitality. It has successful growth on alluvial terrain (Vukićević, 1996). In the parks is rare species, so it's not surprising its small number in the Smederevo fortress.

And in the last group of sublusters following species were located: Eurasian aspen (*Populus tremula* L.), Blue Spruce (*Picea pungens* Engelm.) and White mulberry (*Morus alba* L.*'Pendula'* Dipp.) All three species have similar values for evaluated parameters of bioecological analysis. According to the vitality Eurasian aspen is dominated, but number of its individuals is negligible (only one individual). The reason for this is that *Populus tremula* L. is not long-living species, and as Vukićević (1996) points out adult trees often suffer from rotten core.

CONCLUSION

Smederevo fortress, as a significant cultural and historical property, monument of culture, stated under third degree of protection, also is a valuable tourist destination and an important element of urban core of the city of Smederevo. The special contribution to its representativeness and significance is the wealth of its dendroflora. Species found in the Smederevo fortress are mostly indigenous or allochthonous adaptive to natural environmental conditions, to alluvial habitat, high humidity and moderate climate. But values of parameters of bioecological analysis were not at a high level, especially grades of vitality and decorativeness. The reason for this is a poor measure of maintaining applicable at the area. It is a common that due to poor health of species, any kind of care and maintaining is refused. When a species come to a stage of extinction, they have been removed, which makes dendrofond poorer and creates an image of a space that certainly is not appropriate for monument of this kind. Also, it was difficult to speak objectively about the examined parameters, because all species are not equally presented (in the same percentage).

It is unrealistic to talk about the relationship between deciduous and coniferous species and their impact to the aesthetic value of the space, because there is absolute dominance of deciduous species (coniferous presence is only in two individuals). The reason for this may be a better adaptability of deciduous species in the natural environmental conditions of the researched area.

Overall aesthetic and decorativeness value of the area was rated very poor. Mean value of vitality and decorativeness is the same it reaches 3. This emphasize that the species are very damaged, but with intensive care, ttheir vitality can be recovered. In the terms of decorativenesses of overall composition, it can be concluded that the trees,

at this stage of the current situation, have not emphasized aesthetic values. This can be supported with a fact that only a regular maintenance of dendrofond's health of Smederevo fortress, can provide a good aesthetic value and species longevity.

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ESTETSKA VREDNOST SMEDEREVSKE TVRĐAVE ISKAZANA KROZ PARAMETRE BIOEKOLOŠKE ANALIZE

IVANA BLAGOJEVIĆ, ANA GAČIĆ, JELENA ČUKANOVIĆ,
EMINA MLADENOVIĆ

Izvod

Smederevska tvrđava je utvrđen srednjevekovni grad, površine oko 10,5 ha. Kao centralni gradski park, pripada kategoriji zelenih površina opšte namene. Bioekološka analiza imala je za cilj isticanje parametra važnih za ocenu estetske vrednosti prostora. Klaster analiza je primenjena u cilju determinacije relativno homogenih grupa drveća. Rezultati su pokazali da trenutno stanje biljnog fonda nije na zavidnom nivou. Dalja unapređenja prostora treba da budu usmerena u pravcu isticanja monumentalnosti biljnog fonda, kako bi se i istorija prostora opravdala.

Ključne reči: Smederevska tvrđava, estetska vrednost zelenila, bioekološki parametri.

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YIELD AND WATER USE EFFICIENCY OF IRRIGATED SUDAN GRASS (*SORGHUM SUDANENSE* L.*)* IN THE CLIMATE CONDITIONS OF VOJVODINA

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SUMMARY: In order to study the effectiveness of irrigation on Sudan grass fresh forage yield an irrigation water use efficiency (I_{wue}) and evapotranspiration water use efficiency (ET_{wue}) were determined. A field experiment was conducted on the calcareous chernozem soil in the Vojvodina region, a northern part of the Serbia Republic, during 2005 and 2006 growing season. The experiment was arranged in a randomized complete block design and adapted to conditions of sprinkling irrigation. The trial included irrigated treatment (I - 60-65% of field water capacity - FWC) and a treatment without irrigation (I_o). On average, irrigation did not significantly affect the fresh forage yield of Sudan grass (108.893 t ha^{-1} - 103.314 t ha^{-1}) as the study period had precipitation higher than the long-term seasonal average. Evapotranspiration water use efficiency of Sudan grass, in irrigation conditions (ET_m I_{wue}) ranged from 19.0 to 20.9 kg m^{-3} with an average value of 20.0 kg m^{-3}, while evapotranspiration water use efficiency in conditions without irrigation (ET_a I_{wue}) varied from 18.1 to 25.4 kg m^{-3} with an average value of 21.8 kg m^{-3}. Irrigation water use efficiency (I_{wue}) varied from 2.8 to 3.7 kg m^{-3} with an average value of 3.2 kg m^{-3}. Effect of irrigation on yield of Sudan grass and results of both ET_{wue} and I_{wue} which were similar to those obtained from the literature indicate that irrigation schedule of Sudan grass in the study period was properly adapted to plant water requirements and water-physical soil properties. Determined values of ET_{wue} and I_{wue} could be used for the planning, design and operation of irrigation systems, as well as for improving the production technology of Sudan grass in the region.

Key words: *irrigation, yield, Sudan grass (Sorghum sudanense L.), water use efficiency (WUE).*

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INTRODUCTION

Regarding the intended intensification of livestock production, solutions should be sought in high-yielding, high-quality crops which fit a system of continual feed production (relay cropping). Diversity of use, favorable biological characteristics, relatively modest requirements, high and stable yield and high quality of biomass make Sudan grass an important facet in resolving the problem of rough forage (Ćupina et al., 2002). Being an annual forage crop, Sudan grass is typically used for grazing, production of fresh forage and ensiling. It may be planted at different time interval (main crop, catch crop, double crop), it has a high regrowth rate and it may annually produce two-three cuts. In favorable years, i.e., with adequate water supply, high yields of fresh forage (60-70 t ha^{-1}) may be achieved with minimal cultivation practices (Erić et al., 1995; Erić et al., 2004). In practice, however, yields frequently oscillate on dependence of weather conditions, primarily the amounts and distribution of rainfall. In irrigation, an economic irrigation schedule consistently provides high yields of fresh forage exceeding 100 t ha^{-1} (Pejić i sar 2005).

The importance of analyzing evapotranspiration water use efficiency (ET_{wue}) is illustrated by the efforts of numerous studies that consider the total water use for evapotranspiration towards transpiration use as to the productive part of water to plants (Wallace and Batchelor, 1977; Howell et al., 1990). The parameter ET_{wue} mostly depends on precipitation amount and distribution and establishes whether the growing period is favorable for plant production or not. Irrigation schedules and applied management practices in relation to obtained yields of growing plants substantially influences this coefficient. Wang et al. (1996) pointed out that crop yield depends on the rate of water use and that the factors that increase yield and decrease water used for ET favorably affect the water use efficiency. Howell (2001) indicated that ETwue generally is highest with less irrigation, implying full use of the applied water and perhaps a tendency to promote deeper soil water extraction to make better use of both the stored soil water and the growing-season precipitation. The irrigation water use efficiency (I_{wue}) provides a more realistic assessment of the irrigation effectiveness as many management factors such as fertility, variety, pest management, sowing date, soil water content at planting, planting density and row spacing could affect yield substantially between irrigated and dryland agriculture. The parameter, I_{wue} generally tends to increase with a decline in irrigation if that water deficit does not occur at a single growth period (Howell, 2001).

The main objectives of this study were to: (1) assess the effectiveness of irrigation on Sudan grass fresh forage yield using irrigation water use efficiency (I_{wue}) and evapotranspiration water use efficiency (ET_{wue}) coefficients, (2) and to compare determined values with those obtained from past studies of different climatic conditions, particularly to assist in developing strategies for improved production technology of Sudan grass in the Vojvodina and similar regions.

MATERIALS AND METHODS

The experiments were conducted at Rimski Šančevi, an experimental station of the Institute of Field and Vegetable Crops in Novi Sad (N 45° 19`, E 19° 50`, elev. 84 m) on the chernozem soil of the loess terrace during 2005-2006. The soil of the experiment site is as a highly calcareous loam (Table 1). Structural stability to 0.6 m is good, with

60-71% of soil aggregates larger than 0.25 mm being persistent in water (Pejić et al., 2005). Concerning the physical and water properties (Table 1) this soil is quite suitable for any crop and irrigation system (Živković et al., 1972).

Table 1. Physical and water properties of the soil at the experimental site

Depth (cm)	Textural status (%)			Bulk density (kg m^{-3})	Total porosity (vol.%)	Air porosity (vol.%)	Field water capacity (weight %) (mas.%) 33 kPa	Wilting point (weight %) (mas.%) 1500 kPa	Total available soil water (mm)
	Sand	Silt	Clay						
0-30	34	48	18	1270	54.9	21.9	26.0	10.9	57.5
30-60	29	44	27	1310	48.8	14.1	26.5	11.2	60.0

The experiment was conducted in a system of random blocks and adapted to technical specifications of the sprinkler irrigation system. The criteria used for irrigation in the field experiment included application of water when soil moisture was at 60-65% of field capacity (FC) i.e., irrigation was applied when about half of available water in the soil layer to 60 cm was depleted (Pejić, et al., 2005). The non-irrigated plot was used as control. Irrigation was scheduled by monitoring soil moisture levels at 10 cm intervals down to 60 cm depth. This was estimated by using a gravimetric method at about 10 day intervals depending upon the weather conditions. Maximum evapotranspiration (ET_m) of Sudan grass during growing season was calculated using the bioclimatic method (1) that employs hydrophytothermic index (K) with the value of 0.19 taken from Pejic et al., (2006). After determining the ET_m value the actual evapotranspiration (ET_a) was calculated on the basis of precipitation data and pre-vegetation soil water reserve.

$$ET_m = K\ T \quad (1)$$

Where:

ET_m = monthly maximum evapotranspiration for Sudan grass (mm)

K = hydrophytothermic index for Sudan grass

T = sum of mean daily air temperatures in a given month (°C)

Evapotranspiration water use efficiency (ET_{WUE}) and Irrigation water use efficiency (I_{WUE}) were estimated as Bos (1980 and 1985):

$$ET_{wue} = Y_{irr} \text{ or } Y_{dry}/ET_m \text{ or } ET_a \quad (2)$$

$$I_{wue} = Y_{irr} - Y_{dry}/W_{irr} \quad (3)$$

Where:

Y_{irr} = the yield and ETm for irrigation level

Y_{dry} = the yield and ETa for dryland or rainfed plot

W_{irr} = the amount of water applied by irrigation

The experimental object was the Sudan grass variety NS Srem. Planting in 12.5 cm rows was performed with a portable planter in the second half of April. The seeding rate was 30 kg ha^{-1}. The size of the experiment unit was 12 m^2 (12 x 1 m) replicated four times. The cutting was done when the plants height was about 90 cm, and fresh forage yield (Y) was calculated by t ha^{-1}. The experimental Sudan grass plots received conventional growing technology adjusted to the conditions of irrigation. Statistical processing of data was done by the analysis of variance (ANOVA) and testing the obtained results

by the Fisher's LSD test (P< 0.05 levels between the means).

Precipitation (P) and temperature (T) data were obtained from Rimski Šančevi Meteorological Station (Table 2). To characterize the climate of the experiment area, data gathered by a meteorological station at Rimski Šančevi in a 43-year period (1964-2007) were used. The climate is moderate, with four marked seasons. The mean annual precipitation is 609 mm (361 mm or 59% in the growing season, April - September) and the mean air temperature is 11.2°C (17,7°C in the growing season). Variability of meteorological conditions from one year to another is characteristic for the climate of the Vojvodina Province. This particularly concerns the rainfall, which varies in both, amounts and distribution.

Seasonal precipitation in 2005 and 2006 were 451 and 419 respectively, and therefore the study period had precipitation higher than the long-term seasonal average (1964/2007 – 361 mm) (Table 2).

Table 2. Mean monthly air temperatures and monthly precipitation sum during Sudan grass growing season (Rimski Šančevi)

Year	Month												Seasonal average	
	April		May		June		July		August		September			
	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm
2005	12.7	20.9	17.0	38.1	19.3	135.4	21.3	122.5	18.8	133.9	21.5	0.2	18.4	451.0
2006	15.0	10.3	16.6	70.1	19.7	104.3	23.5	30.9	19.7	124.9	14.6	79.0	18.2	419.5
1964/ 2007	11.2	47	16.6	59	19.6	85	21.3	70	20.7	59	16.8	41	17.7	361.0

Given data indicate that climatic patterns in Vojvodina are changeable and longer-term predictions of precipitation are not possible. That confirms the supplementary character of irrigation in Vojvodina, (Pejić et al., 2011a, Pejić et al., 2011b), i.e. that precipitation can affect the soil water regime and irrigation schedule of growing plants. In 2005 and 2006 the average seasonal air temperature was higher for 0.7 °C and 0.5 °C respectively than the long-term seasonal average (1964/2007 – 17.7 °C, Tab. 2). That difference influenced the water used on evapotranspiration but is still in optimum range for normal growing of Sudan grass.

However, despite its abundance, the precipitation was not favorably distributed, so additional water had to be supplied by irrigation of 210 and 120 mm during 2005 and 2006 respectively (Table 3).

Table 3. Irrigation schedules and irrigation water applied

Year	Irrigation rate (mm)			Irrigation water applied in the season (mm)
	Month			
	May	June	July	
2005	+30 mm 16	60 mm – 04 60 mm – 22	60 mm – 26	210
2006	-	60 mm – 06 60 mm – 21	-	120

+irrigation were performed after sowing to ensure uniform sprouting of plants

+navodnjavanje obavljeno posle setve da obezbedi ujednačeno nicanje biljaka

RESULTS AND DISCUSSION

In Vojvodina, a typical temperate region, Sudan grass is considered to be an irrigation dependant crop because it rarely meets its water requirements from precipitation received during the growing season. In the study period, evapotranspiration rate in irrigation conditions (ET_m) ranged from 505-584 mm and in the rainfed conditions (ET_a) in the range from 469-502 mm (Table 4). The results observed in this research were in agreement with Pejić et al. (2006), who stated that for the Vojvodina region Sudan grass water requirements are 570 mm.

Table 4. Maximum (ET_m) and actual (ET_a) evapotranspiration (mm), maximum (Y_m) and actual (Y_a) yield (t ha^{-1}), evapotranspiration ($ET_{m, a\ wue}$, kg m^{-3}) and irrigation water use efficiency (I_{wue}, kg m^{-3})

Year	ET_m	ET_a	Y_{irr}	Y_{dry}	I_{wue}	ET_{mwue}	ET_{awue}
2005	505	502	95.765	87.983	3.7	19.0	18.1
2006	584	469	122.021	118.644	2.8	20.9	25.4
Average	544	485	108.893a	103.314a	3.2	20.0	21.8

LSD 0.05 = 20.126

The average fresh forage yield increase of Sudan grass due to irrigation was in average 5.4% or 5.579 t ha^{-1}, ranging from 8.8 % (7.782 t ha^{-1}) in 2005 to 2.28% (3.777 t ha^{-1}) in 2006 (Table 4 and 5). On average, irrigation did not significantly affect the fresh forage yield of Sudan grass (I irrigated - 108.893 t ha^{-1} , I_o nonirrigated - 103.314 t ha^{-1}) as the study period had precipitation higher than the long-term seasonal average. The results are in agreement with those given by Nasser and Al-Suhaibani (2006) who obtained pretty the same fresh forage yield of Sudan grass in Saudi Arabia in irrigation conditions (122.393 t ha^{-1}). They concluded that the best results were obtained irrigating Sudan grass in 7 days interval instead of 3 or 11 days interval. In the study period the highest yield was obtained in third cut, both in rain-fed and irrigation conditions in 2005 and 2006 respectively (Table 5).

Table 5. Fresh forage yield (t ha^{-1})

Year		Fresh forage yield- Prinos sveže mase				
		Cut I	Cut II	Cut III	Cut IV	Total
2005	I	26.476	29.706	39.583	-	95.765
	I_o	28.125	29.910	29.948	-	87.983
2006	I	29.500	29.769	44.884	17.868	122.021
	I_o	28.188	28.844	45.634	15.978	118.644

I - irrigated; I_o - nonirrigated.

Table. 6. Time of cutting

Year	Cut I	Cut II	Cut III	Cut IV
2005	29. 06	29. 07	03. 09	-
2006	27. 06	19. 07	18. 08	03. 10

The third cut had the best conditions for Sudan grass growing. The sum of precipitation in August, in both years, (133,9 mm and 124,9 mm in 2005 and 2006 respectively, Tab. 2) was on the rate of Sudan grass water requirement. Forth cut of Sudan grass is rarely possible, even in irrigation, in climate conditions of Vojvodina. Fertile soils such as chernozem have suffered a significant reduction in humus content, in some cases as much as 50% (Bićanić, 1988; Ćupina et al., 2011) thus, forth cut could be used as a green manure to improve fertility, physical and water properties of the soil. Nasser and Al-Suhaibani (2006) also emphasized that in arid climate of Saudi Arabia in first two cuts yield was about 85% of the total obtained forage. According to that study, the growers are advised to take only two Sudan grass forage cuts, using the 7 days irrigation interval. That practice would conserve more than 50% of irrigation water while the reduction in forage may be less than 15% of potential yield.

The best method to describe the role that irrigation has in water use efficiency (WUE) in irrigated agriculture is by expressions given by Bos (1980, 1985). Many researchers have evaluated water use efficiency in different ways (Viets, 1962; Begg and Turner, 1976; Howell, 2001). Consequently, care should be taken when comparing WUE values. Evapotranspiration water use efficiency of Sudan grass, in irrigation conditions (ET_m I_{wue}) ranged from 19.0 to 20.9 kg m^{-3} with an average value of 20.0 kg m^{-3}, while evapotranspiration water use efficiency in conditions without irrigation (ET_a I_{wue}) varied from 18.1 to 25.4 kg m^{-3} with an average value of 21.8 kg m^{-3}. Irrigation water use efficiency (I_{wue}) varied from 2.8 to 3.7 kg m^{-3} with an average value of 3.2 kg m^{-3}. Higher values of fresh forage yield of Sudan grass in irrigation than in rainfed conditions and results of both ET_{wue} and I_{wue} which were similar to those obtained from the literature indicate that irrigation schedule of Sudan grass in the study period was properly adapted to plant water requirements and water-physical soil properties. Determined values of ET_{wue} and I_{wue} could be used for the planning, design and operation of irrigation systems, as well as for improving the production technology of Sudan grass in the region.

CONCLUSIONS

On average, irrigation did not significantly affect the fresh forage yield of Sudan grass (108.893 t ha^{-1} - 103.314 t ha^{-1}) as the study period had precipitation higher than the long-term seasonal average. Evapotranspiration rate in irrigation conditions (ET_m) ranged from 505-584 mm and in the rainfed conditions (ET_a) in the range from 469-502 mm. Evapotranspiration water use efficiency of Sudan grass in irrigation conditions (ET_{mwue}) ranged from 19.0 kg m^{-3} to 20.9 kg m^{-3} with an average value of 20.0 kg m^{-3}, while evapotranspiration water use efficiency in conditions without irrigation (ET_{awue}) ranged from 18.1 kg m^{-3} to 25.4 kg m^{-3} with an average value of 21.8 kg m^{-3}. Irrigation water use efficiency (I_{wue}) varied from 2.8 kg m^{-3} to 3.7 kg m^{-3} with an average value of 3.2 kg m^{-3}. Higher values of fresh forage yield of Sudan grass in irrigation than in rainfed conditions and results of both ET_{wue} and I_{wue} which were similar to those obtained from the literature indicate that irrigation schedule of Sudan grass in the study period was properly adapted to plant water requirements and water-physical soil properties. Determined values of ET_{wue} and I_{wue} could be used for the planning, design and operation of irrigation systems, as well as for improving the production technology of Sudan grass in the region.

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PRINOS I PRODUKTIVNOST UTROŠENE VODE SUDANSKE TRAVE (*SORGHUM SUDANENSE* L.) U USLOVIMA NAVODNJAVANJA U KLIMATSKIM USLOVIMA VOJVODINE

BORIVOJ PEJIĆ, BRANKO ĆUPINA, WANG QUAN-ZHEN,
DJORDJE KRSTIĆ, KSENIJA MAČKIĆ, SVETLANA ANTANASOVIĆ

Izvod

Eksperimentalna istraživanja sa ciljem da se preko obračunatih vrednosti koeficijenata iskorišćenosti vode oceni realizovan režim zalivanja Sudanske trave obavljena su na oglednom polju Instituta za ratarstvo i povrtarstvo na Rimskim Šančevima, na zemljištu tipa karbonatni černozem lesne terase u periodu od 2005-2006 godine. Ogled je postavljen po metodu blok sistema i prilagodjen uslovima navodnjavanja kišenjem. U ogledu su bile zastupljene varijanta sa navodnjavanjem (I - 60-65% od poljskog vodnog kapaciteta – PVK) i kontrolna, nenavodnjavana varijanta (I_o). U ispitivanom periodu, u proseku, prinos sveže zelene mase Sudanske trave bio je signifikantno veći u uslovima navodnjavanja (108.893 t ha^{-1}) u odnosu na kontrolnu, nenavodnjavanu varijantu (103.314

t ha^{-1}). Vrednosti iskorišćenosti vode Sudanske trave u odnosu na evapotranspiraciju u uslovina navodnjavanja (ET_m I_{wue}) kretale su se u intervalu od 19.0 do 20.9 kg m^{-3}, a u uslovima bez navodnjavanja (ET_a I_{wue}) u intervalu od 18.1 do 25.4 kg m^{-3}. Efikasnost iskorišćenosti vode dodate navodnjavanjem (I_{wue}) je bila u intervalu od 2.8 do 3.7 kg m^{-3}. Efekat navodnjavanja na prinos sveže mase Sudanske trave kao i vrednosti ET_{wue} i I_{wue} koeficijenata koji su saglasni vrednostima iz literature ukazuju da je realizovan optimalan zalivni režim Sudanske trave u odnosu na potrebe biljaka za vodom i vodno-fizička svojstva zemljišta. Utvrdjene vrednosti ET_{wue} i I_{wue} koeficijenata mogu biti korišćene u dimenzionisanju zalivnog sistema, a takodje i u unapredjenju proizvodnje ove krmne biljne vrste u klimatskim uslovima Vojvodine .

Ključne reči: navodnjavanje, prinos**,** sudanska trava (*Sorghum sudanense* L.), efikasnost korišćenja vode.

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ECONOMICS AND MANAGEMENT IN HOG RAISING

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ROGOŽARSKI DRAGAN, KRSTIĆ BRANKO[1]

SUMAMARY: In the cattle raising, hog raising is one of the largest and the most important parts. Hog raising is of great importance for Serbia, especially in regions with favorable conditions for the production of corn as the main food for pigs. By analyzing the number of pigs in ten-year period, it can be established that the average number of pigs in the Republic of Serbia was 3,497,000.00 ranged from 3,165,000.00 to 3,832,000.00, with a tendency of decrease of 10,000 pigs per year. The average number of sows and pregnant gilts was 714,600.00 ± 74 730, a first-degree equations true indicates that the average annual reduction in the number of animals was 22,850. Analyzing price movements of pigs in the period 2001 – 2011, we conclude that the price was on average 1.86 ± 0.39 Euros, the average price of pigs being fattened in the same period was 1.32 ± 0.28 Euros, and price of maize was 0.13 ± 0.05 Euros. Analyzing the relationship between corn price and piglets price we get (r_{xy} = -0.45) negative and low correlation coefficient, which indicates a small negative dependence of the price of piglets due to the change of the corn price. The effect of the corn price on the price of the pigs being fattened is almost negligible (r_{xy} = 0, 11). Slightly larger and positive impact has the change in the price of pigs being fattened on increasing the price of piglets (r_{xy} =0.59).

***Key words**: pigs, economics, trends, production results.*

INTRODUCTION

General characteristics of livestock production are very complex, if one takes into account the diversity of the most important factors that may affect production. Number of factors affecting livestock production is large and diverse, which make this production more complicated than farming. In the production of livestock, pig production is one of the largest and most important parts. Hog raising, as economic activity, always

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had great importance in the economic development of a country. Pig production is of great importance for Serbia, especially in regions with favorable conditions for the production of corn as the main feed for pigs (Todorović et.al., 2002). Production capacity of pigs, which are manifested in the rapid growth, high fertility and good feed utilization, is comparative advantages that ensure high participation and importance of hog raising in the development of agriculture. The pig has significant biological characteristics that differentiate it from other domestic animals, which have a significant impact on the cost of production and thus make it the most profitable livestock (Antić, 2001). The pig is very fertile animal (over ten piglets per litter), the normal parturient process is taking place at least twice a year, pigs rapidly grow and thrive, pigs achieved physiological and physical maturity at the age of 5-8 months, and with less than a year gives the first offspring (Tešić et. al., 2002) The fattening of less than three pounds of food gives one kilogram of gain. Under modern conditions of cultivation, the productivity of pigs now reached a level which until recently was considered to be biologically limited. Per sow per year today, it is possible to get 26 pigs, 1800kg and 2000 kg of live weight, 1450 to 1620 halves and up to 900 kg - 1010 kg of meat. The number of fattening pigs per sow per year reaches 25.

MATERIALS AND METHOD

The aim of this study were, in addition to focus to present state of pig production in Serbia in the period 2001 - 2011., economic indicators that show pig production in the same period.

In the first part of this study, we used the statistical data related to the number of pigs in the Republic of Serbia in the period 2001 - 2011. All data are sorted by categories: total number of pigs, piglets, fattening pigs, sows and gilts, boars, breeding, mortality, growth and production of meat and fat. For these data, we calculated descriptive statistical parameters (arithmetic mean, standard deviation, standard error, coefficient of variation and the variation interval. Tendency of the observed parameters, we calculate based on the equation of a straight line. Opting for the best-adjusted line we were performing based on the size of the Pearson correlation coefficient.

In the second part, based on data from the Commodity Exchange in Novi Sad and the ten-year follow-up of production-economic indicators on a farm we calculated the mutual relationship of the main indicators of economic pig production. In the economic analysis of the results, we used the following parameters: the market price of piglets, market price of animals being fattened, the price of corn, euro exchange rate and the price of complete feed mixtures for fattening. All these data were collected on a monthly basis in the period 2001 - 2011. In our analysis, we first calculated the descriptive statistical parameters, followed by regression and correlation analysis we determined relationship and the impact of these factors. Production efficiency was determined based on the cost of weight gain and the market price of hogs.

RESULTS

Analyzing the number of pigs in the ten-year research period, it can be determined that the average number of pigs in the Republic of Serbia ranged from 3,497,000.00

with variation of 3,165,000.00 up to 3,832,000.00 and 5.82% coefficient of variation, indicating that there was not a large variation in the total number of pigs in the study period (Table 1). The tendency of pigs flow has negative flow with an average annual reduction of 10,160 animals, which explains the equations of the first degree ($Y = 3.558\,000\text{-}10.160x_i$) (Fig. 1) (Petrović et. al., 2005) . Based on the data, it is determined that the average number of piglets during the study period was 1,118,000.00 ± 171,700.00 heads, and the number of pigs being fattened ranged from 1.201.000 in 2005, up to 1.775.000 in 2009, with the average number of 1,475,000 ± 208,500 heads. In an analysis of trends and developments in the number of piglets and the number of pigs being fattened, a positive trend with an average annual increase of 37,830 heads and pigs being fattened of 48,050 was noticed. Quantity of breeding animals (gilts and sows, boars) in the study period was very low with a tendency to fall (Popović et.al., 2010) . The average number of sows and pregnant gilts was 714,600.00 ± 74,730, and the equation of the straight line indicates that the average annual reduction in the number of heads was 22,850. The situation regarding breeding boars includes average number of 33,670 heads, with an annual reduction of 3166 heads.

Table 1: Descriptive statistical parameters of the number of pigs in the Republic of Serbia (in thousands of heads)

	$\overline{X}$	SD	Sx	Min.	Max.	CV
Total	3497,00	203,70	61,4100	3165,00	3832,00	5,82
Piglets	1118,00	171,70	57,2400	926,00	1431,00	15,36
Pigs being fattened	1475,00	208,50	69,5100	1201,00	1775,00	14,14
Sows and pregnant sows	714,60	74,73	24,9100	631,00	825,00	10,46
Boars	33,67	9,87	3,2900	21,00	49,00	29,33

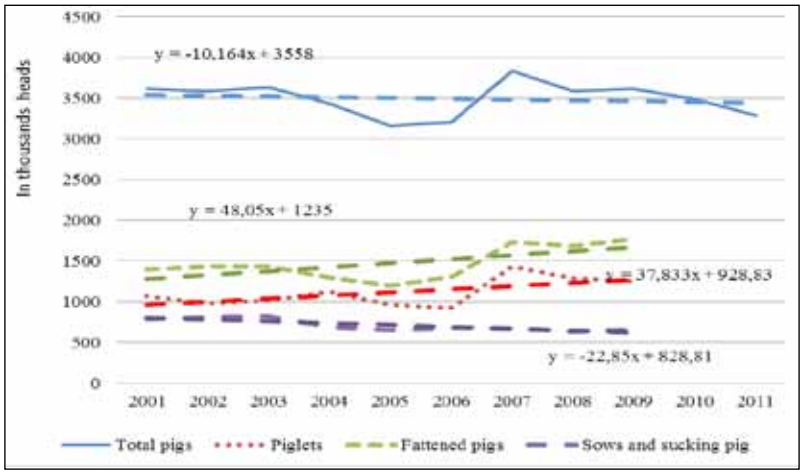

Fig. 1. The tendency of the movement the number of the pigs in the Republic of Serbia

Table 2: Descriptive statistical parameters of the production characteristic of pigs in the Republic of Serbia

	$\overline{X}$	SD	Sx	Min.	Max.	CV
breeding	7.252,0	869,40	274,9000	5.968,00	8328,00	11,99
death	803,90	145,70	46,0700	593,00	981,00	18,12
increase in numbers	429,60	27,93	8,8320	386,00	473,00	6,50
meat production	261,50	13,85	4,3800	242,00	289,00	5,30
fat production	65,80	26,62	8,4170	30,00	101,00	40,45

Using the analysis of the number of fertilized pigs, the average annual reduction in the number of fertilized pigs of 267,900 heads was established, and the average number was 7,252,000 ± 803,900 heads (Table 2) (Popović et. al. 2010). Analyzing the number of fertilized pigs and the number of sows and gilts by year shows that at the annual level only 10:33 ± 0.29 pigs were fertilized per breeding animal. This data refers to the intensity of pig production in the Republic of Serbia. Positive trends in pig farming show minimized mortality and decreased production of fat. The average annual mortality stood at 803,900 ± 145,700, and ranged from 593,000 in 2009, up to 981,000 in 2002, with an average annual reduction of 42,930 heads ($Y = 1.040.000\text{-}42.930x_i$) (Fig. 2). Production of fat records average annual reduction in production of 8,266.60 kg.

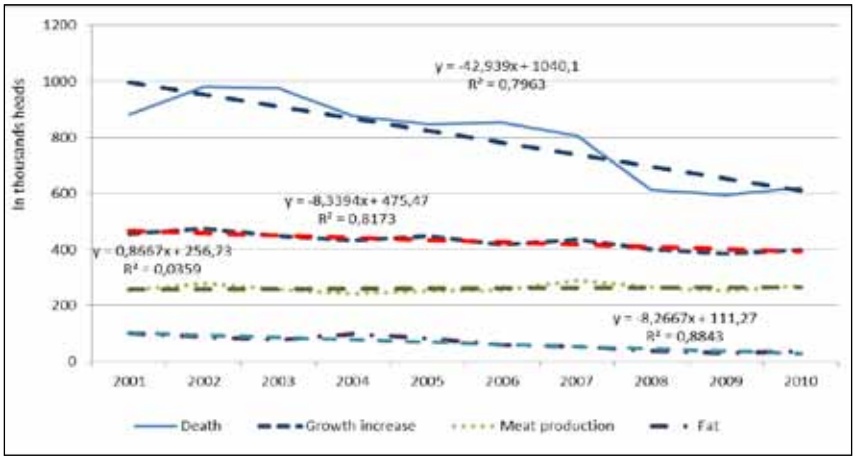

Fig. 2. The tendency of moving the characteristics of pig production in the Republic of Serbia

Table 3: Descriptive statistical parameters of the pig production indicators

Euro	$\overline{X}$	SD	Sx	Min.	Max.	CV
Piglets	1,86	0,39	0,0342	1,18	2.87	21,12
Pigs being fattened	1,32	0,28	0,0240	0,81	2,22	20,99
Corn	0,13	0,05	0,0040	0,06	0,24	34,16

Analyzing the movements of price of the pigs in the period 2001 – 2011 was established that it was an average of 1.86 ± 0.39 Euros with a coefficient of variation of 21.12%. Price of piglets was 1.18 Euros in January 2004, and the highest was 2.87 Euros in November and December 2008, (Table 3). The average price of pigs being fattened in

the same period was 1.32 ± 0.28 Euros, and the coefficient of variation was 20.99%. In April 2007, the purchase price of pigs being fattened was the lowest (0.81 Euro), and in September 2008, was the highest (2.22 Euros). The greatest variation has suffered the price of corn; it was 34.16%, while the average price was 0.13 ± 0.05 Euros. The highest corn price was recorded in July 2011 (0.24 million), while the lowest was in October and November 2005. For the studied period, only the price of piglets has a tendency of slight decrease of 0.009 Euros per kilogram/ per annum. Prices of corn and pigs have a slight tendency to increase - the price of the corn of 0.002 Euros and pigs being fattened of about +0.012 Euros annually. This suggests that during the same period the situation was negative for producers of piglets.

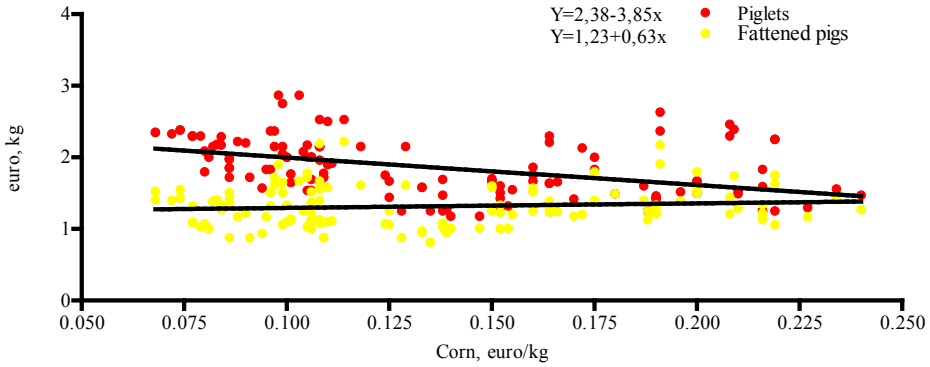

Fig. 3. Regression analysis of the price

Impact analysis of varying the price of corn as the main energy nutrients in pork production to the cost of the purchase price of piglets and fattening pigs was carried out in two directions (Mutavdžić et. al., 2007) . Based on regression analysis and regression coefficients obtained can be found that with the increase in the price of corn, grows the price of pigs being fattened, while the price of piglets continually falls. This is happening because the increase in the price of corn causes a reduction in the number of pigs being fattened, purchase price of fattened pigs increases, demand for pigs for fattening decreases, thus falling price of piglets (Fig. 3). However, this would be very easy to explain we would not take into account other economic parameters(Zekić et. al., 2007). Correlation coefficient will show us the degree of dependence and the strength of connection. Analyzing the relationship between the price of corn and the price of piglets we get (r_{xy} = -0.45), a low and negative correlation coefficient, which indicates a small and negative dependence of the price of piglets in relation to the change of the price of corn. Impact of the price of corn to the price of pigs is almost negligible (r_{xy} = 0.11). Slightly larger and a positive impact has the change in the price of fattening pigs on increased prices of piglets (r_{xy} = 0.59).

DISCUSSION

Basic orientation for further development of pig should be directed to the intensification of production through the application of modern techniques of selection, genetics and reproduction, then quality nutrition and health care, which is accompanied by continuous labeling and identification of animals.

Because we have an experienced and qualified professional staff and a positive experience in the industrial hog raising, pork production in the future, in addition to the quantitative increase, must have a quality improvement, which refers to the increase of the percentage of meat in the halves (Jovanović et. al., 2009). Meaty pigs should dominate in the production programs of future farmers that can meet the high standards and refined taste of consumers, and the payment of pigs must be based on the percentage of meat in the halves according to so-called EUROP system.

In order to avoid cycles of production, which characterized the previous period, which was ruled by the spontaneous production of the individual sector, which still accounts for nearly 83% of pig production, it is necessary to increase farm production where dominant producers should have 50-100 sows (Zekić et. al., 2008). They need to deal with industrial hog raising based on the planned and organized principle of pig production and to be tied with the slaughter industry based on the contract.

CONCLUSION

For pork production in the Republic of Serbia at the moment and with the current level of development, in order to reach production levels of some developed countries, it is necessary:

1) To import purebreds and their crosses with high genetic merits;
2) To complete the process of restructuring and privatization of large agricultural enterprises;
3) To set up production based on contractual relations;
4) To provide favorable long-term credits and investment policy for the supply of raw materials, and facilities;
5) To finally regulate the possibility of exporting pig meat and meat products;
6) To organize a joint approach by organizing associations of producers.

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EKONOMIKA I UPRAVLJANJE U SVINJARSTVU

MIRILOVIĆ MILORAD, TEŠIĆ MILAN, PEJIN IVANA,
ROGOŽARSKI DRAGAN, KRSTIĆ BRANKO

Izvod

U okviru stočarske proizvodnje proizvodnja svinja predstavlja jedan od najvećih i najznačajnijih delova. Svinjarska proizvodnja ima veliki značaj za Srbiju, a posebno za regione koji imaju povoljne uslove za proizvodnju kukuruza kao osnovnog energetskog hraniva za ishranu svinja Analizirajući broj svinja u desetogodišnjem ispitivanom period može se ustanoviti da je prosečan broj svinja u R Srbiji bio 3.497.000,00 sa variranjem od 3.165.000,00 do 3.832.000,00. sa tendencijom smanjenja od 10.000 grla godišnje.. Prosečan broj krmača i suprasnih nazimica bio je 714.600,00±74.730, a jednačina prave prvog stepena ukazuje da je prosečno godišnje smanjenje broja grla bilo 22.850. Analizirajući kretanje cena prsadi u periodu od 2001. do 2011. godine ustanovljava se da je ona bila prosečno 1,86±0,39 eura, prosečna cena tovljenika u istom periodu bila je 1,32±0,28 eura, a cena kukuruza bila je 0,13±0,05 eura Analizirajući vezu između cene kukuruza i cene prasadi dobijamo (r_{xy}=-0,45) negativan i nizak koeficijent korelacije, što ukazuje na malu i negativnu zavisnost cene prasadi u odnosu na promenu cen kukuruza. Uticaj cene kukuruza na cenu tovnih svinja gotovo je zanemarljiv (r_{xy}=0,11). Nešto veći i pozitivan uticaj ima promena cene tovljenika na povećanje cene prasadi (r_{xy}=0,59).

Ključne reči: svinje, ekonimika, trend, proizvodni rezultati.

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DIETARY SUPPLEMENATION WITH NATURAL IMMUNOMODULATORS AND SOWS FERTILITY

DUŠAN ZVEKIĆ, IGOR APIĆ, MLADEN GAGRČIN[1]

SUMMARY: The influence of feeding the sows by diets supplemented with natural immunomodulators on fertility parameters, wer investigated on one large pig farm in AP Vojvodina (Serbia). The diets of pregnant, lactating and weaned sows (in the period weaning-to-fertile estrus) were saplemented with preparations containing premium colostrum, yets and herbal extracts, to stimulate immune sistem. Farrowing rate (AI within ≤ 7 days after weaning), in treated sows was significantly ($p<0.01$) greater (87.6%, 204/233) then in kontrol (untreated) sows (83.7%, 160/205). Average total born and liveborn pigletsper litter, in treated (10.4 and 10,3 resp.) and in control sows (10.2 and 10.0 resp.) was not significant different ($p>0.05$), buth the average stillborn piglets per litter was significantly ($p<0.05$) lower in treated (0.14), compared with control sows (0.21). Our data suggest that the application of natural immunodulators can increase the sows fertility in the intensive pig production.

Key words: *diet, supplementation, natural immunomodulators, fertility, sow.*

INTRODUCTION

In the intensive pigs production technology, the reproductive sows are confronted by many chronic stressors (Hyun et al., 1998). Stressors significantly reduce the immunity of animals (Potočnjak et al., 2006; Kick et al., 2011), which significantly increases the susceptibility to infectious diseases (Sutherland, 2006). The final result is reduction of sows reproductive performance (Floss and Tubbs, 1999; Stančić et al., 2010).

Classical preventive methods of active and passive immunization or treatment of infectious diseases with antibiotics, have significant negative effects in practical application. Namely, these methods increase the cost of production, and cause extra stress

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in treated animals. In addition, vaccination may have a weaker effect, due to the rapid creation of a new viral strains with different immunogenic properties (Gagrčin et al., 2001; Cromwell, 2002). On the other hand, long-term use of antibiotics creates resistant pathogenic microorganisms, and has a negative impact on human health, due to antibiotic residues in animal products (Pugh, 2002). In the recent period, there are attempts to overcoming these negative effects by supplementing the diet with the natural bioactive substances of plant and animal origin, with the immunogenic properties (immunomodulators) (Zvekić, 2006; Pragathi et al., 2011). However, the recent studies about feeding pigs with natural immunomodulators have shown inconsistent results, related to their effects on the sows reproductive performance in the large productive herds (Gallois et al., 2009).

The aim of this study was to determine whether the treatment by natural immunomodulators, may increase the fertility of sows in the herds of intensive pig production.

MATERIALS AND METHODS

Investigations were carried out on one pigs farm, with about 1,200 sows, located in the vicinity of the town of Subotica (AP Vojvodina, Serbia). The sows in the reproductive herd were affected with acute PRRS *(Porcine Reproductive and Respiratory Syndrome)* infection, and in the last two decades, the different forms of PPV *(Porcine Parvo Virus)* infection were detected. These infections had a significant impact on decreasing of sows reproductive performance.

Experimental sows. Total of 476 sows were divided into two groups: 271 treated and 205 untreated (control) sows. Sows in both groups were between 2 and 5 farrowing parity. The first 30 days after artificial insemination (AI), sows are in individual boxes, and then moved to group pens (30 sows in each), up to about 7 days before the scheduled date of farrowing, when it moved to the farrowing facility, with individual pens for sow and litter. Lactation lasts about 35 days. After weaning, sows are moved into group pens (30 sows in each), for estrus detection.

Detection of oestrus and AI. Detection of estrus begins about 24 hours after weaning, by direct contact with sexually mature boar twice daily, at intervals of 10 to 12h. Duble AI was performed, 12h and 24h after detection of standing estrus. Insemination was performed by disposable catheters (Foamtip Safe Blue®, Minitübe, Germany). AI dose volume was 100 mL of fresh liquid diluted semen, with $3x10^9$ progressively motile sperm. BTS Forte semen extender (Minitübe, Germany) were used. Insemination was performed a few hours after the insemination dose formation.

Experimental treatment. In the period from the fertile insemination to the end of gestation, experimental sows were fed classic diets for pregnant sows, supplemented by 0.3% *Swine Guard SHT* immunomodulator. After farrowing, during lactation, sows were fed conventional diets for lactating sows, supplemented by 0.4% *Guard Swine farrowing* preparations. Between weaning and fertile insemination, sows were fed conventional diets, supplemented with 0.4% *Swine Guard SHT* preparations. Control sows were fed identical classic diets, in certain phases of the reproductive cycle, but not containing immunomodulator preparations.

Immunomodulators preparations. "Hokovit" immunomodulator preparations were used (HU Hofmann AG-CU, 4922 Bützberg, Switzerland). According to the manufacturers declaration, these preparations include: (a) the premium colostrum, whose

role is to support auto-antibodies production, (b) yeast extracts, which regulate the digestive processes and (c) herbal extracts, which act as antioxidants and regulators of normal microflora in digestive tract. *Swine Guard SHT* improves the overall health of the sows immune status, increases milk production and food consumption due to better digestion, reduction of metabolic disorders, increases the number and weight of piglets at farrowing. *Guard swine farrowing* significantly increases the quality and quantity of colostrum and milk production, increases the number and weight of piglets at weaning, reduce the incidence of MMA syndrome, increases the appetite of sows in the season of high ambient temperature and prevent diseases caused by staphylococcal and streptococcal infections.

Analysis of data. Descriptive statistics, t-test, analysis of variance (ANOVA) and Duncan's test were done in the software package Statistics 10th.

RESULTS

The distribution of estrus reaction, within the firs 7 days after weaning is present in Table 1.

Table 1. Estrual reaction after weaning

		Treated sows	Control sows
In estrus within 7 days after weaning	n	233	160
	%	86.0A (233/271)	78.0B (160/205)
Av. interval weaning to estrus, days		5.7A	6.4B

^{A,B} Values with different superscript, within the same rows, are statistically significant different ($p<0.01$).

Within the first 7 days after weaning, estrus was detected in significantly ($p<0.01$) greater number (86%) of treated, compared with the control sows (78%). Average weaning-to-estrus interval duration were significantly ($p<0.01$) shorter in treated (5.7 days) than in the control sows (6.4 days).

Farrowing rate, after AI in the first postlactational estrus is present in Table 2.

Table 2. Farrowing rate

		Treated sows	Control sows
Sows inseminated (AI), n		271	205
Farrowing rate, %	A	87.6^{ax} (204/233)	83.7^{bx} (134/160)
	B	65.8^{ay} (25/38)	64.4^{ay} (29/45)
	C	84.5^{ax} (229/271)	79.5^{bx} (163/205)

A – AI in estrus detected ≤ 7 days after weaning; B – AI in estrus detected ≥ 8 days after weaning;

C – For total sows inseminated.

^{a,b} Values with different superscript, within the same rows, are statistically significant different ($p<0.05$).

^{x,y} Values with different superscript, within the same columns, are statistically significant different ($p<0.05$).

The greatest farrowing rate were estimated in the tretated sows, that were AI in estrus detected within 7 days after weaning (C = 87.6%), as well as in the all treated sows (C = 84.5%), compared with control sows (A = 83.7%; C = 79.5%). The differences in these values between the treated and control sows were statistically significant ($p<0.05$). Significantly lower farrowing rate was achieved in the treated (B = 65.8%) and control sows (B = 64.4%) that were inseminated ≥ 8 days after weaning. However, these differences do not differ significantly ($p>0.05$).

Average total born, liveborn and stillborn piglets per litter at farrowing is present in Table 3.

Table 3. Litter size at farrowing

	Treated sows	Control sows	Total
Sows farrowed, n	229	163	476
Av. lactation duration, days	34	33	34
Av. total born piglets per litter, n	10.42[a]	10.23[a]	10.34
Av. liveborn piglets per litter, n	10.28[a]	10.02[a]	10.17
Av. stillborn piglets per litter, n	0.14[a]	0.21[b]	0.16

[a,b] Values with different superscript, within the same rows, are statistically significant different ($p<0.05$).

The average number of total born (10.42) and liveborn (10.28) piglets per litter in treated sows did not differ significantly ($p>0.05$) in comparison with control sows (10.23 and 10.02, resp.). But the averaga number of stillborn piglets per litter was significantly ($p<0.05$) higher in the control (0.21), compared to treated sows (0.14).

DISCUSSION

The various stressogens during pregnancy and lactation, as well as during the period from weaning to fertile insemination, decrease the reproductive performance of sows, by increasing incidence of prolonged weaning-to-estrus interval, rebreedings, abortions and litter size decreasing (Floss and Tubbs, 1999; Potočnjak et al., 2006; Stančić et al., 2010). Reduced immunity increase the susceptibility of animals to infectious diseases (Sutherland, 2006), which are common causes of reproductive disorders (Hogg and Levis, 1997; Gagrčin, 2003; Yeske, 2007; Stančić et al., 2011). Recently, various immunomodulators of plant or animal origin (Zvekić, 2006; Pragathi et al., 2011), or non-specific immunization (Steinmasl and Wolf 1990; Pavičić et al. 2003; Potočnjak et al., 2006), are used in order to increase the immunity of animals subjected to chronic stress in intesive production conditions (Blecha, 2001). Preparations of yeast extract, herbal extract, colostrum, and blood serum is commonly used as a natural immunostimulators (Davis et al., 2004; Gallois et al., 2009; Pragathi et al., 2011; Bonneau and Laarveld, 1999). However, there are not consistent results about effects of natural immunomodulators on sows reproductive performance in the large productive herds (Gallois et al., 2009).

Our results, in a herd with the PRRS and PPV infection presence, show that the addition of natural immunomodulators in the diet can increase the sows reproductive performance. Namely, the number of sows in estrus within 7 days after weaning (86%) and farrowing rate (87.6%) were significantly higher, whille avereage stillborn piglets per litter were lower (0.14) in tretated, compared with control, (untretated) sows (78%, 83.7% and 0.21,

resp.). The results of other authors show that infectious diseases significantly reduce sows fertility (Hogg and Levis, 1997; Gagrčin, 2003; Sutherland, 2006; Yeske, 2007; Stančić et al., 2010). In this regard, based on our results, it could be, indirectly, concluded that natural immunomodulators increase the immunity of the treated sows and increase their reproductive performance. However, detailed studies are required to elucidate the physiological mechanisms of natural imunomodutalatora action and their relations with sows fertility.

CONCLUSION

Based on the results about relations of diets supplementation with natural immunomodulators and sows fertlitz, it can be concluded:

1) Sows treated with natural immunomodulators had a shorter weaning-to-estrus interval, higher farrowing rate and a smaller number of stillborn piglets per litter, compared with control (untreated) sows.
2) These findings suggest that the application of natural immunodulators can increase the sows fertility in the intensive pig production.

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DODAVANJE PRIRODNIH IMUNOMODULATORA U HRANU I FERTILITET KRMAČA

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Izvod

Ispitivan je uticaj ishrane krmača obrocima sa dodatkom prirodnih imunomodolatora, na parametre fertiliteta krmača, na jednoj velikoj farmi svinja u AP Vojvodinini (Srbija). Obrocima suprasnih, laktirajućih i zalučenih krmača, dodati su preparati imunostimulatora, koji su sadržavali biljne ekstrakte, ekstrakte kvasca i kolostruma. Vrednost prašenja, posle VO unutar prvih 7 dana po zalučenju, bila je značajno ($p<0.01$) veća kod tretiranih (87.6%, 204/233) u odnosu na kontrolne (netretirane) krmače (83.7%, 160/205). Prosečn broj ukupno i živorođene prasadi po leglu tretiranih (10.4 i 10,3) i kontrolnih krmača (10.2 i 10.0), nije bio značajno različit ($p>0.05$). Međutim, prosečan broj mrtvo rođene prasadi po leglu, bio je značajno manji ($p<0.05$) kod tretiranih (0.14) u odnosu na kontrolne krmače (0.21). Naši rezultati sugerišu da primena prirodnih imunomodulatora, može povećati fertilitet krmača u intenzivnoj proizvodnji.

Ključne reči: hrana, dodavanje, prirodni imunomodulatori, fertilitet, krmača.

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PERIPARTURIENT METABOLIC PROFILE, BLOOD PICTURE AND BODY CONDITION SCORE IN HEALTHY AND COWS WITH ASSOCIATED PERIPARTURIENT DISEASE*

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SUMMARY: Experiment included 39 cows, 15 healthy and 24 diseased. Ketosis was diagnosed in 13 out of 24 diseased cows, and it was combined with mastitis and metritis (in 10 cows) and displaced abomasum (in 3 cows). The combination of diseases consisting of placenta retention and metritis was present in 8 cows, whereas the combination of displaced abomasum and lameness was present in 3 cows. The cows with associated peripartal diseases have significantly higher concentration of NEFA, BHB, bilirubin, AST and the number of neutrophils in the four weeks postpartum. The concentration of glucose, albumin, urea, calcium, cholesterol was lower in the diseased cows. The hematologic profile of the sick cows was characterized by a decreased number of erythrocytes and lymphocytes with a decrease in the concentration of hemoglobin. A total number of lymphocytes in the diseased cows was lower a week after the calving, but subsequently it considerably increased, exceeding the number determined in the healthy cows. The body condition of the diseased cows significantly declines, exceeding 0.7 of measure unit in the first month whereas the decline of body condition of healthy cows is not so noticeable with the amount of 0.4 of measure unit. In the group of sick cows was found significantly higher proportion of cows that had values of metabolic and hematological profile outside the normal values and that had a significant loss of body condition in early lactation. Cows with associated periparturient disease showed worst adaptation capacity in early lactation which is reflected in metabolic profile, blood pictures and loss in body condition score.

Key words: *dairy cows, peripartal diseases, metabolic profile, haemogram, body condition score.*

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INTRODUCTION

Calving period represents the most important period in a production cycle of a cow when cows are moving from the dry period to the lactation period. Due to the increasing milk production, reduced feed intake and the domination of catabolic hormones, energy balance of cows is negative in this stage. Due to the negative energy balance the cows mobilize their own body reserves to meet their demand for energy which results in an increased lipid mobilization and the release of non-esterified fatty acids (NEFA) into the bloodstream as well as the incresed ketogenesis with the increase in the concetration of beta-hydroxybutyrate (BHB) followed bythe loss of physical condition (Ingvarsten, 2006). Apart from the above-mentioned, a lot of other adaptational changes in the organism which largely depend on the intensity of lipid mobilization and ketogenesis occur, such as: reduced glycaemia, decreased concentration of cholesterol, increased concentration of bilirubin, decreased calcaemia and other changes (Cincović et al., 2011); as well as the reduced number of erythrocytes and the concentration of hemoglobin, the reduced number of leucocytes, but the increased number of neutrophils etc (Belić et al., 2011).

The previously mentioned adaptational processes in the cow bodies result in the development of associated peripartal diseases, and the most frequent are the following: ketosis, metritis, mastitis, rennet-bag dislocation and lameness. Pathophysiological connection can be established between all these diseases and metabolic changes due to the negative energy balance (Drackley et al., 2005; Huzzey et al., 2011; Leblanc, 2010; Ospina et al., 2010).

Due to all the previously mentioned information we assumed that there will be differences in parameters values of the metabolic profile and the haemogram in the first few weeks postpartum with both healthy and diseased cows. This paper is aimed at examining the values of the metabolite, the haemogram and physical condition of the healthy cows and the cows suffering from peripartal diseases.

MATERIALS AND METHODS

This experiment included 39 Holstein-Friesian cows: 15 healthy cows, 24 cows suffering from associated peripartal diseases-having more than one peripartal disease in the first four weeks of lactation (subclinical ketosis, placenta retention, metritis, mastritis, abomasal dislocation, lameness). The cows come from a few farms in Vojvodina and were raised under the same conditions of nutrition and care (free stall system, the diet was based on silage that was given to the cattle piecemeal in TMR mixture).

The blood was taken from the jugular vein in the first, second, third and fourth week postpartum and analyzed the same day in the laboratory. The following biological parameters were examined: NEFA, BHB, glucose, cholesterol, bilirubin, AST, albumin, urea, and Ca. Standard types of kits were used manufactured by Randox (UK), and spectrophotometric measurements were performed on the semi-auto chemistry analyzer Rayto RT 1904c. In addition to all of the previously mentioned, the following haematologic parameters were recorded: the number of erythrocytes, the concentration of hemoglobin, the number of leucocytes, and differential white blood cells line. A hematologic analyzer Hemavet 950c was used.

The body condition score (BCS) was determined according to the 1-5 scoring

system on the recommendation of Elanco Animal Health.

The difference in the values of the metabolic profile, haemogram, and physical condition between the group of healthy cows and the group of cows suffering from various diseases during the four weeks of monitoring was performed using the ANOVA method and LSD test.

RESULTS

Peripartal diseases in dairy cows most frequently occur in combination with ketosis regardless of whether it is subclinical or clinical form. (Fig. 1). Ketosis was diagnosed in 13 out of 24 diseased cows ketosis was diagnosed, which was combined with mastitis and mestritis (in 10 cows) and displaced abomasum (in 3 cows). The combination of diseases consisting of placenta retention and metritis was present in 8 cows, whereas the combination of abomasum dislocation and lameness was present in 3 cows.

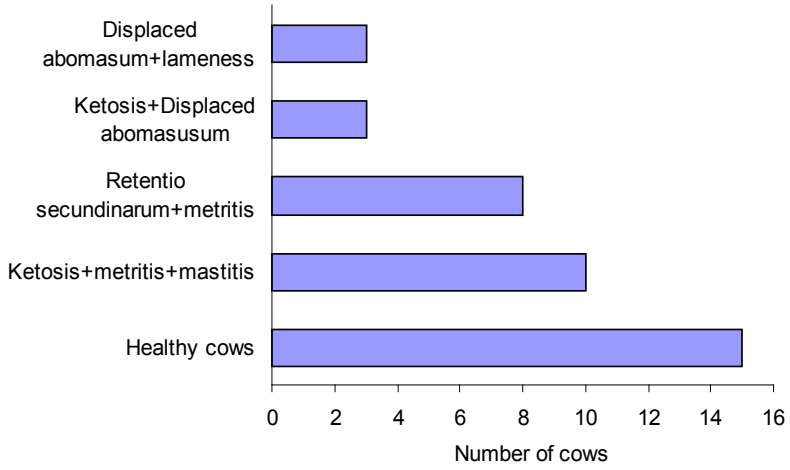

Fig. 1. Frequency distribution of the cows according to the diseases that were diagnosed

The concentration of NEFA I BHB was significantly higher (<0.001) in the diseased cows in all the weeks postpartum (Fig. 2 and 3), but for NEFA the difference disappears in the fourth week postpartum. The week had a significant effect as well (<0.01). The concentration of NEFA and BHB was decreasing in the groups of both healthy and sick cows, but this decline was more intensive in the healthy cows:-0.17:-0.09 mmol/l per week regarding NEFA, , -0.21:-0.17 mmol/l regarding BHB.

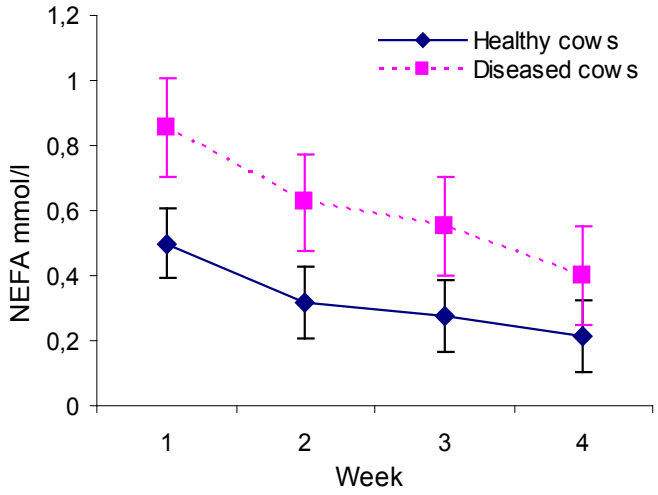

Fig. 2. The Concentration of NEFA in healthy and sick cows

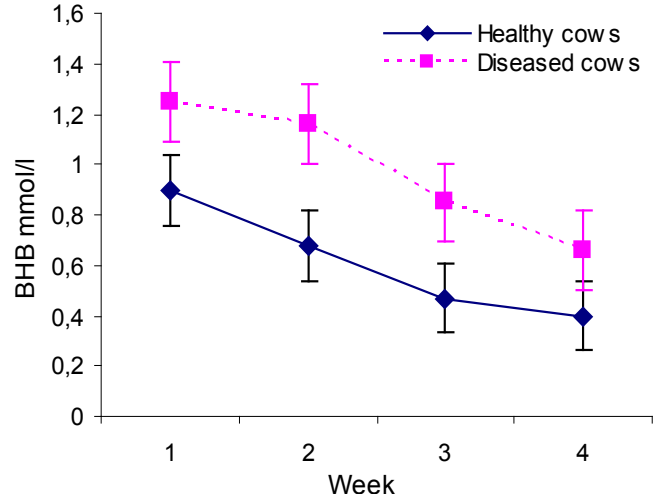

Fig. 3. The Concentration of BHB in healthy and sick cows

The concentration of glucose (Fig. 4) was significantly lower in the diseased cows (<0.001), and the cows from the same group had significantly lower concentration of cholesterol (<0.05) (Fig. 5). The effect that the time had on the development of the values of examined parameters was significant. The change dynamics of the concentration of glucose during the four weeks was approximately identical in both healthy and diseased cows +0.35 mmol/l per week, and the change dynamics of the cholesterol concentration +0.69 mmol/l per week was also identical.

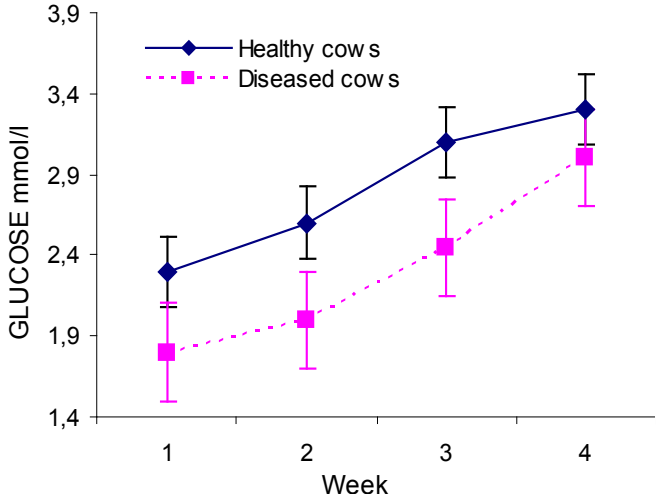

Fig. 4. The concetration of glucose in diseased and healty cows

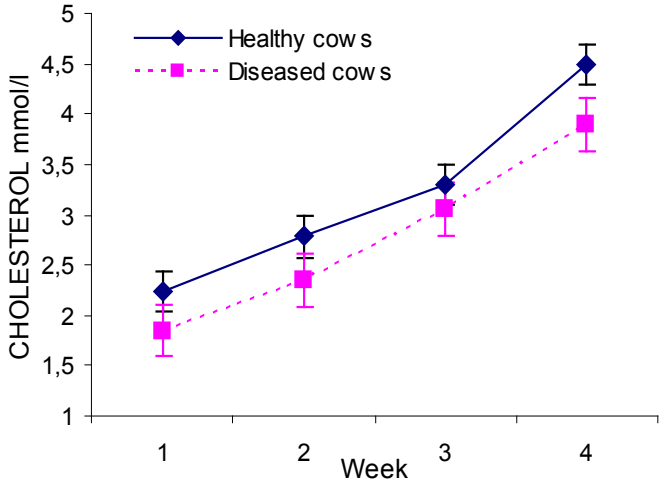

Fig. 5. The concetration of cholesterol in healthy and diseased cows

The concetration of albumin (Fig. 6) in healthy cows significantly increases during the first four weeks pospartum (<0.05), but until the third week the concetration disappeared and then in the fourth week it became significant again, because the albumin concetration in diseased cows declined. The concetration of urea (Fig. 7) was significantly lower in diseased cows in the first week postpartum (<0.01) followed by a gradual decline of that difference. The concentration of urea increased significantly in the first four weeks postpartum (<0.01), and this increase was more intensive in sick cows: +0.6:+0.4 mmol/l per week.

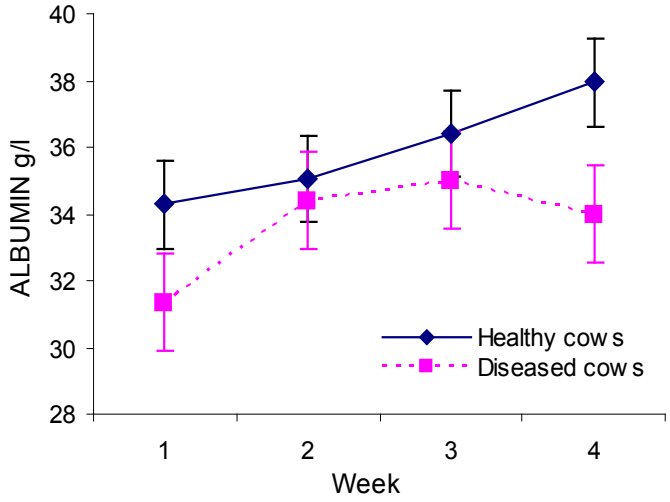

Fig. 6. The albumin concetration in healthy and diseased cows

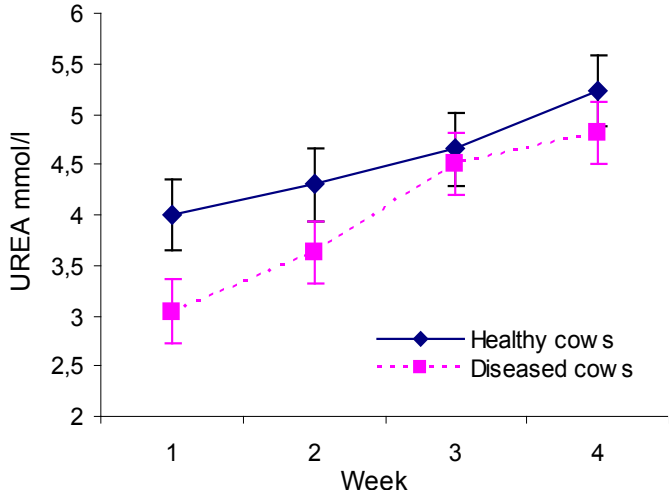

Fig. 7. The urea concetration in healthy and diseased cows

The bilirubin concetration (Fig. 8) was significantly higher in diseased cows during the whole four weeks postpartum (<0.01). Bilrubin concentration was declining significantly during the first four weeks postpartum (<0.01) in both of the groups of cows. The effect of the week on the concentration of AST was statistically insignificant, due to the high variation of values (Fig. 9). The diseased cows showed a significantly higher concentration of this enzyme in the first, second, third and fourth week postpartum.

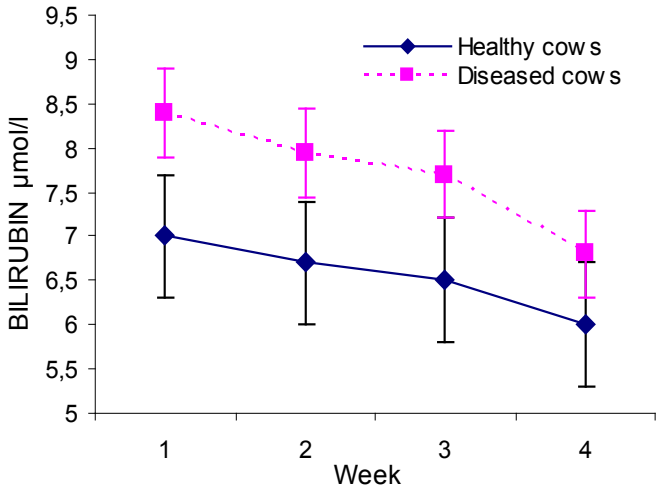

Fig. 8. Bilirubin concetration in healthy and diseased cows

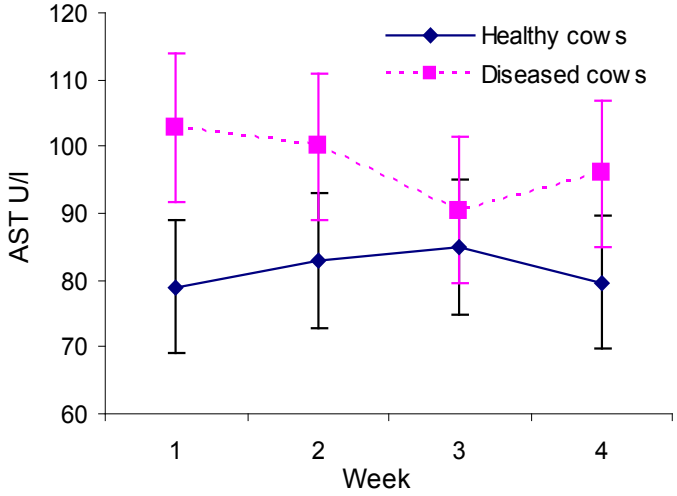

Fig. 9. AST concetration in healthy and diseased cows

Calcium concetration (Fig. 10) was significantly lower in diseased cows (<0.01) in the first and the third week postpartum. The effect of the week was not statistically significant, but the concentration of calcium showed a tendency to change under the influence of the week (<0.1). There was a constant and steady increase in calcaemia in healthy cows, whereas calcaemia in diseased cows increased and declined.

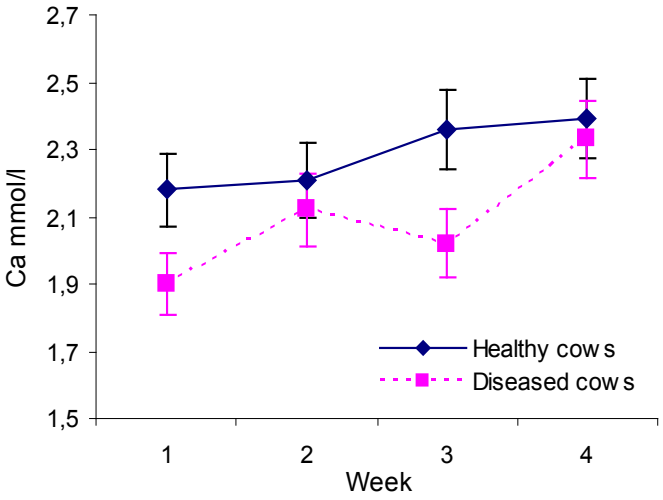

Fig. 10. Calcium concetration in healthy and diseased cows

The diseased cows in the first four weeks postpartum show a tendency towards anemia which is reflected in a notably lower number of erythrocytes and hemoglobin in this group of cows (Fig. 11 and 12). The difference in value of the selected parameters of the red blood cells line is the most noticeable in the first week of partus between healthy and diseased cows but it was observed that this difference gradually declined until the fourth week. The effect of the week on the number of erythrocytes was insignificant, whereas the week during which the blood was sampled had a significant impact on hemoglobin concentration.

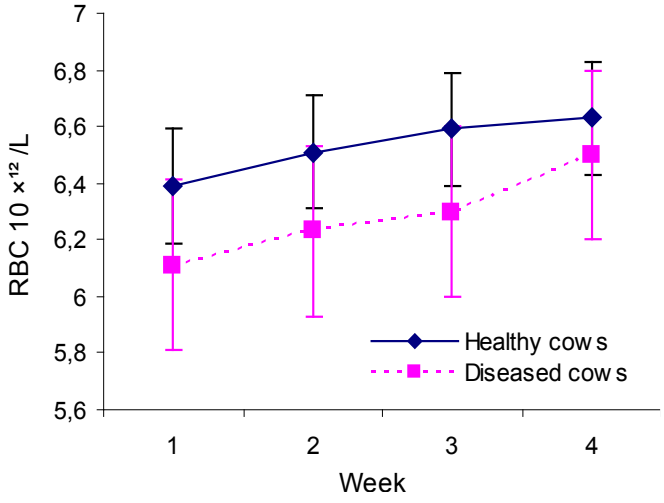

Fig. 11. The number of erythrocytes in healthy and diseased cows

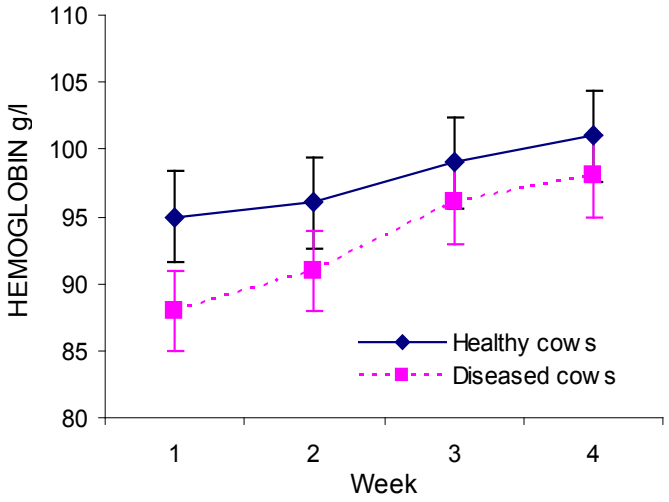

Fig. 12. Hemoglobin concetration in healthy and diseased cows

A total number of leucocytes (Fig. 13) was notably smaller in diseased cows in the first week postpartum. In the second, third and the fourth week an inversion occurs leading to the significant increase in the number of leucocytes in diseased cows. The number of neutrophils is notably higher, whilst the number of lymphocytes is significantly smaller in diseased cows (Fig. 14 and 15). The number of lymphocytes in diseased cows showed the tendency to be higher (<0.1) in cows in the fourth week postpartum.

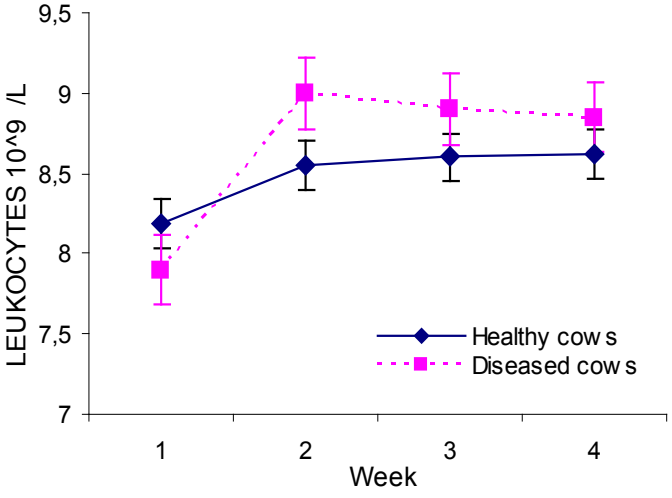

Fig. 13: The number of leukocytes in healthy and diseased cows

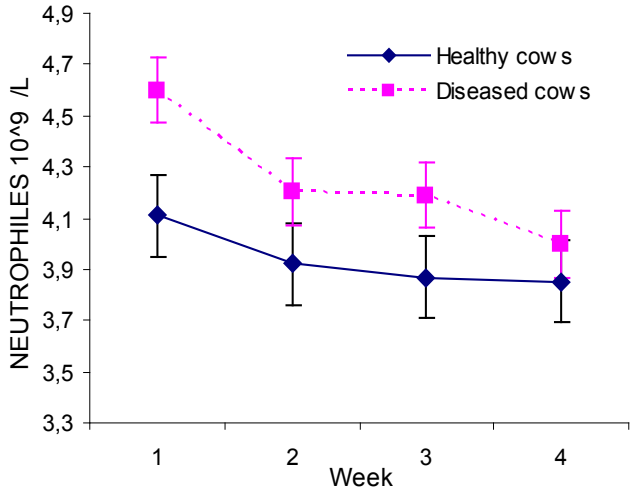

Fig. 14. The number of neutrophils in healthy and diseased cows

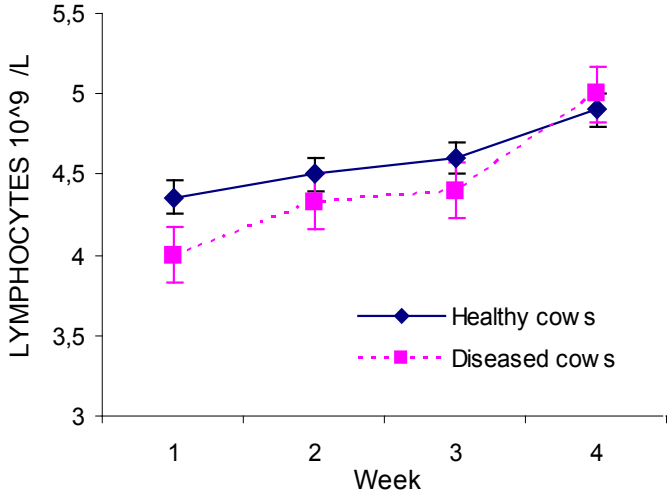

Fig. 15. The number of lymphocytes in healthy and diseased cows

The diseased cows showed significant loss of body condition (Fig. 16) in the first four weeks postpartum (0.7 of measure unit with diseased cows and 0.4 of measure unit with healthy cows) and so the body condition scoring of the cows is significantly lower in the second, third and fourth week postpartum (<0.01).

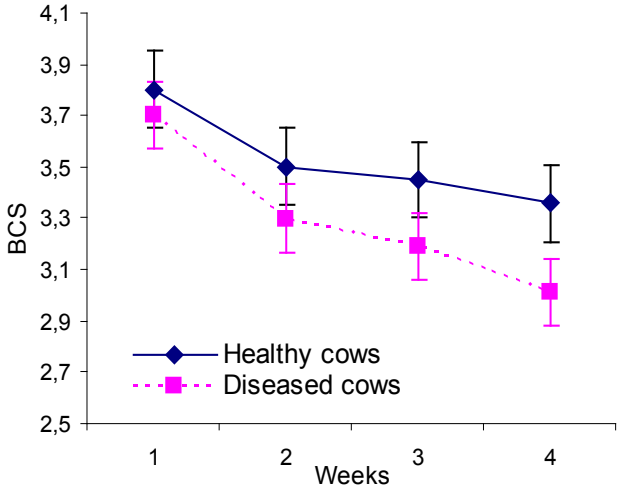

Fig. 16. Body condition of healthy and diseased cows

In the group of sick cows was found significantly higher proportion of cows that had values of metabolic and hematological profile outside the normal values and that had a significant loss of body condition in early lactation. Elevated serum NEFA and BHB, a significant loss of body condition and stress leukogram were the most common signs in diseased cows, while hypoglycemia, hyperbilirubinemia, hypocalcemia and anemia was lower. A certain number of cows in a healthy group had a parameters value outside the reference, but their presence was lower than in a group of sick cows. (See Table 1)

Table 1: Percent of cows with parameters out of reference range in healthy and diseased group during first week after calving

Parametar	Diseased (%)	Healthy (%)	p
NEFA	62,5	26,7	<0,01
BHB	54,2	20,0	<0,01
Glucose	45,83	20,0	<0,01
Bilirubine	45,83	20,0	<0,01
Ca	41,67	13,33	<0,05
RBC	37,5	20,0	<0,05
Hemoglobine	37,5	13,33	<0,05
N:L >1	62,5	26,7	<0,01
BCS loss >0,5	83,3	13,33	<0,01

DISCUSSION

The experiment included 24 diseased cows and 15 healthy cows. There were symptoms of ketosis combined with mastitis, metritis and displaced abomasum. It is well-known that a high concentration of NEFA during the period before calving and a

high concentration of BHB (subclinical ketosis) during the period after calving increases the chances of developing abomasum dislocation (Cameron et al., 1998; Geishauser et al., 2000; LeBlanc et al., 2005). The increased concentration of NEFA increases the risk of occurrence of placenta retention (LeBlanc et al., 2004). Subclinical ketosis increases duration and seriousness of the disease (Sordillo et al., 2009). These results are in agreement with our results showing that the diseased cows have a significantly higher concentration of NEFA and BHB in the first four weeks after calving. The average NEFA concentration in diseased cows was 0.9 mmol/l and BHB concentration was 1.3 mmol/l in the first week postpartum. These results match the previously obtained marginal values. Prepartum values of NEFA indicating the risk of the occurrence of peripartal diseases range from 0.2 to 0.5 mml/l and regarding postpartum period the values range from 0.57 to 1 mmol/l (Oetzel, 2004; Ospina et al., 2010 Chapinal et al., 2011; Cincović and Belić, 2012) which is in accordance with the results we obtained. The same authors discovered that the concentration of BHB is important for the development of peripartal diseases if it exceeds 1.5 mmol/l, which is higher than the values obtained in this paper. Metabolic and hematologic findings from cows during the peripartal period depend on the intensity of lipid mobilization (González et al., 2011). During the peripartal period, due to the liver strain caused by ketones, various metabolic adaptations occur such as decreased glycemia, decreased albumin production in the liver, decreased cholesterol concentration, increased concentration of AST and bilirubin, and decreased urea concentration (Cincović et al., 2011; Đoković et al., 2009). This kind of situation is characteristic of the cows that are prone to fatty infiltration of the liver and ketosis. A significantly higher concentration of NEFA, BHB and AST was found along with decreased concentration of calcium and glucose which is in agreement with our results (Vanwinden et al., 2003). NEFA concentration in combination with leukocyte profile is important model to predict uterus and mammary gland health (Belić et al., 2012). One big research which included more than one thousand cows indicated that there is a significant variation in relation to the mean value of albumin, glucose, cholesterol and calcium in the cattle with a high incidence of peripartal diseases (Kida, 2002).

An increase in the number of leucocytes in cows suffering from metritis happens on account of the increase in the number of neutrophils. The increase in the number of neutrophils can be explained as the consequence of inflammation development, but this also occurs as a reaction to the increased concentration of cortisols during the labor. It is important to mention that the functionality of neutrophils declines during the peripartal metabolic stress which predisposes cows to metritis (Hammon et al., 2006). The reduction in the number of erythrocytes and the reduced concentration of hemoglobin which dominates in the cows prone to metritis can be explained as a consequence of bleeding from the inflamed injuries of the uterus. Haemogram of the cows with placenta retention which precedes metritis indicates leucocytosis and anemia (Ahmend et al., 2009), which was confirmed by our obtained results. The leukogram was obtained along with monocytosis by examining haemogram at the moment of diagnosing endoemetritis (Hanafi et al, 2008) which means that peripartal findings might be useful in estimating the occurrence of metritis. The increase in the number of leucocytes during the peripartal period in dairy cows suffering from mastitis is in accordance with the previously obtained results (Barnouin and Chassagne, 2000; Holtenius et al., 2004). The cows with lameness have significantly altered hematological parameters in comparison with the observation of healthy cows which is reflected in the decreased number of

erythrocytes and the reduced hemoglobin concentration while the total number of leukocytes and neutrophils was significantly higher (Meimandi Parizi and Khalafizadeh, 2006), which matches the results we obtained. A decreased number of leukocytes in diseased cows in the first week postpartum is an interesting finding which is considered to be a consequence of a significant effect of fatty acids, ketone and oxidative stress on immunological stress (Sordillo et al., 2009). Increased ration between neutrophils and lymphocytes occurs when the animals are under stress primarily due to the effect of the cortisols (Bertoni et al., 2003).

The body condition of the diseased cows significantly declines, exceeding 0.7 of measure unit in the first month whereas the decline of body condition of healthy cows is not so noticeable with the amount of 0.4 of measure unit. This change can be linked with higher catabolism which puts a strain on the diseased cows. The previous results indicated that cows losing from 0.5 to 1.0 of measure unit BCS (body condition score) showed that they carry a higher risk of the occurrence of metritis (Butler and Smith, 1989).

In the group of sick cows was found significantly higher proportion of cows that had values of metabolic and hematological profile outside the normal values and that had a significant loss of body condition in early lactation. Ospina et al. (2010a) were found that in herd in which more than 15% of animals sampled had prepartum NEFA concentration ≥0.30 mEq/L was 75%, BHBA ≥12 mg/dL was 40%, and postpartum NEFA ≥0.70 mEq/L was 65%. These results require further research on the impact of the metabolic profile in an independent sample of cows.

High values of NEFA and BHB can lead to significant changes in the metabolic profile and blood picture in healthy cows during early lactation, and many parameters can be outside of reference (Cincović et al., 2012). Therefore the monitoring of metabolic profile and blood test must be done in parallel with the clinical observation of cows in early lactation.

CONCLUSION

The cows suffering from peripartal associated diseases show significant changes in metabolic and hematologic profile in the first four weeks postpartum. During this period the body condition of the diseased cows shows more intensive decline. In group of diseased cows exists higher percent of cows with metabolic and hematological parameters which were out of reference for cattle. Cows with associated periparturient disease showed worst adaptation in early lactation which is reflected in metabolic profile, blood pictures and loss in body condition score.

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PERIPARTALNI METABOLIČKI PROFIL, KRVNA SLIKA I TELESNA KONDICIJA ZDRAVIH I KRAVA SA ZDRUŽENIM PERIPARTALNIM BOLESTIMA

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Izvod

Cilj ovog rada je bio da se ispitaju vrednosti metaboličkog profila, krvne slike i telesne kondicije u prve četiri postpartalne nedelje kod zdravih krava i krava sa različitim peripartalnim bolestima. U ogled je uključeno 39 krava: 15 zdravih i 24 obolelih krava. Ketoza je nađena kod 13 krava u kombinaciji sa mastitisom/metritisom (10 krava) i dislokacijom abomasuma (3 krave). Retencija palcente sa metritisom postojala je kod 8 krava,a kod 3 krave je nađena kombinacija dislokacije sirišta i jake šepavosti. Kod 13 od 24 obolelih krava dijagnostikovana je ketoza, koja je bila u kombinaciji sa mastitisom i metritisom (kod 10 krava) i sa dislokacijom abomasuma (kod 3 krave). Krave sa združenim peripartalnim bolestima imaju značajno višu koncentraciju NEFA, BHB, bilirubina, AST i broj neutrofila u prve četiri postpartalne nedelje. Koncentracija glukoze, albumina, uree, kalcijuma i holesterola je bila niža kod bolesnih krava. Hematlološki profil obolelih krava odlikovao se nižim brojem eritrocita i limfocita uz sniženu koncentraciju hemoglobina. Ukupan broj leukocita kod bolesnih krava bio je niži u prvoj nedelji posle partusa, da bi potom značajno porastao iznad broja utvrđenog kod zdravih krava. Telesna kondicija obolelih krava pokazuje intenzivniji pad (0.7:0.4 jedinice) u prve četiri postpartalne nedelje. U grupi bolesnih krava nađena je značajno veća proporcija krava čije se vrednosti metaboličkog profila i krvne slike bile izvan referenrnih, a veći je bio i gubitak telesje kondicije. Krave sa združenim peripartalnim bolestima pokazuju lošiji adaptacioni kapacitet što je pokazano kroz vrednosti metaboličkog profila, krvne slike i telesne kondicije ispitivanih krava.

Ključne reči: mlečne krave, peripartalne bolesti, metabolički profil, krvna slika, telesna kondicija.

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CHANGES IN THE LIPID PEROXIDATION INTESITY IN AUXIN TREATED CHERRY ROOTSTOCKS SOFTWOOD CUTTINGS*

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SUMMARY: In order to investigate the effect of phytohormones exogenous auxins on the level of the mechanical injury induced oxidative stress, intensity of lipid peroxidation (LP) was measured in rootstocks of cherry softwood cuttings. Basal parts of the cuttings and leaves of five rootstocks (Mahaleb 1 and 2, Gisela 5, European ground cherry (EGC) and "Oblačinska" sour cherry) were sampled 0, 2, 4 and 6 days after cutting. Cuttings were treated with 0.5% solutions of three auxins: α-naphthylacetic acid (NAA), indolebutyric acid (IBA) and combination of these two (INCIT K). Results obtained for the LP intensity varied depending on the rootstock, plant organ and auxin applied. The best LP-lowering effect of auxins occurred in all genotypes when treated with NAA. In the leaves, the LP peaked on the 2nd day while the best LP-lowering effect was recorded in European ground cherry rootstocks on the 4th day, independently of auxin applied. The highest MDA production in leaves was recorded in Mahaleb 2 and European ground cherry rootstocks treated with IBA (60-90% higher then control) two days after the cuttings were made. It has been established that in the most of the rootstocks examined auxins showed lowering effect on LP which points to their positive effect, not only on rooting of softwood cuttings, but on their antioxidant protection system, as well.

Key words: *sour cherry cuttings, oxidative stress, auxins, lipid peroxidation***.**

Original scientific paper / *Originalni naučni rad*

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INTRODUCTION

Abiotic stress factors, such as UV light and other forms of radiation, photooxidation, air pollution, drought, herbicides, certain injuries, hyperoxia, ozone, temperature fluctuations and other stresses are known to induce free radical formation in most aerobic organisms (Malenčić et al., 2010).

At early times after wounding, plants transiently produce active oxygen species (AOS), including the superoxide anion in damaged tissue and hydrogen peroxide, both locally and systematically (Orozco-Cardenas, Ryan, 1999). Production is maximal at several minutes after wounding for superoxide and at 4-6 h for hydrogen peroxide, and then declines (Orozco-Cardenas, Ryan, 1999). AOS can react with unsaturated fatty acids causing peroxidation of membrane lipids of plasmalemma and other cell organelles, which affect the regular metabolism by damaging the cellular components and leads to loss of membrane integrity (Smirnoff 1998).

It has been shown that wounding triggers an increase in the endogenous levels of the plant hormones, such as jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA), ethylene (ET) that mediate wound-activated gene expression through unified signal transduction pathway. In contrast, phytohormones gibberellic acid (GA) and auxins/ indole-3-acetic acid (IAA) levels are affected after wounding, due to decreased biosynthesis or by oxidation during oxidative stress (Normanly, 2010; Blomster et al., 2011). The endogenous levels of auxin decline upon wounding and recovery of the initial levels of active auxins has been proposed as mechanism to limit the duration of the response to wounding (Rojo et al., 1998).

Auxin plays crucial role as a negative regulator of stress-induced morphogenic response, caused by prolonged stress exposure (Blomster et al., 2011). Following wounding, the regeneration of plant tissue is controlled by auxin produced by the young leaf directly above the wound site (Taiz and Zeiger, 2006). The phytohormone auxin plays a central role in the control of cell and plant growth. It can stimulate or inhibit cell expansion, stimulate cell division, promote differentiation of vascular tissues, inhibit shoot branching, and promote lateral root formation (Marchant et al., 2002; Aloni et al., 2003). The synthetic auxin analogue, 2,4-D, is especially active in inducing cell division, and indeed somatic embryogenesis (Pasternak et al., 2005).

According to different authors (Hirt, 2000; Kovtun et al., 2000), auxin and AOS have antagonistic effects on cell cycle progression and gene activation and it has been proposed that a possible and emerging candidate for an intermediate function in both, stress and growth responses, seems to be the auxin (Pasternak et al., 2005). The combination of an inhibitory effect of oxidative stress on the cell cycle (Reichheld et al., 1999), and a stimulatory effect on auxin levels (Pasternak et al., 2002), point that AOS and auxin are regulators of plant development during stress (Potters et al., 2007; 2009; Tognetti et al., 2011).

In order to investigate the effect of exogenous auxins on the mechanical injury induced oxidative stress in softwood cuttings, the level of lipid peroxidation was measured in the basal parts of the cuttings and leaves of five cherry rootstocks.

MATERIAL AND METHODS

Five cherry rootstocks [Mahaleb 1 and 2, Gisela 5, European ground cherry (EGC) and "Oblačinska" sour cherry] were used for the experiment. Mother trees were grown at the nursery of Institute for Fruit science, Viticulture, Horticulture and Landscape architecture, at Rimski Šančevi, Novi Sad. Stems were cut early in the morning, placed into freezer bags and transported on ice to laboratory, in order to avoid additional stress during the sampling process. Cuttings were treated with 0.5% solutions of three auxins: α-naphthylacetic acid (NAA), indolebutyric acid (IBA) and combination of these two (INCIT K), for 60 min. Control was represented with cuttings treated with distilled water. After the cuttings have been made samples were transferred to specialised cutting substrate "Steckmedium"' (Klasmann-Deilmann, GmbH 49744 Geeste Germany) with addition of slow-released fertiliser, and placed in greenhouse with fogging system. Fogging was regulated automatically and fogging intervals lasted about 30 s, followed by 60 s pause, in order to achieve 95% humidity.

As a measure of lipid peroxidation (LP) intensity, the amount of malondialdehyde (MDA) was determined by the MDA or thiobarbituric acid-reactive-substances (TBARS) assay. MDA is formed through autooxidation and enzymatic degradation of polyunsaturated fatty acids in cells. This secondary end product of the oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) via an acid-catalyzed nucleophilic-addition reaction yielding a pinkish-red chromagen with an absorbance maximum at 532 nm (Hodges et al., 1999). MDA or TBARS assay has been used extensively since the 1950s to estimate peroxidation of lipids in membrane and biological systems. The TBARS assay remains popular due to its simplicity, lack of expense, and rapidity with which large numbers of samples can be processed with minimal manipulation.

LP intensity was measured as TBARS production, spectrophotometrically. For this assay, plant material - basal parts of the cuttings and leaves, were first homogenized and then extracted in 10% trichloroacetic acid (TCA) in ratio 1:5 (w/v) and centrifuged at 12000 x *g* for 30 min at 4 °C. One cm^3 of supernatant was incubated with 4 cm^3 20% TCA containing 0.5% TBA for 30 min at 95 °C. The reaction was stopped by cooling on ice for 10 min and the product was centrifuged at 10000 x *g* for 15 min. The absorbance of the TBARS was measured at 532 nm and their concentration was determined using the MDA extinction coefficient of 155 mM/cm and expressed as nmol MDA/g fresh weight.

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

RESULTS AND DISCUSSION

Formation of lipid peroxides, their degradation, and the roles of these hydroperoxides in cellular metabolism has recently attracted interest. The most accurate approach to measure lipid peroxidation is to directly quantify the primary hydroperoxide products. However, these are extremely difficult to measure as a result of their lability and require lengthy procedures. Consequently, the determination of MDA, a secondary end

product of oxidative lipid degradation, has become the system of choice for estimating LP. Although there are methods available for directly quantifying MDA, such as with gas chromatography or high-performance liquid chromatography, simpler derivative-type methods, such as the TBARS assay, offer a relatively facile and rapid spectrophotometric technique with reduced sample manipulation.

Results obtained in this study varied depending on the rootstock, plant organ and auxin applied. The level of LP in the basal parts of the cuttings and leaves of different cherry rootstocks is shown in Figures 1-6.

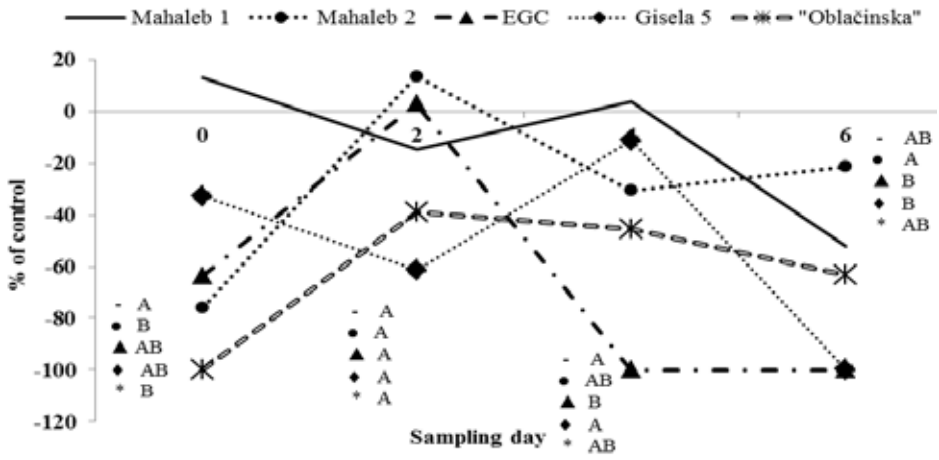

Fig. 1. Changes in LP levels in leaves of different cherry rootstocks treated with NAA.

The values marked with the same letter do not differ significantly at $P<0.05$.

All cherry rootstocks reacted in the same manner after mechanical injury was made, but some differences concerning auxin applied are visible. The accumulation of TBARS occurred in the leaves of all genotypes between 0 and 2nd day, but it seems that auxin application had positive lowering effect on LP intensity in the following days. This effect was noticeable in leaves of all rootstocks investigated when NAA was applied, during the whole experiment. The positive effect of NAA was especially pronounced between days 2 to 6, in the most of the genotypes (Fig. 1). Similar happened in Mahaleb 1 and 2, and EGC between days 2 to 4 when treated with IBA (Fig. 2). Same as for NAA, Gisela 5 and "Oblačinska" rootstocks showed no increase in LP in their leaves compared to control when IBA was applied, as well. Application of INCIT K seems also to help plants and their antioxidant protection systems to cope with the stress because in all rootstocks, 6 days after the cutting, LP intensity was significantly lower compared to control (Fig. 3).

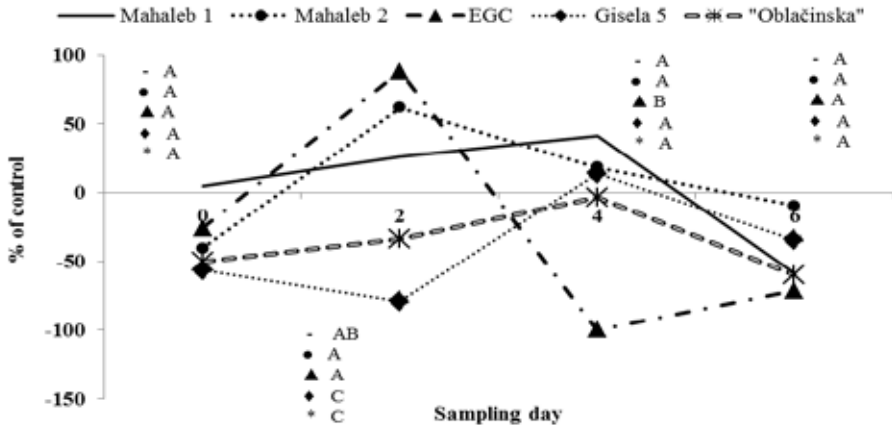

Fig. 2. Changes in LP levels in leaves of different cherry rootstocks treated with IBA.
The values marked with the same letter do not differ significantly at P<0.05.

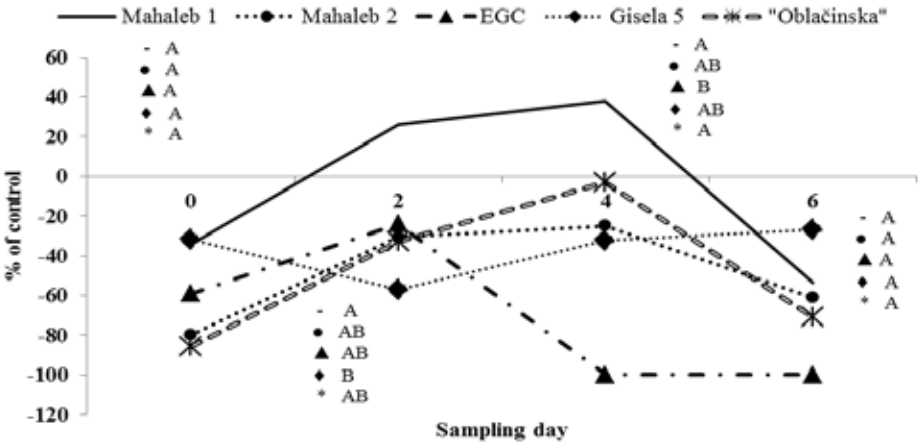

Fig. 3. Changes in LP levels in leaves of different cherry rootstocks treated with INCIT K.
The values marked with the same letter do not differ significantly at P<0.05.

Contrary to leaves, LP intensity in the basal parts of the cuttings was significantly higher. As expected, the LP occurred predominantly in the tissue where cuttings have been made – in their basal parts (Figs. 4-6). Still, rootstocks reacted differently – Mahaleb 1 was at least affected by the injury and its LP level was the lowest on the beginning of the experiment (day 0). The best LP-lowering effect was detected once again with the phytohormone NAA (Fig. 4).

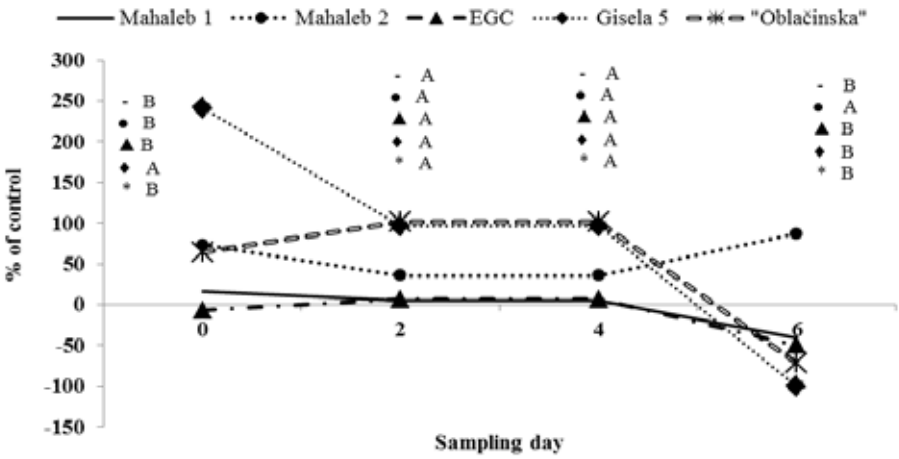

Fig. 4. Changes in LP levels in softwood cuttings of different cherry rootstocks treated with NAA.

The values marked with the same letter do not differ significantly at P<0.05.

Rootstock EGC reacted similarly to NAA, having LP level same as in control cuttings. Mahaleb 2 rootstock was the only one that showed significant damage in membrane structure after NAA was applied, having LP level 30-80% higher then control. IBA also helped rootstocks to lower their TBARS accumulation and all rootstocks had their LP levels on the same or lower level then control (Fig. 5). Application of INCIT K affected LP intensity in the basal parts of rootstocks investigated quite differently; in Mahaleb 2 and "Oblačinska" oxidative damage could not be prevented and LP level was about 100% higher compared to control (Fig. 6). On the other hand, in the rest of the rootstocks this auxin helped in lowering the peroxidation of cellular components including cell membranes.

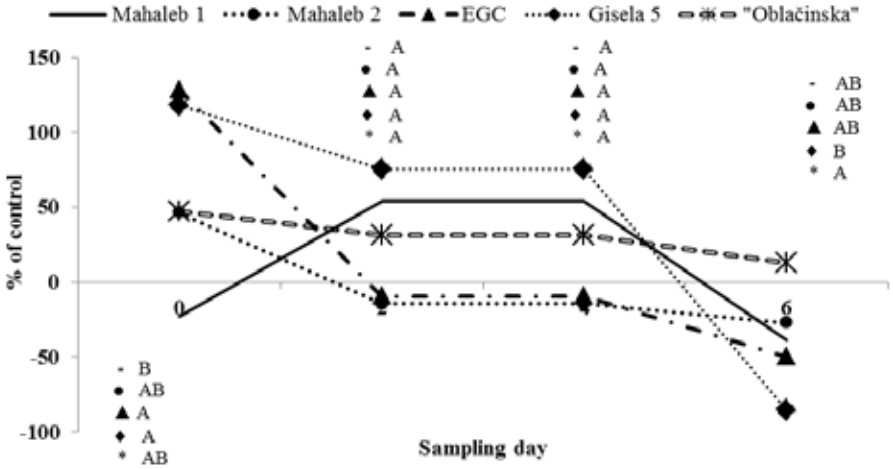

Fig. 5. Changes in LP levels in softwood cuttings of different cherry rootstocks treated with IBA.

The values marked with the same letter do not differ significantly at P<0.05.

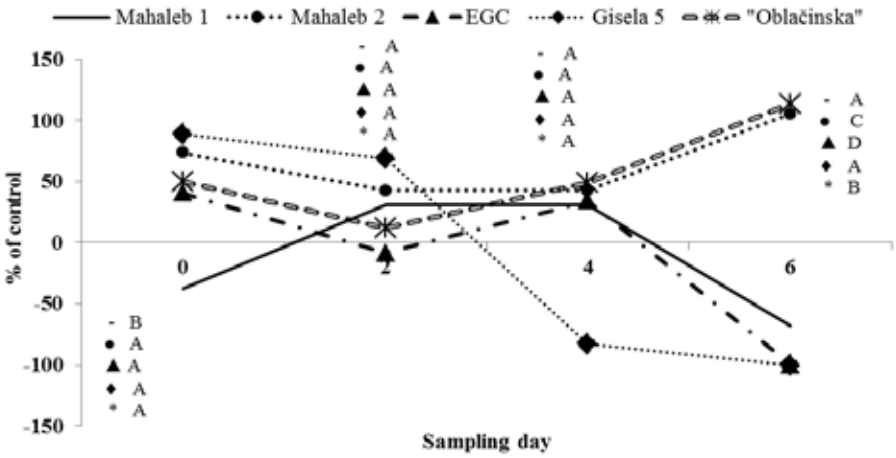

Fig. 6. Changes in LP levels in softwood cuttings of different cherry rootstocks treated with INCIT K.

The values marked with the same letter do not differ significantly at $P<0.05$.

It has been established that in the most of the sour cherries examined auxins showed lowering effect on LP which points to their positive effect, not only on rooting of softwood cuttings, but on their antioxidant system, as well. LP-lowering effect of the auxins may be due to their interaction with other hormones involved in the process of LP that is tightly connected with cell death responses, such as auxin antagonist JA, which regulates cell death responses (Blomster et al., 2011).

Comparison of the rootstocks investigated showed that Mahaleb 1, EGC and Gisela 5 reacted positively to application of all auxins, having the lowest production of end-products of the LP. This good regenerative characteristic should be acknowledged and used in the fruit softwood cutting production. In the same time it should be mentioned that phytohormone NAA showed the best antioxidant and regenerative effect and affected beneficially most of the rootstocks which recommends it for rooting of softwood cuttings.

CONCLUSION

Results obtained for the LP intensity varied depending on the rootstock, plant organ and auxin applied. The best LP-lowering effect of auxins occurred in all genotypes when treated with NAA. In the leaves, the LP peaked on the 2nd day while the best LP-lowering effect was recorded in European ground cherry rootstocks on the 4th day, independently of auxin applied. The highest MDA production in leaves was recorded in Mahaleb 2 and European ground cherry (EGC) rootstocks treated with IBA (60-90% higher then control) two days after the cuttings were made. It has been established that in the most of the rootstocks examined auxins showed lowering effect on LP which points to their positive effect, not only on rooting of softwood cuttings, but on their antioxidant protection system, as well.

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PROMENE INTENZITETA LIPIDNE PEROKSIDACIJE U REZNICAMA PODLOGA ZA VIŠNJU I TREŠNJU TRETIRANIM AUKSINIMA

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Izvod

U radu je ispitan nivo oksidativnog stresa izazvanog mehaničkom povredom biljke prilikom proizvodnje reznica, u bazalnom delu i listovima pet podloga za višnju i trešnju (Magriva 1 i 2, Gizela 5, Stepska (EGC) i "Oblačinska" višnja). Istovremeno, reznice su tretirane 0.5% rastvorima tri auksina (α-naftilsirćetna kiselina, NAA, indolbuterna kiselina, IBA i kombinacija ovih hormona, INCIT K), u periodu od 60 min, kako bi se utvrdio efekat auksina na antioksidantni status reznica višnje. Uzorci su uzimani nakon 0, 2, 4 i 6 dana. Kontrolu su činile reznice držane u vodi bez hormona. Intenzitet lipidne peroksidacije (LP) je meren kao produkcija malondialdehida (MDA), spektrofotometrijski na 532 nm, i izražen je u nmol MDA g^{-1} sveže mase.

Dobijeni rezultati su varirali u zavisnosti od podloge, organa biljke i primenjenih auksina. Utvrđeno je da je intenzitet LP u bazalnom delu bio najveći prvog dana, nakon ozleđivanja, a da su svi ispitivani auksini snižavali produkciju MDA u narednim danima. Najbolji efekat pokazala je Magriva 1 tretirana sa NAA. U listovima, LP je bila najveća 2. dana, a najbolji efekat na smanjenje pokazali su primenjeni auksini u Stepskoj višnji (4. dana). Najveća produkcija MDA u listovima zabeležena je u Magrivoj 2 i Stepskoj višnji tretiranih sa IBA, 2. dana nakon odsecanja reznica (60-90% više od kontrole). Utvrđeno je da u većini podloga pod dejstvom ispitivanih hormona dolazi do smanjenja intenziteta LP što ukazuje na pozitivan efekat primenjenih auksina, ne samo na ožiljavanje reznica nego i na njihov antioksidantni sistem.

Ključne reči: reznice, oksidativni stres, auksini, lipidna peroksidacija.

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INTESTINAL PARASITES OF POULTRY IN INTENSIVE FARMING WITH SPECIAL EMPHASIS ON *BLASTOCYSTIS SP.*

TAMAŠ ŠILI, VESNA LALOŠEVIĆ[1]

SUMMARY: The prevalence of most parasitic diseases of poultry is significantly reduced in commercial farming systems as a result of improved conditions of keeping, hygiene and management. However, parasitic diseases are still of great importance in intensive farming systems on deep litter or in "free range" breeding. A large number of parasites can cause disruptions in productivity, such as reduced weight gain, poor feed conversion, reduced egg production and even fatal outcome in severe infestation. In the practical part of the survey we examined poultry feces of different categories and age, from different poultry farms. Research has proven the presence of intestinal parasites Heterakis gallinarum, Ascaridia galli, Eimeria spp. and Blastocystis spp. in poultry fecal material. About all of this parasites, except Blastocystis we know more or less everything. For the reason Blastocystis is an emerging parasite in poultry production our discussion is mainly based on this zoonotic protozoa.

Key words: *intestinal parasites, poultry, Heterakis gallinarum, Blastocystis.*

INTRODUCTION

Poultry production/farming is spread throughout the world, both in extensive, and in intensive form. Unlike some products of animal origin, production, marketing and consumption of poultry products is related to less social and religious taboos. These factors have significantly influenced that poultry products become the most important source of protein for human nutrition worldwide. Expansion of poultry production, compared to other food industries of animal origin, suitable for easy industrialization, rapid turnover, low cost production and establishing an effective prophylaxis of diseases. Important factors in the continued development of poultry production in many countries include: market acceptability and attractiveness of poultry meat, competitive

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Original scientific paper / *Originalni naučni rad*
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price and relative ease of mastering and application of new technologies, such as health-care systems.

There are basically two systems of poultry farming: the modern commercialized-intensive systems and traditional-extensive systems. Extensive poultry farming systems are characterized by low inputs in the field of breeding, nutrition and disease control and low outputs. Intensive system includes high inputs but also high outputs. Closing the chain of production, improving methods of sanitation and disinfection, pest control, and production under the principle "all in all-out", as well as prophylactic use of vaccines and antibiotics have significantly reduced the occurrence of disease in the modern poultry industry. However, widespread and uncontrolled use of antibiotics has led to the emergence of microorganisms resistant to many antibacterial and antiparasitic drugs. The development of resistant pathogens, like E. coli, Salmonella, Eimeria spp. etc. leads to significant problems in the commercial poultry sector. Particular importance is given to zoonotic diseases such as salmonellosis and campylobacteriosis. Besides all this concern about drug residues in poultry products is increasing among consumers.

The prevalence of most parasitic diseases of poultry was significantly reduced in commercial farming systems as a result of improved housing conditions, hygiene and management. However, parasitic diseases are still of great importance in deep litter systems, free-range and organic production systems. In poultry, a large number of parasites can cause disruptions in productivity, such as reduced weight gain, poor feed conversion, reduced egg production and even death in severe infestations. In addition, the parasites can greatly reduce the organism's resistance to various diseases, reduce the immune response after vaccination, and lead to exacerbation of pre-existing pathology. If we take into account the fact that in our country poultry farming is done mainly in systems with deep litter, or due to economic reasons little importance is given to biosecurity measures, antibiotics and other drugs are used unrestrained, there are all the conditions present for development, reproduction and harmful effects of parasites, even in intensive poultry farming.

Aim of the research was to compare two different industrialized poultry production systems from the aspect of presence or absence of intestinal parasites. One of our goals was also to make an insight in the prevalence of Blastocystis sp. in poultry, and try to explain some related epidemiological and clinical aspects.

MATERIAL AND METHODS

We conducted a cross-sectional prevalence study of intestinal parasites in various poultry production systems from four farms on the territory of Vojvodina, during spring-autumn 2010. The study included farms with deep litter production systems and a farm with 2/3 floor grids in the houses.

In the first stage we compared two farms, one with deep-litter production system and one with 2/3 floor grids. We collected 20-20 fecal samples from both farms. A hazelnut sized homogenized fecal samles were suspended in 20-30 ml technical glycerine in test tubes and centrifugated at 1500 rpm for 4-6 minutes. After completing the spin one drop of supernatant was transferred with a plastic tube on glass slides. Prepared samples were then observed at low and high magnification under a light microscope.

In the second stage we collected 12 fecal samples from poultry of different categories and ages, from all four farms. A collective sample consisted of 50 g of feces,

which was uniformly collected from a number of places within one or more objects. As a diagnostic procedure we used the method of direct microscopic examination of native preparations, now without flotation-concentration. Fecal samples were well mixed and homogenized. We poured into the test tubes about 20-30 ml of normal saline and then transferred into them a small amount of homogenized feces and then we mixed it using a wooden stick. A drop of the sample thus prepared was placed on glass slides using a plastic tube. A number of slides were prepared without staining, and some of the preparations we stained by Giemsa and finally covered with glass cover slips. In this way, native preparations were obtained which were then observed under light microscopy at low and then high magnification.

RESULTS AND DISCUSSION

Preparations that were prepared by flotation concentration revealed that the incidence of intestinal parasites is largely expressed in a system with deep litter, while our study did not prove the presence of any forms of intestinal parasites in the system with the grid floor. From 20 samples of feces from the system with deep litter in 6 cases there was a smaller or larger number of nematode eggs Heterakis gallinarum, while in one sample besides Heterakis gallinarum eggs we also found Ascaridia galli eggs. Our null hypothesis, that there are no differences between the two production systems from the aspect of intestinal parasites appearance, is rejected with 99% of confidence based on the conducted t-test (t_0=5,824, $t_{(40,99)}$=3,551; t_0>t). In the second stage, light microscopy revealed that out of 12 examined, eight samples were positive for some type of intestinal parasites (Table 1).

Table 1. The results of direct light microscopy (stage two of the research)

Sample no.	Category	Age	Farming system	Parasites found
1	Layer parent stock (in lay)	37 weeks	deep litter	Blastocystis sp., Heterakis gallinarum
2	Layer parent stock (in lay)	87 weeks	deep litter	Blastocystis sp.
3	Broiler parent stock (in lay)	77 weeks	deep litter	-
4	Pullets (in rearing)	10 weeks	deep litter	-
5	Pullets (in rearing)	4 weeks	deep litter	Eimeria spp.
6	Broilers	7 weeks	deep litter	Blastocystis sp.
7	Broiler parent stock (in lay)	28 weeks	2/3 floor grid	Blastocystis sp.
8	Broiler parent stock (in lay)	60 weeks	2/3 floor grid	Blastocystis sp.
9	Broilers	5 days	deep litter	-
10	Pullets (in rearing)	6 weeks	deep litter	-
11	Pullets (in rearing)	17 weeks	deep litter	Blastocystis sp.
12	Commercial laying hens	33 weeks	deep litter	Blastocystis sp.

One sample was positive for Heterakis gallinarum and Eimeria spp., while as many as 7 samples were positive for *Blastocystis*. Microscopy demonstrated a high prevalence of Blastocystis in poultry from 3 out of 4 farms. Age, production category and farming system had no effect on the prevalence of *Blastocystis* in poultry. *Blastocystis* is equally

present in younger and older categories of poultry, except that the prevalence in laying poultry (over 18 weeks of age) was above 80% (5 of 6 samples were positive), whereas in rearing it was much lower, only about 30% (2 of 6 samples were positive). *Blastocystis* was present also in heavy and light provenance poultry, in both housing systems. Number of *Blastocystis* organisms in fecal material was approximately the same on all three farms, or for all categories of poultry, and amounted to approx. 4-10 per field, at magnification of 400 times. The cells varied in their size from approximately 5-50 μm. The cells were generally spherical or oval shaped. The cytoplasm of most cells was noticeable as a thin zone around the central vacuole. By light microscopy organelles were poorly visible.

Despite the ubiquity of the genus *Blastocystis* in the intestinal tract of humans and range of animals, very little is known about this organism (Zierdt, 1991; Boreham and Stenzel, 1993). Information about the taxonomy, life cycle, transmission, host range and pathogenicity of *Blastocystis* is not definitive. Most of the presently accepted data have derived from human isolates of Blastocystis hominis (Lee and Stenzel, 1999).

Blastocystis hominis was first reported as a yeast in human faecal samples. Following a series of physiological and morphological comparisons carried out in the 1960s *Blastocystis* was placed among the protozoans, but only recently it was classified as a protozoa after an analysis of small-subunit rRNA sequences placed it firmly as a member of the Stramenopiles along with other organismas such as diatoms, brown algae, slime nets, and water moulds (Silberman et al., 1996). For over 80 years the general opinion was that *Blastocystis hominis* is an intestinal parasite restricted to humans and other primates (Zierdt et al., 1988), but in the past 20 years *Blastocystis hominis* like organisms have been found in variety of animals such as mammals, birds, reptiles, amphibians and occasionally in insects. These include *Blastocystis galli* from chickens (Belova and Kostenko, 1990), *Blastocystis anatis* from ducks (Belova, 1991) and *Blastocystis anseri* from geese (Belova, 1992). Although molecular studies on *Blastocystis* isolates from humans and animals showed that the parasite is genetically polymorphic, most of the isolates from humans and animals are identical or very close to each other based on some genetic indices (Yoshikawa et al., 1996, 1998, 2003, 2004a; Abe et. al., 2003; Arissue et al., 2003; Noël et al., 2003). Besides that, current epidemiological and experimental data demonstrate the poor host specificity of *Blastocystis* and proves its transmission from human-to-human, animal-to-human, human-to-animal and animal-to-animal (Parkar et al., 2007). As a result previous nomenclature restricted to species such as *Blastocystis galli* for isolates from chickens, *B. anatis* from domestic ducks, *B. anseri* from domestic geese, etc. is proven inefficient. For these reason, a consensus published on 2007 proposed the use of the term *Blastocystis sp.* followed by a subtype (from 1 to 10) for mammal and avian isolates, including those isolated from humans (Stensvold et al., 2007a). Therefore, as suggested by Yoshikawa et al. (2004b) most of *Blastocystis* isolates from humans and other animals have been accepted as zoonotic parasites.

What we know about the morphology and life cycle of *Blastocystis sp.* mainly originate from studies conducted on *Blastocystis hominis* (Figure 1.), but as noted *B. hominis* is no longer known as a separate species so all the collected data about morphology and life cycle is applicable on *Blastocystis sp.*

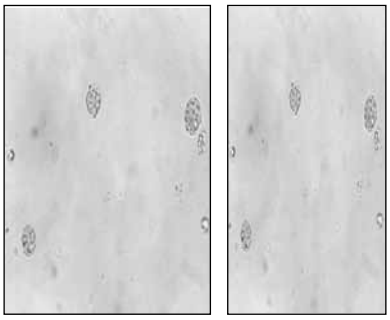

Fig. 1. *B. hominis*, human feces, native preparation, magnification 400x, orig.

Reports of the morphology of *B. hominis* from culture samples commonly have noted three major forms, vacuolar, granular, and ameboid of the organism (Boreham and Stenzel, 1993; Zierdt, 1991; Zierdt et al., 1967). The vacuolar form has been considered to be the typical *Blastocystis* cell form (Zierdt, 1991). The granular form of *B. hominis* has an ultrastructure similar to that of the vacuolar form, apart from having morphologically and cytochemically different central vacuole contents (Dunn et al., 1989; Tan and Zierdt, 1973). Vacuolar and granular forms usually are spherical cells, although irregularly shaped cells may be present in culture samples (Stenzel and Boreham, 1996). Vacuolar forms vary greatly in size, with the average diameter of cells usually being between 4 and 15 ηm (Zierdt, 1991). Granular forms often are slightly larger than the average vacuolar forms, and diameters of 10 to 60 ηm (Zierdt et al., 1967). The vacuolar and granular forms display a thin peripheral band of cytoplasm surrounding a large central vacuole. The organelles usually are found in thickened areas of cytoplasm, often appearing at opposite poles of the cell. These areas of cytoplasm may protrude into the central vacuole (Dunn et al., 1989; Zierdt, 1991) or extend outward to give the cell an irregular outline (Dunn et al., 1989).

Most organelles (Mitochondrion-like organelles, Golgi complex, one or more nuclei) appear to be simple representations of their type (Stenzel and Boreham, 1996). There are several reports about different forms of *Blastocystis*, like multivacuolar and avacuolar forms, ameboid form and a cyst form, but the results are insufficient for a definitive conclusion, although recent studies suggest that the cyst form is likely to be the infective form of *Blastocystis* (Stenzel and Boreham, 1996). This two authors also suggest that binary fission is the only plausible method of reproduction for *Blastocystis* that has been demonstrated. Plasmotomy, endodyogeny, schizogony, and sporulation are not supported by morphological data.

The prevalence of *Blastocystis sp.* infection is high among laboratory rats (60%), pigs (70-95%) and birds (50-100%) (Tan, 2004). The higher prevalence of Blastocystis infection in birds can be correlated to the difference in behavior between birds and mammals, because birds like chickens often eat feed contaminated with feces so it is reasonable to speculate that the chicks are more frequently ingesting the contaminated fecal cysts rather than mammals (Yoshikawa et al., 2004a), and it is known that the primary transmission route is fecal-oral. Also, backyard and free-range chickens easily contaminate drinking water, and by The World Health Organization publication on drinking water quality (2011) Blastocystis sp. is one of the pathogens that must be considered as a waterborne zoonoses.

Animals exhibiting a high prevalence of *Blastocystis sp.* infections may represent an extensive reservoir for infection of humans (Noël et al., 2005). It is proven that experimental animals, such as chickens, can be infected with human-isolate subtypes, thus indicating the zoonotic potential of some human *Blastocystis* isolates (Iguchi et al., 2006). Zoonotic transmission of B. hominis has been speculated since epidemiological studies suggested a conection between close contact with animals and blastocystosis in humans. The mode of transmission is mainly unclear but may be associated with animal contact and with the ingestion of food and water (Lee et al., 2012) contaminated with cysts from reservoir hosts. The studies of Doyle et al. (1990) and Salim et al. (1999) reported that people who work closely with animals do stand at risk of aqcuiring *Blastocystis* infection, and that 44% of examined patients harboring *B. hominis* as the sole enteric pathogen had a history of previous exposure to animals.Thus the high prevalence of *Blastocystis* in chickens represents a significant source for human infection.

The clinical significance of *Blastocystis* in chickens is contradictory because the parasites were isolated from animals showing some clinical symptoms, but also from clinically completely healthy individuals.

In our study we isolated *Blastocystis sp.* from poultry that has been completely healthy, without any symptoms that would indicate intestinal infection, but also from a flock with mild sub-acute to chronic diarrhea. In flocks from which we isolated *Blastocystis* parasites, we did not observe any disturbances in productivity, growth and body weight maintenance. There are several opinions about the mechanism of pathogenesis. Studies have focused on immunological reactions of epithelial cells to proteases secreted by *Blastocystis*. *Blastocystis* initially down-regulates and then up-regulates production of the inflammatory cytokine IL-8 in epithelial cells (Long et al., 2001), causing gastrointestinal symptoms such as enteritis, colitis and ileitis.

Most clinical methods are based on finding the organism in stool specimens. Variations on this method include concentration, staining, culturing and molecular diagnosis by polymerase chain reaction (PCR) testing (Termmathurapoj et al., 2004). The last one is the most efficient diagnostic technique used, although is more costly, it is known to be more sensitive than the direct smear and xenic culture (Stensvold et al., 2007b). PCR is important not only for diagnosis but also for subtyping, thus some new modified PCR techniques (Santín et al., 2011) can play a great role in explaining the complexity of this genus, its share in human and animal disease, as well as its zoonotic potential. By Roberts et al. (2011) microscopy detects only 48% of the positive samples. If the use of PCR is not feasible for as a diagnostic method, it is recomended that at least two different diagnostic techniques be used for detection of *Blastocystis*. Because of mentioned we need to be cautious when interpreting prevalence reports, especially for studies that rely solely on microscopy.

The requirement for treatment of *Blastocystis* infections remains controversial. In the absence of conclusive evidence of pathogenicity of the organism, treatment with potentially dangereus drugs and the inability to undeniably prove the real causes of symptoms is a significant problem. The treatment should be applied with caution, only after a thorough clinical examination of other possible causes of symptoms. In vitro studies on the effects of drugs, were conducted exclusively in Blastocystis homminis cultures. From 10 antiprotozoal drugs tested, metronidazole proved to be most effective by preventing the production of IL-8. Antibacterial drugs such as ampicillin, penicillin, streptomycin, gentamicin, colistin, vancomycin and antifungal amphotericin B, do not

show any effectiveness against parasites. Considering the present data the most likely route for transmission of *Blastocystis* is the fecal-oral. Thus, control measures must include good hygienic practice, appropriate hygiene in common and sanitary facilities and education to prevent fecal contamination of the enviroment and ingestion of contaminated material (Stenzel and Boreham, 1996).

CONCLUSION

The first stage of are research clearly demonstrates the advantages of keeping poultry on grid floor, and the role of such a system in preventing the development and spread of parasitic diseases.

Based on the results of this research, especially its second phase, as well as the results obtained by other authors we can say that our current knowledge of *Blastocystis* and the subsequent disease that it causes is not enough to determine the importance of parasites in animals. Current literature has to be taken into consideration with a certain caution, because it contains numerous unclear and unconfirmed data. In recent years there has been progress in defining the morphology and life cycle of the parasite, thanks to successful experiments on rats, mice and birds. However, a more detailed knowledge of the biology of organisms is essential for defining effective methods of diagnosis, treatment and control. In future research it is important to determine the infective stage of the parasite and the stages in the life cycle as a suitable target for therapy. It is necessary to accurately determine the function of cell organelles, which can help determine the target point for therapy.

From the clinical aspect, more evidence is needed before we declare *Blastocystis sp.* as the cause of disease in animals. The case studies are of limited value in research, because it is almost impossible to rule out other causes of infections and non-infectious, non-specific symptoms associated with *Blastocystis* infection. Epidemiological findings are significantly limited. It is necessary to determine the true prevalence in different populations of poultry, then determine whether there are different strains that cause symptomatic or asymptomatic disease, and verify the exact mode of transmission. Due to the current lack of incontrovertible data about this organism it is difficult to predict the status of this parasite in the period of 5-10 years, but it is certain that by thorough research may lead to new surprising conclusions.

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CREVNI PARAZITI U INTENZIVNOM ŽIVINARSTVU SA POSEBNIM OSVRTOM NA BLASTOCYSTIS SP.

TAMAŠ ŠILI, VESNA LALOŠEVIĆ

Izvod

Prevalenca većine parazitskih bolesti živine signifikantno je redukovana u komercijalnim sistemima uzgoja, kao rezultat poboljšanih uslova držanja, higijene i upravljanja. Međutim, parazitske bolesti su i dalje od velikog značaja kod intenzivnih sistema uzgoja i eksploatacije na dubokoj stelji, odnosno kod slobodnog komercijalnog uzgoja na ispustima, tzv. „free range" a veliki broj parazita može prouzrokovati poremećaje u produktivnosti, kao što su smanjen prirast, loša konverzija hrane, smanjenje nosivosti pa čak i smrtni ishod kod težih infestacija. U praktičnom delu rada izvršen je koprološki pregled fecesa živine različitih proizvodnih kategorija i uzrasta sa farmi na kojima se vrši odgoj i eksploatacija u intenzivnoj formi. Istraživanje je dokazalo prisustvo crevnih parazita Heterakis gallinarum, Ascaridia galli, Eimeria spp. i parazita Blastocystis sp. u fekalnom materijalu živine. Sve što je vezano za ove parazite, osim za Blastocystis sp. nam je manje ili više poznato. Pošto je Blastocystis parazit čija je uloga u živinarstvu tek u nastajanju, diskusija je uglavnom posvećena ovoj zoonotskoj protozoi.

Ključne reči: crevni paraziti, živina, Heterakis gallinarum, Blastocystis.

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TURKISH HAZEL (CORYLUS COLURNA L.) SEEDLING CHARACTERISTICS AS ROOTSTOCK FOR HAZELNUT CULTIVAR GRAFTING

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SUMMARY: In the Faculty of Agriculture plant nursery at the University of Novi Sad, Serbia, Common hazel (Corylus avellana L.) cultivars were grafted on the Turkish hazel (Corylus colurna L.) rootstock. To produce high-quality planting material, seeding success, growth and development of one-year-old seedling was followed. This paper presents results of Turkish hazel A_1, B_1, B_4, B_7 and C_3 genotype nut germination from sowing performed in 2009. The results indicate high percentage of germinated nuts and a high plant survival rate. One-year-old seedlings measured during a vegetation rest period in 2010 and two-year-old seedlings measured in 2011 were better developed compared to the seedlings examined in the previous years, indicating that the application of the drip irrigation system in seedling development yields positive results.

Key words: *Turkish hazel, Corylus colurna L., field germination, one-year old seedlings, two-year old seedlings.*

INTRODUCTION

In nursery production, biological growth properties of the root and trunk are mostly manifested during the first year of plant development. Consequently, most studies in this field focus on the evaluation of the growth indicators in one-year and two-year-old Turkish hazel (*Corylus colurna* L.) seedlings. For example, Harris et al. (2005) examined the root length and the above-ground part of the Turkish hazel, Green Ash and Lilac seedlings. The authors found the correlation between the measured parameters and the total length of the root system, which was measured with rhizotron.

Original scientific paper / *Originalni naučni rad*

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In an earlier study, Harris et al. (2001) investigated one-year-old scions of Turkish hazel. Their findings indicate that the root growth in plants taken out in the fall is favorable when compared to those that have been kept in cold storage and planted in the spring. Based on these results, the authors recommend autumn planting for rootstocks without any irrigation.

In the nursery at Rimski Šančevi, autumn sowing of seeds for the production of rootstocks of different fruit species, including Turkish hazel (Ninić-Todorović, 1990), is performed annually, as the nursery has a cold chamber for storing graft branches as well as suitable storage of stratified seeds.

With application of optimal agrotechnical measures, in 2010 and 2011, we established remarkable growth of seedlings of specific genotypes, which surpassed those used in earlier studies (Ninić-Todorović et al., 1994; 2003; 2006; 2007).

MATERIAL AND METHODS

Turkish hazel fruits were collected at the time of physiological independence of cup stems from the mother tree. The key fruit maturity indicators are yellow-green cup and light brown nut color (Ninić-Todorović, 1990). Nut sowing was performed in the middle of October 2009, using genotypes A_1, B_1, B_4, B_7 and C_3 collected in the nursery of JKP "Gradsko Zelenilo" in Novi Sad. The optimal spacing and seeding depth—7 cm for proper development of seedlings of Turkish hazel in the first two years—was ensured. Sampling for the purpose of growth indicator testing in the one-year-old seedlings was carried out at the end of the vegetation period in 2010. Height and diameter at the base of two-year-old seedlings were measured *in situ* during the period of vegetative rest in 2011.

Measurements of the seedling root crown diameter and tree diameter at the base of two-year-old seedlings were conducted using a micrometer of 0.01 mm precision, while tree height was measured using a standard metric ruler. To determine the seedling root system morphological characteristics, we used a software package for image analysis "ImageJ''.

Data processing was performed using the statistical package STATISTICA 10 (StatSoft,Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The best nursery production results were obtained from the October seed sowing, as indicated by high field germination. According to long-term meteorological data related to the area where the nursery at Rimski Šančevi is located, low temperatures and frosts typically occur in November. Consequently, seeds sown in October have ample time to go through the period of warm stratification before the first appearance of frost. In November, once frost appears, physiological drought period and subsequent ripening stages of the embryo commence. The period from October, when the nuts are sown, until May, when the seedlings emerge, is optimized to overcome the prolonged dormancy of Turkish hazel seeds.

Nut classification is performed by collecting of cup from each tree separately. The seed material is carefully handled during processing and storage. Based on the indica-

tors of morphological characters (Ninić-Todorovic, 1990; 2009), the nut size is uniform within each sample.

The results (Table 1) indicate a high percentage of field germination and high plant survival rate. The values ranged from 76.62% for germination and 91.30% for survival (genotype A_1) to 88.20% and 95.40% (genotype C_3), respectively, after the first growing season. Thus, these results indicate that favorable plant selection and management enables successful development of seedlings in the first and second year of growth *in situ*.

Table 1. Field germination and survival of plants moment

Genotype	Field germination (%)	Survival percentage (%)
A_1	76.62	91.30
B_1	78.19	85.60
B_4	80.30	93.70
B_7	84.50	95.30
C_3	88.20	95.40

In the conditions of higher field germination and plant density in the first vegetative cycle, the number of surviving seedlings was higher than in the set with fewer plants, characterized by lower germination energy. Lower germination causes development of unfavorable physiological, morphological and technological properties in nuts. In the year when field germination analysis was performed, protection from rodents was also successful.

Table 2 presents mean values of parameters examined for one-year seedlings of Turkish hazel, errors in arithmetic means and standard deviation. Average tree height values of one-year-old Turkish hazel seedlings ranged from 28.60 cm for genotype A_1 to 42.93 cm for genotype C_3. Genotype A_1 was characterized by the lowest mean root length (29.13 cm), the lowest root crown diameter (5.76 mm) and the lowest number of the first line roots (22.60). Maximum average values of the root length and the root crown diameter were observed in genotype C_3, while the maximum number of the first line roots (31.93) was observed in genotype B_7. Trunk and root weight, two very important parameters for the examination of one-year-old seedling growth, proved to be highly variable compared to other examined parameters. The highest values of the trunk and root weight were measured for genotype C_3 (15.04 g and 17.03 g), whilst the lowest trunk weight was found in genotype A_1 (7.22 g), and the lowest mean root weight was observed in genotype B_4 (10.17 g).

Table 2. Morphometric characteristics of the one-year old seedlings of Turkish hazel (*Corylus colurna* L.) in 2010. year

Genotype	Tree hight (cm)	Root lenght (cm)	Root crown diameter (mm)	Number of first line roots	Trunk weight (g)/	Root weight (g)
	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx
A1	28.60±1.67*	29.13±0.74*	5.76±0.37	22.60±0.89	7.22±1.01	12.01±1.34
B1	28.66±1.66*	29.26±0.80*	7.34±0.48	28.36±0.82	8.70±1.13*	10.48±1.12
B4	36.37±2.38	31.41±0.81	8.81±0.44	24.86±1.08	8.67±1.17*	10.17±1.12
B7	40.48±1.47	32.09±1.01	9.46±0.29	31.93±1.05	13.22±0.90	14.75±1.02
C3	42.93±1.54	38.70±0.96	10.29±0.33	31.46±1.13	15.04±1.32	17.03±1.42
x±Sx	35.60 ± 0.92	32.12 ± 0.48	8.33 ± 0.21	27.89 ± 0.53	10.60 ± 0.55	12.92 ± 0.57
SD	11.30	5.89	2.66	6.55	6.74	7.08

*Marked effects are significant at $p<0.050$.

According to previous studies (Ninić-Todorović et al., 1994), the height of one-year-old seedlings ranged from 17 to 20 cm, root length ranged from 34 to 38 cm, the root crown thickness ranged from 5 to 7 mm, number of first line roots ranged from 40 to 50, trunk weight ranged from 1.6 to 2.6 g and root weight ranged from 2.6 to 3.2 g. Seedlings were developed without irrigation and with minimal care measures, which caused the root system to be longer and the above-ground part lower, i.e. such care regiment resulted in low development of all growth indicators. The root system of one-year-old seedlings is of taproot type with many thin first line roots. These findings suggest that stimulation of root development should be performed at the end of the growing season by cutting off half of the taproot length.

The examined growth indicators of one-year-old Turkish hazel seedlings, which were developing during the vegetation period in 2004, were published in Ninić-Todorovic et al., (2007). The seed material for this study was collected at the location Futoški Park, and the height of one-year-old seedlings ranged from 26.77 (B_4) to 49.93 cm (B_9), root length ranged from 29.13 (B_5) to 38.70 cm (B9), root crown diameter ranged from 7.34 (B_1) to 12.22 mm (B_8), number of first line roots ranged from 22.60 (B_5) to 31.93 (B_7), trunk weight ranged from 7.24 g (B_5) to 19.89 g (B_8), and root weight ranged from 10.48 g (B_1) to 21.85 g (B_8).

The examination of the growth parameters was carried out in very dry years (Table 2), whereby drip irrigation system was applied and proved to be extremely effective for the development of Turkish hazel seedlings. According to the results published by Ninić-Todorovic et al., (1994), two-year-old seedlings were characterized by height of 45-60 cm, 50-60 cm root length, root crown thickness of 9-12 mm, 20-35 first line roots, 4-12 second line roots, 16-25 g tree mass, and root mass of 30-40 g. These growth indicators suggest that in conditions without irrigation, seedlings were much less developed compared to the specimens analyzed in 2011, for which two indicators were measured *in situ* and the results presented in Table 3.

Table 3. Morphometric characteristics of the two-year old seedlings Turkish hazel (*Corylus colurna* L.) in 2011

Genotype	Tree high (cm)	Diameter at the base of the tree (mm)
	$x \pm S_x$	$x \pm S_x$
A_1	80.78±2.16*	13.77±0.45*
B_1	92.01±4.26	15.50±0.47
B_4	102.14±5.93	15.55±0.60
B_7	86.52±5.85*	15.52±0.72
C_3	100.14±98	16.60±0.81
$x \pm S_x$	92.32±2.70	15.39±0.34
SD	10.46	1.31

*Marked effects are significant at p<0.050.

After examining the root crown height and diameter of two-year-old Turkish hazel (*Corylus colurna* L.) seedlings, significant differences between genotypes are evident. Tree height ranges from the minimal value in genotype A_1 (80.78 cm) to a maximum value in genotype B_4 (102.14 cm). The total average value for the tree height was 92.32 cm.

The average values for the root crown diameter in two-year-old seedlings were uniform for all genotypes, except genotype C_3, for which high values of this parameter were recorded (16.60 mm). At the level of all genotypes, low standard deviation (SD = 1.31) was observed. These results indicate that the seedlings of Turkish hazel genotypes B_4, B_7 and C_3 were developed in the first year as a rootstock for grafting hazelnut cultivars. Grafting was performed at the beginning of April 2012, using the English method of connecting to two-year-old seedlings in order to obtain highly grafted hazelnut seedlings.

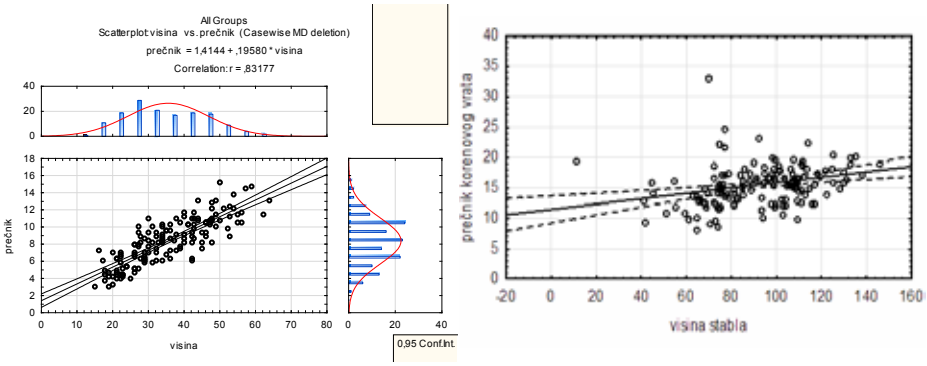

Fig. 1 and 2. Correlation between the height and diameter of root crown for a one-year old seedlings of Turkish hazel (*Corylus colurna* L.)

The correlation analysis of tree height and root crown diameter of one-year-old seedlings indicated high and positive correlation between the two characters. The ratio of tree height and root crown diameter of one-year-old and two-year-old Turkish hazel seedlings was r = 0.6078 r = 0.5492, respectively. The calculated correlations suggest that the growth of the above ground parts of seedlings followed the growth and development of the root system.

Grafting of hazelnut cultivars on Turkish hazel rootstock has been successfully performed in Serbia since 1989, with the key results published by Ninić-Todorović (1990; 2000), Ninić-Todorović et al. (1994; 2003; 2006), Korać at al. (1995, 1996, 2000), Cerović et al. (2007) and others.

CONCLUSION

Morphological and physiological characteristics of the nut, primarily achieved through germination, have directed the technological processes towards achieving high productivity and economy of production of hazelnut seedlings grafted on Turkish hazel (*Corylus colurna* L.) as a rootstock.

In the study year, the evaluated nut samples have demonstrated a high percentage of germination and plant survival rate.

When the seedlings were irrigated by "drop by drop" irrigation method, good development and growth indicators were measured. Tree height of one-year-old plants was in the range of 28.60 (A_1) to 42.93 (C_3) cm, which is in accordance with the results obtained for field-germinated nuts and corresponding plant survival rates. Root length and root crown diameter were the lowest for genotype A_1 (29.13 and 5.76 mm), and the highest for genotype C_3 (38.70 and 10.29 mm). Maximum average number of first line roots was measured for genotype B_7 (31.93), whereas maximum trunk weight of 15.04 g and 17.03 g root weight corresponded to genotype C_3. Two-year-old seedlings were twice as tall as the plants evaluated in the test years without irrigation. The maximum tree height (100.14 cm) and the greatest thickness at the base (16.60 mm) were measured for genotype C_3.

Grafting of hazelnut cultivars on Turkish hazel rootstock has many advantages in raising orchards. As Turkish filbert does not form offshoots from the roots, the use of advanced breeding measures is facilitated, such as pest and disease protection and mechanized harvesting. Turkish hazel planting material is suitable for organic production.

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KARAKTERISTIKE SEJANACA MEČJE LESKE (CORYLUS COLURNA L.) KAO PODLOGA ZA KALEMLJENJE SORTI LESKE

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Izvod

U rasadniku Poljoprivrednog fakulteta u Novom Sadu, na Rimskim Šančevima razvijena je tehnologija kalemljenja kultivara leske na mečjoj leski (*Corylus colurna* L.) kao podlozi. Praćen je uspeh setve, rast i razvoj jednogodišnjih i dvogodišnjih sejanaca u cilju proizvodnje kvalitetnih podloga i sadnog materijala. U radu su prikazani rezultati klijavosti orašica genotipova A_1, B_1, B_4, B_7 i C_3 mečje leske iz setve obavljene 2009. godine. Orašice su pokazale visok procenat klijavosti i momenat preživljavanja biljaka. Jednogodišnji sejanci mereni u toku mirovanja vegetacije 2010. godine i dvogodišnji sejanci mereni 2011. bili su razvijeniji u odnosu na ispitivanja sejanaca ranijih godina. Primena sistema za navodnjavanje kap po kap, dala je dobre rezultate pri razvoju sejanaca u ispitivanim godinama.

Ključne reči: mečja leska, *Corylus colurna* L., terenska klijavost, jednogodišnji sejanci, dvogodišnji sejanci.

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PREVALENCE OF DIROLIRARIOSIS IN PET DOGS IN NOVI SAD*

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SUMMARY: The aim of this study is to reveale the prevalence of dirofilarial infections in pet dogs. From the year 2010 to the year 2012, a total of 77 blood samples were collected from privately owned pet dogs in Novi Sad. All samples were examined by wet blood smears, the modified Knott test and heartworm antigen test. Circulating microfilariae of both Dirofilaria (D.) immitis and D. repens were found in dogs. Prevalence values for D. immitis and D. repens were 9,09% and 12,99%, respectively. Results of this study, compared with results of previous investigations, shows increase of infection with D. immitis and decrease of infection with D. repens. Further investigations are required with higher number of samples to confirm this findings.

Key words: *D. immitis, D. repens, prevalence, pet dogs.*

INTRODUCTION

Nematodes of the genus *Dirofilaria (D.)* are currently considered emerging agents of parasitic zoonoses in Europe. Two main filarial infections occur in domestic and wild carnivores in Europe: *D. immitis*, the etiological agent of canine and feline heartworm disease and *D. repens*, the main etiological agent of subcutaneous filarial infections. Climate changes, the existance of animal reservoirs (domestic and wild canides), and global movement of dogs have caused an increase in the spreading of these mosquito-borne nematodes. *Dirofilaria* infections have spread from the traditionally endemic/hyperendemic region of southern Mediterranean toward northern and eastern areas during recent years in Europe (Genchi et al., 2011).

The life cycle of both parasites consists of five larval stages developing both within an intermediate mosquito host (from microfilaria L1 to infective L3 larva), that also

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act as vector, and in a definitive vertebrate host (from L3 larva, through L4 and L5 larvae to the adult stage).

Dirofalaria immitis resides preferentially within the pulmonary arteries, but it may also be found in the right ventricle. As the number of heartworms increases they enter the right atrium, and eventually migrate into the caudal vena cava. Large number of parasites may obstruct the caudal vena cava and blood flow to the right atrium (caval syndrome). Adult heartworm can live up to 7 years in dogs. The presence of circulating microfilariae in blood of dogs implies the presence of adult heartworm in host. Dogs with microfilariae in their blood represent reservoirs of infection for other dogs (Ware, 2011).

Heartworm disease can be a serious and potentially fatal disease of dogs. The stage of disease depends on number of adult parasites in pulmonary arteries, the duration of infestation and host's immune response. Adult parasites damage the pulmonary arteries endothelium and induce the arterial thrombus genesis, which result in pulmonary infarction and area of consolidation around the affected vessels. Vascular and parenchymal damages increase pulmonary vascular resistance and lead to pulmonary hypertension. Pulmonary hypertension results in increased right ventricular afterload, right ventricular hypertrophy and cor pulmonale. Besides, microfilariae in blood represent circulating antigens, and may cause immune complex deposition in glomeruli or joints (Ware, 2011; Atkins, 2009; American Heartworm Society Canine Guidelines, 2010). The clinical signs of heartworm disease are mostly those of respiratory and cardiovascular system clinical signs, such as cough, haemoptysis, dyspnoea and decreased exercise intolerance.

Dirofalaria repens is a parasite of the subcutaneous connective tissues, mainly in dogs. The adult parasite resides in the subcutaneous connective tissues; females produce larvae (microfilariae) in the natural host organism and release them into the circulation. Infection with *D. repens* is generally asymptomatic. Main clinical manifestations of *D. repens* infection are nodular multifocal dermatitis and pruriginous papule (Scott and Vaughn, 1987; Haliwell and Gorman, 1989). However, in cases where dogs found to be massively infected with adult worms and with high microfilaremia in blood, gross and histopathological changes in many organs, like spleen, liver, kidney, heart, lungs and brain were reported (Kamalu, 1991). Due to all of these, *D. repens* infection of dogs is an underestimated problem in veterinary medicine (Džaja et al., 2008).

Both *Dirofilaria* species are zoonotic. Recently the case of human *D. immitis* infection was found, and confirmed by molecular analysis in Italy (Avellis et al., 2011). In Europe, human *Dirofilaria* infections are caused mostly by *D. repens* (Genchi, 2012). Though most *D. repens* infections are benign in humans and the immature worm is localized in subcutaneous tissues, the localization in the lung and in other deep tissues can mimic a tumor (Pampiglione and Rivasi, 2000). Reports suggest that full development of *D. repens* in human hosts is possible and contradict the commonly accepted belief that human dirofilariasis is caused exclusively by immature worms (Simon et al., 2012).

First cases of dirofilariosis in Serbia were found in dogs during the autopsy (Milosavljević and Kulišić, 1989). During last two decades researches on the topics of seroprevalence, diagnostic procedures, therapy and clinical cases in both dogs and humans, were performed (Lalošević et al., 2004; Tasić et al 2008; Đorđević et al., 2010; Spasojevic Kosic et al 2011; Pavlović et al., 2012, Gabrielli et al., 2012). The aim of this study is to determine the prevalence of *D. immitis* and *D. repens* infections in pet dogs in Novi Sad.

MATERIAL AND METHODS

From the year 2010 to the year 2012 pet dogs from Novi Sad were tested for dirofilaria infections. This research was done in 77 privately owned pet dogs. At the moment of testing, dogs were at least 7 months old, exposed minimally to one mosquito season and without history of treatment with macrocyclic lactones. Blood sample was collected from each dog for examining microfilariemia and antigen testing.

Microfilariemia

Techniques for detecting circulating microfilariae include microscopic examination of fresh blood smears and modified Knott test. Detection and enumeration of circulating microfilariae (mf) of both *D. immitis* and *D. repens* were carried out by the modified Knott test (Bazzochi et al., 2008). Sample of venous blood (1 ml) was mixed with 10 ml of 2% buffered formalin and centrifuged for 5 min at 200 x g. One hundred microliters of sediment was mixed with equal parts of a 1: 1000 methylene blue stain. An aliquot of 20 ml of stained sediment was placed on a slide, and examined under a microscope. The number of mf was multiplied by 10 and expressed as mf/ml. Morphological characteristics of microfilariae, such as length, width, cephalic and caudal ends, were assessed in order to differentiate microfilariae of two *Dirofilaria* species (Genchi et al., 2007).

Antigenemia

Detection of circulating *D. immitis* antigens was carried out by commercial kit SNAP Heartworm RT Test (Idexx Laboratories) according to manufacturer's instruction.

RESULTS AND DISCUSSION

Out of 77 dogs 7 dogs were infected with *D. immitis*, while 10 dogs were infected with *D. repens* (Table 1). No dogs were infected with both parasites.

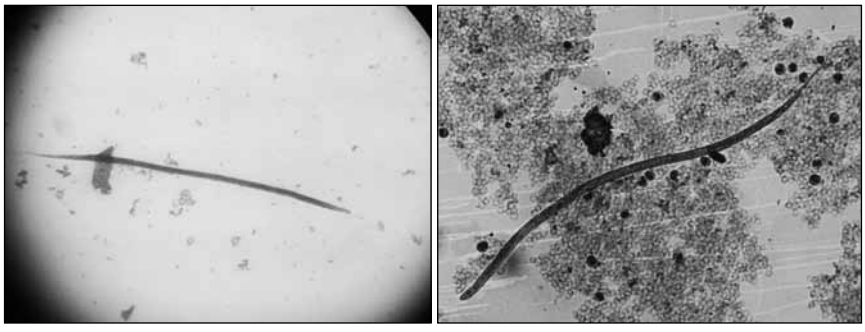
The diagnosis of heartworm infestation in dogs is based on identifying the microfilariae of *D. immitis* in a blood sample and on finding adult heartworm antigen in blood, serum or plasma. Techniques for detecting circulating *D. immitis* microfilariae (the first stage larvae L1) include microscopic examination of fresh blood smears, the Knott concentration test and the filter test. In present study native blood smears were negative in five dogs, while Knott test revealed the existance of microfilariae. The diagnosis of adult heartworm is enabled by detecting a circulating adult heartworm antigen, or occasionally by identifying worms in the pulmonary arteries or right heart using echocardiography. The diagnosis of *D. repens* in dogs is based only on identifying the microfilariae of *D. repens* in blood, and differentiation of these microfilariae from those of *D. immitis* (Fig. 1). No antigen detection test for filarial subcutaneous dirofilarial infection is available nowadays.

Table1. Results of microfilariemia and antigenemia of *D. immitis* and microfilariemia of *D. repens* in naturally infected dogs

D. immitis			D. repens	
Dog	microfilariemia	antigenemia	Dog	microfilariemia
1	7500 mf/ml	++	1	not counted
2	1200 mf/ml	+	2	800 mf/ml
3	230 mf/ml	+	3	20 mf/ml
4	0 mf/ml	+	4	50 mf/ml
5	1500 mf/ml	+	5	1750 mf/ml
6	242 mf/ml	+	6	680 mf/ml
7	1400 mf/ml	-	7	90 mf/ml
			8	160 mf/ml
			9	124 mf/ml
			10	160 mf /ml

(- negative antigen test; + low antigen level; ++ high antigen level)

In this study 5 dogs had positive heartworm antigen test with circulating microfilariae of *D. immitis*, in one dog the test was positive without circulating microfilariae, and in one dog circulating microfilariae of *D. immitis* was diagnosed with negative heartworm antigen test. Definitive diagnosis of heartworm disease is made by positive Knott test and positive antigen test. Heart worm is also diagnosed by negative antigen test if microfilaria of *D. immitis* are detected by Knott test. In case of very low heartworm burden antigen test could be negative. It takes only one female and one male to produce microfilariae and two worms may be below the limit of detection for an antigen test in an animal. Occult infection with *D. immitis* is established in case of positive antigen test and negative Knott test. Animals with adult worms may not have circulating microfilariae for several reasons: immature adults are present in the pulmonary arteries, there is only a single sex of parasite present, the worms are sterile or there is a immunologic destruction of microfilariae by host.



A **B**

Fig. 1. Microfilariae *D. immitis* (A) and *D. repens* (B).

They can be differentiate according to their length and width, cephalic and caudal end.

By using recognized methods in the diagnosing of dirofilariosis, in this study group of dogs prevalence of *D. immitis* and *D. repens* were 9,09% (7/77) and 12,99% (10/77) respectively. The investigation covered a sensitive population of dogs, old enough to develop the adult form of the parasite, as well as dogs with clinical manifestations that may arise as a consequence of dirofilaria infections (cough, fatigue, weight loss, skin lesions and nodules).

Studies of dirofilariosis in Novi Sad at first showed an infection only with *D. repens*, while *D. immitis* was undiagnosed (Tasić et al., 2008). In time period form the year 2004 to the year 2010, the prevalence of dirofilariosis in military dogs was 14% (Pajković et al., 2010), but the authors did not differentiate microfilariae. In pet dogs in Novi Sad prevalence varies from 7% in the year 2004 to 11% in 2009 (Savić et al., 2012), but in this study antibodies against *Dirofilaria* were detected. Among pet dogs in Novi Sad, prevalence of 5% (3/60) was determined by using only heartworm antigen testing (Pavlović et al., 2012). Compare to the study of Tasić et al (2008), which has provided full diagnostic protocol for dirofilarial infections, our study shows increase of infection with *D. immitis* and decrease of infection with *D. repens*. Further investigations are required with higher number of samples to confirm this findings.

Recently in Romania (Popescu et al., 2012), Russia (Guzeeva et al., 2012), Poland (Masny et al., 2011), Slovakia (Iglódyová and Miterpáková, 2012), Czech Republic (Dobešová and Svobodová, 2011), Latvia (Stepanjana, 2011), Hungary (Kucsera et al., 2012) and Croatia (Pilat et al., 2012) are published new data about the prevalence of *D. repens* and *D. immitis* in dogs and human patients.

Because of well known zoonotic potential of dirofilarial infections, it would be interesting to investigate prevalence of dirofilarial infections in both pet dogs and their owners. Further researches, including human population and entomological investigations, are needed in order to define appropriate control programs for dirofilarial infections in our country.

CONCLUSION

This study revealed a 9,09% prevalence of *D. immitis* infection and 12,99% prevalence of *D. repens* in pet dogs in Novi Sad. Because of the serious pathological changes which dirofilarial infections may cause in dogs, veterinarians should implement in their practice the prevention, diagnosis and treatment of these diseases. Due to the risk to public health, diagnostic testing of dogs and therapy of infected animals, as well as prophylactic treatment for healthy animals, are needed in order to reduce the spread of infection to other dogs and humans.

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PREVALENCA DIROFILARIOZE KOD PASA KUĆNIH LJUBIMACA U NOVOM SADU

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Izvod

Cilj ovog rada je da pokaže prevalencu infekcije dirofilarijama kod pasa kućnih ljubimaca. Od 2010. do 2012. godine pregledano je 77 uzoraka krvi prikupljenih od pasa iz Novog Sada. Uzorci krvi od svakog psa su pregledani nativnim krvnim razmazom, modifikovanim Knotovim testom i dokazivanjem postojanja antigena odraslog parazita. U krvi pasa su dijagnostikovane mikrofilarije i *D. immitis* i *D. repens*. Prevalenca infekcije *D. immitis* iznosila je 9,09%, dok je za *D. repens* prevalenca bila 12,99%. Rezultati ovog istraživanja, u odnosu na prethodna istraživanja, pokazuju povećanje infekcije pasa sa *D. immitis* i smanjenje infekcije sa *D. repens*. Dalja ispitivanja, na većem broju pasa, su potrebna kako bi se potvrdile procene ovog istraživanja.

Ključne reči: *D. immitis*, *D. repens*, prevalenca, psi kućni ljubimci.

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COPROCULTURE ASSAY FOR ESTIMATION OF *IN VITRO* LARVAL REDUCTION OF SHEEP GASTROINTESTINAL NEMATODES BY NEMATOPHAGOUS FUNGUS, *DUDDINGTONIA FLAGRANS**

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SUMMARY: Gastrointestinal nematodes in sheep represent serious problem in pastoral systems due to various production losses and significant cost in sheep industry worldwide. One of control options for these parasites in grazing sheep is application of nematophagous fungi to reduce number of available infective larvae, where Duddingtonia flagrans is one of the most promising candidates. In order to estimate effect on larval reduction in vitro, coproculture assay was performed with theoretically generated dose of chlamydospores added to faeces. Total reduction rate of larvae in fungus group compared to control was 32.45%, but without statistically significant difference in larval yields at 0.05 confidence level. Observed reduction percentage was lower in our study compared with results of other researchers. Possible reasons for low efficacy of fungus obtained in our study compared to other results are very complex and some factors are discussed.

Key words: *Duddingtonia flagrans, larval reduction, sheep, biological control, coproculture assay.*

INTRODUCTION

The importance of gastrointestinal nematodes (GIN) of sheep, beside various losses with significant cost for sheep industry, is much higher nowadays because of increase of resistance to antihelmintics routinely used for helminth control (Simin et

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al, 2011). Growing public demand for food without drug residues imposes introduction of innovative ways of controlling clinical and subclinical parasitism on pastoral farming systems, and one of options is biological control of free living stages of GIN using nematophagous fungi with *Duddingtonia flagrans* as one of most promising candidates (Lalošević et al, 2009; Simin et al, 2011).

Chlamydospores of fungus given *p/o* to animals need to survive passage through the gastrointestinal tract (GIT), to develop structures (traps) for capturing nematode larvae in feces and to catch these larvae before they leave feces and reach the surrounding vegetation and soil. To achieve this, the authors recommend the use of protocols that involve dosing with very high concentration of chlamydospores. According to this approach, higher concentration of chlamydospores increases the likelihood to catch a higher number of larvae. That is why the great effort was made to determine the optimal *p/o* dose for maximal reduction of larvae in sheep and goat feces, but clear and consistent dose-dependent effect was not confirmed (Ojeda-Robertos et al, 2008b).

In majority of experiments, the dosage for naturally infected sheep (and goats) was per kg body weight, and the most common dose used and recomended by other researchers was $5x10^5$/kg body weight (BW) (Chandrawathani et al, 2004; Epe et al, 2009; Fontenot et al, 2003; Larsen et al, 1998; Paraud and Chartier, 2003; Paraud et al, 2005; Peña et al, 2002).

In previous search for optimal dose, researchers failed to determine the final number of chlamydospores in feces, so Ojeda-Robertos and collegues (2008a) developed a technique for counting chlamydospores in feces, which allowed studies of the effect of different doses of fungus on final number of spores in feces and it's effectiveness in reducing the number of larvae in coprocultures.

Grønvold et al. (2004) and Ojeda-Robertos et al. (2009) determined that digestibility of *p/o* administered chlamydospores is about 90%, which means that only about 10% of total number of administered spores survive the passage through sheep GIT . If lamb of approximately 20 kg body weight produces an average of 1200 g of feces per day (da Silva et al, 2009), the final number of chlamydospores per gram of faeces (CPG) after passing through the GIT of sheep can be calculated. For the most used dose ($5x10^5$/kg BW), this number is approximately 830 CPG.

The aim of this study was to perform coproculture assay for determination of *in vitro* efficiency of biological control agent *D. flagrans* in reducing the number of larvae of naturally infected sheep with different levels of FEC by simulating the quantity of spores per gram of faeces which would survive passage through GIT of sheep, if animals were dosed with most commonly used dose of $5x10^5$/kg BW. This approach will provide insight into the effectiveness of fungus when dosing is based on the usual number of spores per kg body weight, which would be helpful for generating effective doses for *in vivo* studies.

MATERIAL AND METHODS

Faecal samples were taken directly from the rectum of seventeen naturally infected sheep randomly selected from flock of 120 Merinoland ewes grazing near the town of Srbobran (45.34° N; 19.48°E) in South Bačka region, Vojvodina province. 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 in the following

two days. Samples were analyzed for determination of faecal egg counts (FEC) according to procedure described in Simin et al. (2012) and ten positive samples with different level of infection were selected for coproculture assay. Each sample was weighted and divided to two equal volumes (7 g) and added to experimental (with *D.flagrans*) and control group (without fungus).

D.flagrans (obtained from collection of Universite catholique de Louvian, Belgium) was cultured on potato decstrose agar (PDA) for four weeks at 25ºC in standard plastic petri dishes. For collection of chlamydospores, five ml of sterile physiological saline was added to surface of culture in each petri dish, scraped with sterile platinum loop and collected to conical glass flask (volume 250 ml). Separation of chlamydospores of *D.flagrans* from mycelia was achieved by mixing, washing and sieving technique according to Ojeda-Robertos et al. (2005). After that procedure, satisfying separation of chlamydospores was achieved with only few chlamydospores in chains left as confirmed by microscopic examination.

Quantification of chlamydospores in the suspension was done by counting their number in ten subsamples of 5 µl taken after through mixing of the flask each time before sampling. Suspension was stored in refrigerator at 4ºC until use.

Feacal samples of sheep in experimental group was thoroughly mixed with required number of chlamydospores by calculating appropriate volume of the suspension. Control faecal samples were mixed with equal volume of distilled water.

Coprocultures were made according to Fontenot et al. (2003) and Terill et al. (2004), with some modifications.

Briefly, 200 ml plastic cups were cut at half, and faecal samples were mixed with suspension of *D.flagrans*/distilled water in the lower half. After mixing, six small holes were perforated in the bottom of every cup; cups were covered with cheesecloth and turned upside down in new 200 ml plastic cup, previously filled with 10 ml of distilled water. The cultures were incubated at 25ºC for ten days and mixed twice during that time. After incubation, the cups were filled with warm water and left overnight for baermanisation. The next day, cups with faecal samples were removed, level of water reduced to 50-60 ml without disturbing the sediment that contained larvae. The contents were then transferred to disposable plastic wine glasses with small reservoir at the base of the cup, and left in refrigerator for two hours. Again, water level was reduced to 1 ml, larvae were enumerated in ten subsamples of 5 µl, and data expressed as number of larvae per gram of faeces (LPG= larval counts x 20/ grams of faeces in culture).

Larval yield was calculated as the ratio between LPG and FEC x 100 (Paraud et al, 2005). Percentage of reduction (PR) of larvae was calculated according to Terill et al. (2004): PR= 100 – (mean larval yield in fungus group x 100/ mean larval yield in control group).

Two hundred and fifty larvae from control group were identified to the level of genera according to van Wyk et al. (2004) in order to determine which parasites infect sheep at that farm.

The comparison of larval reduction between samples was calculated with parametric t-test using Microsoft Excel 2007. The statistical significance of the variables was tested at the 0.05 confidence level.

RESULTS AND DISCUSSION

Larval populations after constant coproculture temperature (25ºC) during ten days comprised of L_2 and L_3 larvae (Table 1.) and all larval counts were included in analysis. The results showed that total reduction of larval yield in experimental group was 32.45% compared to control, with the mean larval yield in the fungus treated group lower (10.98%; 95 % CI: 6.94 to 15.01) than in control group (16.25%; 95% CI: 9.51 to 22.99), but not statistically significant at 95% confidence level (p=0.07).

Table 1. Larval populations after coproculture in both groups

Fungus group		Control group	
L_2 (%)	L_3 (%)	L_2 (%)	L_3 (%)
5.38	94.62	4.84	95.16

According to identification keys provided by van Wyk et al. (2004), GIN that parasitise sheep at this farm belong to *Haemonchus contortus*, *Trichostrongylus spp.* and *Teladorsagia circumcinta* (54.8, 41.6 and 3.6 % of identified L_3 larvae, respectively). FEC values from ten sheep included in the experiment, LPG values, larval yield and individual larval reduction percentages are shown in Table 2. In four out of ten sheep faecal cultures there was not reduction of larvae following fungal treatment. Individual reduction rates ranged from 5.26 to 86.49 % in six remaining samples.

Table 2. FEC, LPG, larval yield values in both groups and individual larval reduction percentage

		LPG		Larval yield (%)		
Samples included in the study	FEC (epg)	Fungus group	Control group	Fungus group	Control group	Reduction percentage
1	100	20.00	22.86	20.00	22.86	12.50
2	1110	48.57	25.71	4.38	2.32	0.00
6	90	17.14	17.14	19.05	19.05	0.00
10	70	5.71	14.29	8.16	20.41	60.00
11	80	11.43	20.00	14.29	25.00	42.86
13	160	14.29	17.14	8.93	10.71	16.67
14	290	40.00	34.29	13.79	11.82	0.00
15	690	42.86	42.86	6.21	6.21	0.00
16	490	51.43	54.29	10.50	11.08	5.26
17	320	14.29	105.71	4.46	33.04	86.49
Average value	340	26.57	35.43	10.98	16.25	-

Total reduction of infective larvae in our study (32.45%) was low when compared to results obtained by other authors. For comparison of results, it is important to highlight that there are various designs of coproculture assays. There are a few available studies where authors directly mixed different quantities of different *D.flagrans* fungal

units (chlamydospores or conidia) with faeces of domestic ruminants that contained eggs of GIN: calves (Fernández et al, 1999; Grønvold et al, 2004), sheep (Silva et al, 2011) and goats (Sanyal et al, 2008). Some researchers preferred to dose animals *p/o*, and then to perform laboratory procedures of larval recovery (studies in sheep: Larsen et al, 1998; Peña et al, 2002; Fontenot et al, 2003; in goats: Terill et al, 2004; Ojeda-Robertos et al, 2005; Paraud et al, 2005, and in both species Waghorn et al, 2003), or to add already developed L_3 larvae to fungal cultures (Morgan et al, 1997; Mendoza de Gives et al, 1999). For that reason, only results where direct mixing was performed will be compared and discussed.

In calves, Fernández et al. (1999) added 6250 CPG of four different isolates of *D.flagrans* to study the effect on reduction of *Cooperia oncophora* (FEC=250 epg) larvae on different temperature regimes, and observed reduction percentage from 63.3 to 84% for these isolates at 20ºC constant temperature. Grønvold et al, (2004) mixed increasing concentrations of chlamydospores ranging from 250 to 200000 CPG, and established reduction from 93% for only 250 CPG to 99% by increase of CPG (FEC=1050 epg, *C. oncophora*) at similar constant temperature.

In naturally infected sheep, Silva et al. (2011) mixed 1000 conidia with 20 g of *H. contortus* infected faeces and obtained 85.82% of reduction (FEC level not shown) at 26ºC for 7 days. Campos et al. (2009) showed that conidia can also survive GIT passage, though reduction rate was significantly lower when compared to chlamydospores (23.89 vs. 61.23%). Since we used chlamydospores in our study, it is hard to compare effect with conidia even at the similar concentration (830 compared to 1000 fungal units).

True comparison can be made only with results of coproculture assay by Sanyal et al. (2008) in goats, although sheep were included in our investigation. Sheep and goats are parasitized with same species of GIN (Taylor et al, 2007) but goats do not develop strong immune response to nematode infection unlike other domestic ruminant hosts (Paraud et al, 2006). In their research, Sanyal and colleagues have mixed three levels of chlamydospores (1000, 10^4, and 10^5 CPG) with three levels of worm eggs (FEC=100, 500 and 1000 epg). We have extrapolated the reduction percentage from their data, and the values for 1000 CPG which was similar with our dose, were ≈17% for FEC=100 epg and ≈50% for FEC=500 and 1000 epg. These results were not much higher than ours (Table 2).

Reduction of larvae was different between studies, and it depended of initial FEC value and number of mixed units of fungal material. It is clear that increase of CPG results in higher reduction rate (Grønvold et al, 2004; Sanyal et al, 2008), but larval density is also important stimulus for trap formation of *D.flagrans* (Morgan et al, 1997; Sanyal et al, 2008). The percentage of larval development in control sheep cultures in our study (2.32-33.04%) was higher than percentage found by Ojeda-Robertos et al. (2005; 0.9-11.1%) and lower than the results of Terill et al. (2004; 3.9-100%) in goat coprocultures. Regardless of that, equal/ similar values of larval yields in four samples where reduction of larvae was absent showed that both tested groups were cultured under same conditions and that equal opportunity was provided for larval development. This makes fungal efficiency more obvious, even at low level of 32.45%. According to Larsen (2000), if species of known predacious fungi are tested in laboratory experiments, the researchers are more or less guaranteed some sort of positive response, depending on dose and number of nematodes involved. Also it is possible that isolate of *D.flagrans* used in our study is less effective than others, since Fernández et al. (1999)

showed different reduction rate of four isolates of fungus tested under same conditions.

Two samples yielded more larvae in fungus compared to control group. This is possible due to unequal distribution of helminth eggs in the faeces, where coefficient of variation of mean epg may range from 22 to 270% (Paraud et al, 2005) so different yields may be obtained. Although temperature is standardized variable in studies, other uncontrolled biotic or abiotic factors like consistency of faeces and thus oxygenation for example, may have influenced the larval development and variability (Paraud et al, 2005). Composition of larval species is different comparing to results obtained in august at the same farm, when there was 92%, 4%, 4% (n=100 larvae) *H.contortus, Trichostrongulus spp.* and *Chabertia ovina,* respectively (Simin, unpublished results). *H.contortus* is still predominate species, with 55% larvae in culture. This is very important fact, considering that this bloodsucking parasite is the most pathogenic species of sheep GIN (Sutherland and Scott, 2010), and that 5000 adults may cause loss of 250 ml of blood per sheep (Taylor et al, 2007). It must be repeated that the ultimate goal in dosing grazing animals with nematophagous fungi is reduction of larval number on pasture and thus lowering infection rate. Some field studies experienced great success with reduction of larvae up to 99% (e.g. Chandrawathani et al, 2003) while other obtained unsatisfying results (e.g. Rocha et al, 2007; Silva et al, 2010). *In vitro* studies are conducted in order to determine the best i.e. optimal performance of fungus species in certain conditions. Data obtained from these studies make good starting point for field application as well as reconsideration of study design if failure occurs.

In Serbia, we have recently started to test impact of *D.flagrans* on sheep GIN: the first trial tested effect on eggs (Lalošević et al, 2011) and this is our first study which examined effect on larvae. This coproculture assay design which theoretically generated final concentration of chlamydospores for most commonly used dose of $5x10^5$/kg BW is based on data obtained by others. Both Grønvold et al. (2004) and Ojeda-Robertos et al. (2009) independently determined 10% survival rate of chlamydospores after passage through sheep GIT, making survival rate constant variable. Total daily faecal output, which was one of variables included in calculation of final number of CPG in our study, is very variable and is directly related to dry matter intake and digestibility of the consumed herbage (Smith and Frost, 2000). It is measurable, but special faecal collection harness for sheep is required, if one wants to weight faeces for sheep at pasture. While our faecal collection harness is under construction, for lamb total faecal output, we have selected data from da Silva et al. (2009) since they have measured total faecal production in animal while grazing. One of the reasons for low level of larval reduction in our *in vitro* study may be to miscalculated number of final CPG concentration, due to different amount of total daily faeces produced by adult sheep at conditions at this farm.

Further studies are needed to investigate complex factors that influence reduction percentage of larvae. In the following experiments, all parameters will be measured in order to gain better insight for effect of fungus in given circumstances.

CONCLUSION

In this preliminary investigation, *in vitro* larval reduction percentage of GIN in naturally infected sheep by nematophagous fungus *D.flagrans* was estimated using coproculture assay, and the total reduction percentage was 32.45%. Although low, the results of larval reduction from this experiment are not discouraging. Since *Haemon-*

chus contortus is very present species at this farm, the fact that there can be even 30 % less larvae available at the pasture, may be the difference of life and death for some individuals, especially young, old and reconvalescent sheep. Also, this may be valuable fact for preventing and lowering subclinical losses, which are costly in grazing sheep due to parasitic gastroenteritis (West et al, 2009).

Further research is already in progress with modified protocols in order to improve *in vitro* efficiacy of this biocontrol agent, since the final aim is successful (*in vivo*) application in grazing sheep naturally infected with these parasites and measure of the effect of *D.flagrans* on pasture infectivity.

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TEST KOPROKULTURE ZA PROCENU REDUKCIJE LARVICA ŽELUDAČNO-CREVNIH NEMATODA OVACA IN VITRO PRIMENOM NEMATOFAGNE GLJIVE, DUDDINGTONIA FLAGRANS

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Izvod

Želudačno-crevne nematode ovaca predstavljaju značajan problem primenom sistema napasanja zbog različitih proizvodnih i značajnih finansijskih gubitaka u ovčarskoj industriji širom sveta. Jedna od mera koje se primenjuju u kontroli ovih parazita kod pašnih ovaca je primena nematofagnih gljiva u smanjenju broja raspoloživih infektivnih larvica, među kojima je vrsta *Duddingtonia flagrans* kandidat koji najviše obećava. U cilju procene efekta redukcije larvica u *in vitro* uslovima, urađen je test koprokulture

sa teorijski generisanom dozom hlamidospora koja je dodata u feces. Ukupna redukcija larvica u oglednoj grupi je iznosila 32,45% u poređenju sa kontrolnom, ali nije bilo statistički značajne razlike u prinosu larvica na nivou značajnosti 0,05. Redukcija dobijena u našem ogledu je niža u odnosu na rezultate drugih autora. Potencijalni razlozi za nisku efikasnost gljive u ovom istraživanju u poređenju sa drugim rezultatima su veoma složeni i neki faktori su analizirani u diskusiji.

Ključne reči: *Duddingtonia flagrans,* redukcija larvica, ovce, biološka kontrola, test koprokulture.

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LYSINE CONTENT IN DIFERENT HYBRIDS OF MAIZE IN PIG'S DIETS

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SUMMARY: The aim of this study was to examine the content of lysine in maize, as one of the main compound of feeding mixtures for pigs. Sixteen commercial samples of maize hybrids, were analyzed for lysine content which was highly correlated with the level of protein, r = 0.86. Obtained results were compared to ones established by EVONIK. Analytical methods based on acid hydrolysis and detection by liquid chromatography was used by applying previously validated conditions. Limit of detection (LOD) for lysine in maize was 0.035%, while the limit of quantification (LOQ) was 0.106%. Lysine content was ranged from 0.142% to 0.297% with the average value of 0.223% and standard deviation of 0.049%.

Key words: *lysine, maize hybrids, pigs.*

INTRODUCTION

Dietary amino acids have crucial signification for health, development, growth, reproduction and lactation in animals. Some animals, like non-ruminants cannot synthesize essential amino acid and they must be taken in with food. Inadequate settlement for pig with protein and amino acids is particularly harmful if it occurred in early stages (Kovčin et al., 2001).

Pig diets mostly involve a portion of amino acid that is not biologically available to the animal. This is because most proteins are not fully digested and the amino acids are not fully absorbed, and also because not all absorbed amino acids are metabolically available. Diets vary considerably in the proportions of their amino acids that are biologically available. The amino acids in some proteins such as milk products are almost fully bioavailable, whereas those in other proteins such as certain plant seeds are much

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Original scientific paper / *Originalni naučni rad*

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less so (Southern, 1991; Lewis and Bayley, 1995; Žilić, 2010). A few essential amino acids tend to be limiting in typical swine diets. Lysine is usually the most-limiting (or first-limiting) amino acid. It means that if a diet is formulated to supply the correct amount of lysine, then generally the levels of other essential amino acids will be adequate (Myer et al., 1996). If diet does not insure the proper amount of lysine and other essential amino acids, pigs will grow slower than the pigs that receive the appropriate concentration of amino acids, also will develop less muscle and more fat. Higher amino acid levels allow the animal to deposit greater amounts of lean tissue rather than fat. Estimation of the proportions of amino acids in ideal protein for growing swine have been derived from an examination of various types of data, including the composition of pig tissue, the composition of sows' milk, and combinations of individual estimation of amino acid requirements. Therefore, the overall ideal pattern will change as the proportions of maintenance and new tissue synthesis change. In addition, there are changes in the amino acid content of pig tissue as the pig grow from birth to market weight (Kyriazakis et al., 1993; Susenbeth, 1995; Mahan and Shields, 1998).

Pigs require a diet with a balanced pattern on necessary amino acids in which protein supplements usually represents 20% of diet, but make up approximately up to 35% of the cost of diet. Lower cost of protein reduced quantity or concentration of amino acids in diet which reduce the margin of safety. Information about quantity of amino acids and quantity required by the pig is mandatory for efficiently amino acids optimization (Mosenthin et al., 2000). In theory, any deviation of pattern of amino acids in ideal protein will lead to a reduction in animal performance, at least in terms of the efficiency of dietary protein utilization. In practice, however, swine seem to be relatively tolerant to quite wide variations in the pattern of amino acids, as long as all amino acid requirements are met. Knowing the composition of amino acids in proteins is used to assess the feeding value of dietary proteins. In order to meet the protein requirements, such as amino acids requirements, the diet must include enough essential amino acids in the right proportion. The fast and accurate determination of the amino acids from the raw materials used in animal feeding is necessary for a correct estimation of the feeding value of the protein (Maros et al., 2011). Requirements vary depending on the species and age of animals. The effects of maize lysine content would be most evident in a swine finisher diet because of the relatively high proportion of maize typically used.

According to Serbian regulations governing the quality of animal feed, complete feed mixture for pigs are divided into several categories and for each lysine and protein content is specified. For a compete mixture for (feeding) piglets lysine content ranges from 1% - 1.3%, and 18% - 22% of protein content. In a complete mixture for pigs these values are from 0.55% - 0.8% for lysine, and 13% - 16% for protein. In pigs it requires extra protein and lysine (Službeni glasnik Republike Srbije, 2010).

The aim of this study was to examine the content of lysine in different hybrids of maize used for making diets for pigs. Different maize hybrids were tested for protein content and that content was correlated to the lysine content.

MATERIAL AND METHODS

Material. Acetonitrile (LC grade) and methanol (LC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Borate buffer, OPA and FMOC reagents and amino acid mixture standard solutions (10, 25, 100, 250 and 1000 pmol μl^{-1}) were obtained

from Agilent Technologies (Waldbronn, Germany). Hydrochloric acid, used for preparation of 6 M and 0.1 M HCl, was obtained by Lach-Ner (Neratovice, Czech Republic). Sodium phosphate monobasic was purchased from Acros Organics (New Jersey, USA). LC grade water was produced by Heming ID-3 system (Belgrade, Serbia), while cellulose membrane filters (pore size 0.22 μm) were purchased from Agilent Technologies (Waldbronn, Germany).

Equipment. The analysis was performed using the Agilent 1260 Infinity Liquid Chromatography System, equipped with μ-degasser binary pump, standard autosampler, ZORBAX Eclipse-AAA column and DAD detector.

Samples. There were 16 commercial maize hybrid samples of known producer in order to determine lysine content. *Sample preparation:* Samples milled in a laboratory mill. After that, 0.6 g to 0.7 g of sample was measured (equivalent to the nitrogen content of 10 mg) in a vacuum hydrolysis tube, and then hydrolyzed with 6 M HCl. Hydrolysis was carried out with Reacti-Therm™ apparatus with constant stirring for 6 hours at 150 °C. After hydrolysis, samples were allowed to reach the room temperature and then evaporated to dryness using the Reacti-Therm™ at 70 °C in a stream of nitrogen. The residue was quantitatively transferred into a 50 ml volumetric flask with 0.1 M HCl. The solution was filtered through quantitative filter paper, and then additionally filtered using a cellulose membrane filter (pore size 0.22 μm). This method was previously validated (Jajić et al., 2012).

HPLC determination. Chromatography conditions were in accordance with the Agilent method (Henderson et al., 2000) with the exception of mobile phase A preparation where 5.678 g Na_2HPO_4 was dissolved in 1 L water and then adjusted with 6 M HCl to pH 7.8. Hydrolyzed samples and standard mixtures of amino acids were automatically derivatized with OPA and FMOC reagents using the appropriate injector program. After derivatization, sample or standard mixture was injected on ZORBAX Eclipse-AAA column (5 μm, 150 × 4.6 mm) at 40 °C. Mobile phase A was 40 mM Na_2HPO_4, adjusted with NaOH to pH 7.8, and mobile phase B acetonitrile-methanol-water (45:45:10, v/v/v). Separation was performed at a flow rate of 2 ml min^{-1} with a gradient program, during the 26 min period of analysis. All data were analyzed to test the correlation.

RESULTS AND DISCUTION

Lysine is usually used as a reference for other essential amino acids in the ideal protein concept due to its simplicity of analysis in feedstuffs.

Table 1. Lysine content in maize samples

Sample	Lysine (%)	Protein (%)
1	0.241	7.49
2	0.188	6.47
3	0.235	6.67
4	0.255	7.54
5	0.257	7.22
6	0.196	6.25
7	0.256	8.28
8	0.242	7.54
9	0.194	7.03
10	0.279	8.78
11	0.198	6.62
12	0.241	7.60
13	0.237	7.60
14	0.231	7.50
15	0.234	7.91
16	0.290	8.83
Average	0.236	7.46
Standard deviation (%)	0.030	0.796
Coefficient of variation (%)	12.57	10.67

Therefore, estimating accurately the requirement of lysine is essential for the setting of other indispensable amino acids (Baker, 1997).

In this paper there were 16 samples of hybrid maize, which further will be used for purpose of making swine mixture, and was controlled by the proportion of lysine as an important parameter for feeding gilts. In complete mixtures it ranges from 0.55% to 1.3% and in supplementary mixtures lysine value is 2.0% - 3.3% (Službeni glasnik Republike Srbije, 2010).

Samples of maize were analyzed and the results are presented in Table 1. As can be seen, lysine content ranges from 0.188% in sample number 2, to 0.290% in sample number 16. Average value for lysine in maize samples, obtained from 16 samples was 0.236% with standard deviation of 0.030% and coefficient of variation of 12.57%. The results were compared with results presented by EVONIK (EVONIK Industries, 2010), which study included 918 samples.

As can be seen, in Table 2, lysine content was ranged from 0.14% to 0.37% with the average value of 0.24%. By comparing these results, it can be concluded that lysine content of maize samples in both studies was quite similar. Namely, both, the lysine content and coefficient of variation, correspond to results presented by EVONIK.

Table 2. Lysine content in maize samples (EVONIK Industries, 2010)

EVONIK	Average (%)	0.24
	RSD (%)	9.80
	Range (%)	0.14-0.37

Furthermore, in maize samples, protein content was determinated (Table 1) and then compared to lysine content. Protein content was ranged from 6.47% in sample

number 3 to 8.83% in sample number 16 with standard deviation of 0.79% which indicates the consistency of test results. The correlation coefficient (r) was 0.86 which represent strong positive correlation which is represented in figure 1.

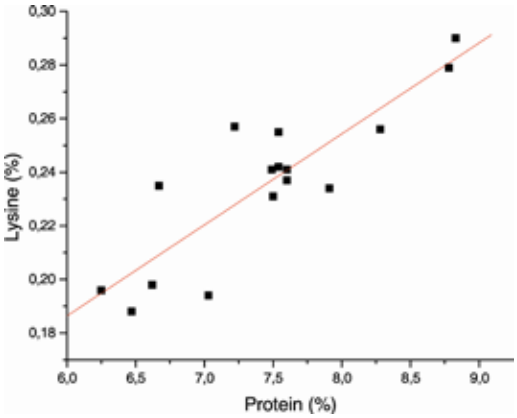

Fig. 1. Correlation between lysine and protein content

CONCLUSION

Significance of lysine in pig nutrition, as an essential amino acid is large, so it is necessary to take care of its entries, and if it is supplemented, dose it according to age. This study illustrated that lysine content corresponds with protein contents in maize hybrids. Also, it represented agreement between obtained results and results that EVONIK presented. Average value for lysine in maize samples, was 0.236% with standard deviation of 0.030% and coefficient of variation of 12.57%, and correlation coefficient (r) was 0.86 which represent strong positive correlation to protein content. The results were corresponding to results presented by EVONIK, which average is 0.24%, and value of coefficient of variation was 9.80%. Also, one of the recommendations is that in the preparation of meals for pigs as a laboratory controlled mixtures, needs to be devoted more attention to lysine ratio, than the protein content.

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SADRŽAJ LIZINA U RAZLIČITIM HIBRIDIMA KUKURUZA ZA ISHRANU SVINJA

IGOR JAJIĆ, SAŠA KRSTOVIĆ, BILJANA PERIŠIĆ, DEJAN BEUKOVIĆ, MIRKO IVKOVIĆ, MIROSLAV UROŠEVIĆ,

Izvod

Cilj ovog rada bio je ispitivanje sadržaja lizina u kukuruzu, kao veoma značajnoj aminokiselini u ishrani svinja. Sadržaj lizina je analiziran u šesnaest komercijalnih hibrida kukuruza, pri čemu je dobijena visoka korelacija (r = 0,86) u odnosu na sadržaj proteina. Dobijeni rezultati sadržaja lizina su poređeni sa Evonikovim. Analitička metoda je zasnovana na kiseloj hidrolizi, a kvantifikacija je izvršena tečnom hromatografijom, prema ranije validovanoj metodi. Granica detekcije za lizin u kukuruzu iznosi 0,035%, dok je granica određivanja 0,106%. Sadržaj lizina u analiziranim uzorcima se nalazio u opsegu od 0,142% do 0,297%, a srednja vrednost je iznosila 0,223%. Vrednost standardne devijacije je iznosila 0,049%.
Ključne reči: lizin, kukuruz, svinje.

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RESUMPTION OF OVARIAN ACTIVITY AFTER CALVING AND INFLUENCE ON REPRODUCTIVE PERFORMANCE IN DAIRY COWS (A REVIEW)*

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SUMMARY: During the past few decades, continued genetic progress for milk production, coupled with new reproductive technologies performed in high-producing dairy cows, has led to significant decreasing of dairy cows fertility all over the world. Delayed anoestrus post partum is one of the major causes of fertility decreasing and economic losses in intensive milk production. The aim of these paper is to review the causes and possibility for reducing its impact on subsequent reproductive performance in dairy cows.

Key words: *ovarian aktivity, anestrus, post partum, dairy cow.*

INTRODUCTION

The duration of the interval between two successive calving (i.e. calving interval) is the main parameter of reproductive efficiency in dairy cow herds. This period should, optimally, last 12 to 13 months, because, in that situation, the maximum milk and calves production per cow per year can be achieve (Crowe, 2008). However, in the practical intensive production conditions, calving interval often lasting over 14 months. As the duration of pregnancy is biological constant, the duration of this period is significantly influence by the period from calving to successful conceptions establishment, i.e. service period duration (Stančić and Košarčić, 2007).

From the standpoint of reproduction, duration of service period is directly determined by the interval from calving to first ovulatory estrus resumption, and by the

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Review scientific paper / *Pregledni naučni rad*

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period from first to successful insemination (Bousquet et al., 2000). The first ovulation, in healthy cows, is usually occurs between 15 and 30 days post partum. However, at about 70% of cows, this ovulation is silent, i.e. ovulation without manifestation of external estrus signs (Stančić 1989; Crowe, 2008). For practice, it is very important to record the first ovulation after calving, in order to monitoring the following cyclical ovarian activity and estrus manifestations, which can be used for insemination. This is important because the cow should be fertile inseminated within 90 days post partum, in order to achieve optimum length of calving interval of 12 months. However, during the past few decades, numerous studies have reported the significant decrease in dairy cows reproductive performance, primarily as a result of delayed resumption of ovarian activity post partum (Thacher et al., 2006; Savović, 2010; Gautam et al., 2010; Gvozdić et al., 2011). The aim of this paper is to review the recent findings about post partum ovarian activity resumption in high milk producing dairy cows.

REPRODUCTIVE PERFORMANCE IN HIGH MILK PRODUCED HERDS

In recent decades, a permanent decline of reproductive performance is estimated in high milk producing dairy cows herds all over the world (Dobson et al., 2007). Reduction in reproductive efficiency is manifested by prolonged post partum anoestrus (Thacher et al., 2006), increasing the number of cows with silent estrus of irregular lasting, as a consequence of shortened luteal phases in the early cycles post partum (Lucy, 2007), reduced successful conception rate after first insemination (Lucy, 2001), increasing the number of cows with abnormal preimplantational embryos development, as well as increasing the various uterus diseases (Furchon et al., 2000, Bouchard and du Tremblay, 2003), which leads to increased embryonic and fetal mortality (Lucy, 2007). The substantial increase in the appearance of regular and irregular returns to estrus, and increasing the number of required successful insemination per conception (i.e. AI-index), is direct results of embryos and fetuses mortality (Sheldon and Dobson, 2003; Savović, 2010; Stančić et al., 2011). In the most developed European countries, successful conception rate after first postpartal insemination, decrease from 55% to 45%, in the period since 1990. to 2000. year (Bousquet et al., 2004). Increasing number of insemination per successful conception (i.e. AI-index) is the direct result of conception rate decrease. For example, Lucy (2001) states that past 20 years, AI-index decrease from average 1.75 insemination per successful conception, to over 3 insemination per conception. This parameter is very important for assessing the degree of fertility of cows, because it is inversely proportional to the successful conception rate and service period duration (Kossaibati and Esslemont, 2000). It has been suggested that in high-yielding dairy herds, there is marked incidence of prolonged anoestrus post partum increasing and that this is the main factor that reduce reproductive performance and economic looses in high-yielding dairy herds (Peter et al., 2009). Direct consequence of prolonged anoestrus after calving is increasing the service period duration. Therefore, the control of service period duration is the most important factor for increase the reproductive performance in dairy cow herds. This control include interaction of three basic factors: (1) normal resumption of cyclic ovarian activity post partum, (2) effective detection estruses post partum and (3) high successful conception rate after first insemination post partum (Iglesia et al., 1996; Crowe, 2008; Garcia et al., 2011).

Milk production in the contemporary high-yielding dairy herds are usually ranges

between 8,000 and over 10,000 kg per cow per year. This is the result of very intensive selection to the high milk yield, as well as advances in feeding and housing technology. Most research shows that the decline in reproductive efficiency of cows is directly related to high milk production, as well as a variety of stressful factors, resulting from the technology of housing cows in very cramped conditions (Rodriguez-Martinez et al., 2008). In addition, application of modern biotechnology for stimulation and control of certain reproductive functions, and treatment of reproductive disorders, also have a significant impact on the reduced reproductive performance of high- yielding dairy cows. This, in particular, relates to the use of hormonal drugs (gonadotropins, progestogens and luteolitics), used for the induction and synchronization of post partum oestrus (Grafenau et al., 1998; Grafenau et al., 1999; Stančić and Košarčić, 2007; Grafenau et al. , 2008; Gvozdić et al., 2011).

PHYSIOLOGOY OF OVARIAN ACTIVITY ESTABLISHMENT *POST PARTUM*

During the period of pregnancy, in the cows blood circulation dominate high concentrations of progesterone, originate from gestational corpus luteum and placenta. High concentrations of progesterone, via the hypothalamus, inhibit the release of gonadotropins (Follicle hormone - FSH and Luteinizing hormone - LH) from the pituitary, resulting in the absence of ovarian activity, in terms of follicular growth and ovulation (McDonald, 1989). Therefore, cow exhibit gestational anoestrus. However, about 20 days before normal term of parturition, blood progesterone concentrations rapidly decline, especially during the 2 to 3 days before parturition. This decrease in progesterone concentration is a consequence of GnRh-ACTH secretion (gonadotropin releasing hormone adrenocorticotropic hormone) from the fetal hypothalamus, which stimulate ACTH secretion from the fetal pituitary. ACTH stimulates the secretion of cortisol from the fetal adrenal cortex, which inhibits the synthesis of progesterone in the placenta and stimulates the synthesis of estrogen and $PGF_{2\alpha}$ in the placenta. Estrogen and $PGF_{2\alpha}$ stimulate the secretion of oxytocin from the mother neurohypophysis and inhibition of secretion of progesterone from the gestational corpus luteum. These events stimulate the myometrial contractions and beginning of parturition (Peters and Ball, 1987).

Resumption of cyclic ovarian activity (follicular growth and ovulation), under the influence of pituitary gonadotropins (FSH and LH) secretion, normally occurs within the first 2 to 4 weeks post partum (Webb et al., 2004). The growth of ovarian follicles begins usually 7 to 10 days post partum, as a result of pituitary FSH secretion increasing, about 3 to 5 days after calving. Tonic releasing of pituitary LH release (i.e. LH-surge), lead to final growth of dominant follicles and ovulation (Peters and., 1994; Stančić et al., 1995; Ginther et al., 996; Crowe et al. 1998). After the first ovulation post partum, cyclic corpus luteum formating and cyclic ovarian activity resume. Over 80% of cows normally resume cyclic ovarian activity within the first 35 days after calving (Reist et al., 2000). Generally, in normal cows, the first ovulation, on average, occurs 15 days, second 32 days and the third 53 days after calving (Wattiaux, 1996; Walker, 1997). However, it is normal that the first ovulation post partum is silent, with no external signs of estrus, in the about 76% of cows. Second estrus is visible in about 50%, and the third estrus after calving is visible in more than 90% of cows (Walker, 1997; Stančić and Košarčić, 2007; Crowe, 2008).

PROLONGED *POST PARTUM* ANESTRUS

In high producing dairy cows, described endocrine mechanisms of cyclic ovarian activity resumptin, may be delayed for shorter or longer period after calving, leading to increasing the period from calving to first ovulation. As a consequence, postpartum anoestrus period will be prolonged (Thacher et al., 2006). If ovulation does not occur within the first 35 days post partum, and if the first estrus is not manifest within the first 50 days post partum, the postpartum anoestrus is prolonged (Stančić, 1989; Walker, 1997). Also, if ovulation occurs within the first 35 days after parturition, and no luteal activity (increase in concentration of progesterone) ≥ 14 days after ovulation, it is also considered to be prolonged anoestrus post partum. Today, the prolonged postpartum estrus was defined as absence of increasing concentrations of progesterone in blood plasma or milk, within the first 50 days post partum (Gautam et al., 2010). According to some studies, between 20 and 48% of dairy cows manifested prolonged postpartum anestrus (Rhodes et al., 2003). In one of our research, performed on a large dairy farm in Vojvodina, within the first 60 days after calving, the first estrus was recorded in only 36% of cows that had no peripartal problems, while this value was about 10% lower in cows with disorders during and immediately after calving (Savović, 2010; Stančić et al., 2011). Some studies show that prolonged period for ovarian function resumption, occurs in about 75% of high milk production cows, which is usually a consequence of prolonged luteal phase or delayed first ovulation after calving (Shrestha et al., 2004).

The interval from calving to first ovulation, may be significantly prolonged, influenced by numerous zootechnological factors, such as inadequate nutrition, poor body condition, poor housing conditions, high milk production, calving parity and inadequate ambiental climate factors (Santos et al., 2009). Prolonged anoestrus post partum also can be the result of post partum disorders and diseases of infectious and non-infectious etiology (Peter et al., 2009). Basically, all these factors influence the prolonged inhibition of pituitary FSH and LH secretion, which delays the normal development and ovulation of dominant follicles. Although these factors do not affect the initial disturbance of ovarian follicles growth post partum, it was shown that they can significantly disrupt and/or prolong the ultimate growth, maturation and ovulation of dominant follicles (Peter et al., 2009).

The rapid weight losses, as a result of significant negative energy balance in the cows body, due to intensive increase in milk production during the first 2 to 3 months of lactation, is one of the most common causes of prolonged post partum anestrous (Crowe, 2008; Erdeljan et al., 2011). Negative energy balance is directly related to the inhibition of Gn-RH release from the hypothalamus, which results in reduced and/or delayed ovulatory LH surge release from pituitary (Mwaanga and Janowski, 2000). This inhibition is associated with a low content of IGF-I (insulin-like growth factor), in cows with a negative energy balance (Zulu et al., 2002).

Dystocia, retention of placenta and uterine infection are the most common pathologic causes of prolonged postpartum anestrous. These disorders is usually caused by prolonged duration of gestational corpus luteum (i.e. corpus luteum persistent), as a result of impaired synthesis of $PGF_{2\alpha}$ an luteolitic hormone in the inflamated endometrium (Stančić, 1995; Fourchon et al., 2000; Gröhn and Rajala-Schultz, 2000; Bell and Roberts, 2007; Savović, 2010; Stančić et al., 2011; Gvozdić et al. 2011).

The formation of follicular cysts in the early post partum period, also signifi-

cantly prolonged interval from calving to first ovulation (Kesler and Garverick, 1982). Follicular cysts are defined as follicular structures with a diameter of at least 2.5 cm, which persist for at least 10 days in the ovary, in the absence of corpus luteum. They can be pure follicular cysts (i.e. enlarged unovulated follicle) or luteinizing follicular cyst (partial lutinization of follicle theca interna), in contrast to the corpus luteum cyst. The concentration of progesterone in blood plasma of cows with follicular cysts was low (<1.0 ng / ml), but was significantly increased in cows with luteal follicular cysts. Clinically, cows or heifers with follicular cysts, is usually anoestrual or nymphomanic, depending on the level of follicular wall luteinization (luteal follicular cysts) and the size and number of pure follicular cysts. Cows with cystic corpus luteum usually manifested a normal estrous cycle (Kesler and Garverick, 1982). Although many follicular cysts, formed in the early postpartum period, regresses spontaneously, their occurrence in 6 to 19% of animals, they represent a significant problem of reproduction in herds of dairy cows. Namely, it is often difficult to assess whether performed a more expensive treatment, or waiting for the cyst spontaneously regresses (Gossen and Hoedemaker, 2006). Occurrence of follicular cysts etiology is not understood, but it is known that all the factors that can disrupt the physiological mechanism for release of Gn-RH from the hypothalamus, may lead to follicular cyst. The relatively successful treatment of follicular cysts with Gn-Rh preparations supports this assumption. Cystic follicles are significantly more common in some breeds (Holstein Friesian and Jersey), and less in some others (Guernsey and Ayrshire). There is also considerable variation depending on the occurrence of cysts in some line of bulls within the same race (Coleman, 2008).

Some studies suggest that a prolonged interval from calving to first estrus is genetically determined in high milk producing breeds (Jamrozik et al., 2005). It was found that prolonged anestrous is more common in some breeds (genotypes), for example Holstein Friesian, but mechanism of action of genotype on this phenomenon is unknown (Mwaanga and Janowski, 2000). In the high producing dairy cows herds, a manifestation of external signs of estrus is not always clearly expressed, and this period is short, often as a result of metabolic imbalances due to high milk production or as a result of some pathological conditions, such as mastitis, endometritis, laminitis, etc. (Veselinović et al ., 2004; Van Eerdenburg et al., 2002; Diskin, 2008). Quite often it happens that signs of estrus are not registered, because of inadequate estrus detection technologies on the farm, or because the estrus signs are weak and/or short manifested (Garcia et al., 2011). Clearly expressed outward signs of estrus, particularly standing reflex, are a very good indicator of high reproductive efficiency of cows. In addition, estrus detection is significant for determine the optimal moment of insemination, which is one of the primary factors of high herd reproductive efficiency (Garcia et al., 2011).

CONCLUSION

Numerous studies clearly show a significant reduction in reproductive efficiency of high-yielding dairy cows herds worldwide. The main reasons are the increased occurrence of prolonged post partum anestrous and increased number of rebreedings (unsuccessful insemination), as a result of significantly increased of embryonic and fetal mortality rate. Direct result is the increasing of service period and intercalving interval duration. The ultimate result is significant reduction of milk and calf production per cow per year. The problem of prolonged post partum anestrous in herds of high

producing dairy cows is very complex, as a consequence of the interaction between multiple genetic and paragenetic factors. Therefore, it is not possible to defined a unique and practical useful method to solving this problem. However, the earlier records of prolonged postpartal cyclic ovarian activity resumption or prolonged absence of luteal activity after the first ovulation, which occurred within the normal (physiological) post partum period, is the crucial factors to significantly shorten the service period and, consequently, increase the reproductive efficiency in high-yielding dairy cows herds.

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REUSPOSTAVLJANJE OVARIJALNE AKTIVNOSTI POSLE TELENJA I UTICAJ NA REPRODUKTIVNU PERFORMANSU VISOKO MLEČNIH KRAVA (PREGLED)

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Izvod

Tokom nekoliko zadnjih decenija, genetski napredak u proizvodnji mlek, povezan sa primenom novih reproduktivnih tehnologija, doveo je do značajnog smanjenja fertiliteta u zapatima krava visoke mlečnosti širom sveta. Produžen postpartalni anestrus je jedan od glavnih uzroka smanjenog fertiliteta i ekonomskih gubitaka u intenzivnoj proizvodnji mleka. Cilj ovog rada je da se prikažu uzroci i mogućnost smanjenja uticaja ovog faktora na sledeću reproduktivnu performansu visoko mlečnih krava.

Ključne reči: ovarijalna aktivnost, anestrus, post partum, muzna krava.

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THE PRESENCE OF TENCH AND ALLOCHTHONOUS FISH SPECIES IN SOME WATER COURSES OF VOJVODINA*

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SUMMARY: Based on the data obtained through complex ichthyological research in waters of Vojvodina in the last thirty years, the presence of tench-Tinca tinca was monitored, as well as the presence of the following allochthonous fish species: Ictalurus nebulosus, Lepomis gibbosus, Carassius gibelio, Ctenopharyngodon idella, Hypophthalmichthjs molitrix, Aristichthys nobilis and Pseudorasbora parva as an important disturbing factor of the above mentioned indigenous species. The results show significant decrease in number, and even extinction of tench, while the presence of imported species is increasing, especially brown bullhead, pumpkinseed sunfish and Prussian carp.

Key words: *Tinca tinca, allochthonous fish species, Vojvodina.*

INTRODUCTION

Tench – *Tinca tinca* L. 1758 is one of the rarest and most endangered freshwater fish species today, both in Serbia and other parts of Europe. It has almost completely disappeared from the waters of Vojvodina, and that is why it is subject of the Rulebook on declaration and protection of protected and strictly protected wild species of plants, animals and fungi published in the Official Journal of the Republic of Serbia, no. 5/2010 from 5 February 2010. Furthermore, permanent close hunting season was declared for this fish species, based on the Law on Fisheries, Official Journal of the Republic of

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Review scientific paper / *Pregledni naučni rad*

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Serbia, no. 17 from 13 March 2009. It has also disappeared from fish farms, although its contribution was significant. It was bred in polyculture with carp, and during the 1960s it was the second most important species in carp fish ponds (Ćirković et al., 2008; 2009; 2012). After hydro-ameliorative measures, regulation of natural water flows, building embankments and dams, disappearance or reduction of flood plains around rivers, draining many standing waters, habitat of this species has been reduced drastically, and this trend continues (Maletin et al., 2008a).

Apart from the above mentioned, the remaining populations have almost completely disappeared due to introduction of allochthonous fish species. The greatest impact have had so called American complex species (black bullhead-*Ictalurus nebulosus* and pumpkinseed sunfish-*Lepomis gibbosus*) and Chinese or "Far East" complex (Prussian carp-*Carassius gibelio*, grass carp-*Ctenopharyngodon idella*, topmouth gudgeon-*Pseudorasbora parva*, silver carp-*Hypophthalmichthys molitrix* and bighead carp-*Aristichthys nobilis*) (Lenhardt et al., 2011). Black bullhead, pumpkin seed sunfish and topmouth gudgeon have direct negative impact on tench populations because they feed on fish roe, and the first two species also feed on tench young (Cakić et al., 2004). Prussian carp has an exceptionally great negative impact, because its specific reproduction (gynogenesis) disturbs tench spawn, it feeds on fish roe, and it is also direct competitor of tench in terms of diet and habitat. When large production, rapid increase in numbers, exceptional adaptability and aggressiveness of the Prussian carp are added to the above, it can be concluded that this species has almost completely ousted tench from its habitat (Maletin et al., 2005). Grass carp, through habitat destruction, also has indirect but great impact on tench populations. This species feeds on large amounts of water macrophytes (Ćirković et al., 2011), and it can thin out the water vegetation to such an extent that it can hinder tench spawn. In aquaculture, bighead carp and silver carp are in direct competition with tench for food because, before the introduction of these two species, undigested food discharged from digestive tract of the common carp was directly used by tench.

MATERIAL AND METHODS

The ichthyology material was collected in the periods of high, low and medium water levels with an electrofishing device and nets of various mesh diameters starting from 1980 in running and standing waters of Vojvodina, as well as in canal network of Danube-Tisa-Danube Hydro-system. It was determined by using the keys Simonović (2006).

RESULTS AND DISCUSSION

For the last thirty years, complex ichthyological research has been done in the waters of Vojvodina, the part of which was monitoring of the presence of tench-*Tinca tinca*, as well as the presence of the introduced species which are, apart from habitat degradation, its main disturbing factor. From the abundance of data, it can be seen that in most of the waters in Serbia, which are tench's natural habitats, this species is registered in a very small proportion. In the Koviljsko-Petrovaradinski rit Special Nature Reserve, the presence of tench was registered in the period between 1979 and 1996, and

later it disappears (Maletin et al., 1997a). Since then, at the same site, the proportional contribution of brown bullhead-*Ictalurus nebulosus*, pumpkinseed sunfish-*Lepomis gibbosus* and Prussian carp-*Carassius gibelio* has increased, which is, apart from the above mentioned authors, also stated at various points, brown bullhead was present with 37.50% (Okruglica), more than 42.57% at Šlajz and up to 48.63% at Dunavac (Lujić et al., 2012). The significant presence of pumpkinseed sunfish at this site was registered in 1980 (13.47%) (Budakov et al., 1983), and with even greater proportional contribution it was registered in 1999 and 2008 (20.09% and 26.39% respectively at Arkanj and 37.16% at Šlajz) (Lujić et al., 2012). In 1996, when tench was last recorded at this site, Prussian carp was present with 41.05% (Maletin et al., 2008b) and in 2000, its presence in the total catch at Tonja has risen up to 66.66% (Maletin et al., 2001). The significant presence of this imported species was also registered in 2006 at Arkanj (31.72%) (Maletin et al., 2008a), as well as in 2008 at Okruglica (37.50%) (Maletin et al., 2011). Tench was being registered in canals of Danube-Tisa-Danube Hydro-system with very small proportional contributed up to 2006, when it disappears (Maletin et al., 1997b; Maletin et al., 2008b). At this site, as it was mentioned before, the disappearance of tench also coincides with the increased presence of the above mentioned allochthonous species, especially Prussian carp, the proportional contribution of which moved from 41.51% in 1983 (Budakov et al., 1984) to 75.60% in 2006 (Marković et al., 2010). The similar state was registered at Bajski canal, where Prussian carp was present with 44.41% in 1996 (Maletin et al., 1997b), as well as at Vrbas-Bezdan, Odžaci-Sombor, Novi Sad-Savino Selo, Bački Petrovac-Karavukovo and Banatska Palanka-Novi Bečej canals, where its proportional contribution moved between 42.80% and 62.50% in the period between 1996 and 2006 (Maletin et al., 2011). The most significant tench presence in the waters of Vojvodina from the mid 1970s was registered in the Obedska Bara Special Nature Reserve in 1976 and it was 11.06% (Lujić et al., 2012). Since then, its number has decreased, and at the same time, according to these authors, the presence of Prussian carp, the proportional contribution of which was 61.22% at this site in 1982, has increased. Concerning this imported species which, as it has previously been mentioned, directly endangers tench, the most drastic situation is in the Stari Begej-Carska Bara Special Nature Reserve, where the presence of Prussian carp has moved from 20.55% in 1984 (Kostić and Maletin, 1992) to 84.31% in 2007 (Marković et al., 2010). Tench was registered at this site in 1984 and 1988 with low proportional contribution (Kostić and Maletin, 1992). Concerning watercourses in Banat, the presence of tench in the Tamiš River has not been registered since 1998 (Maletin et al., 2002). At this site, the significant presence of pumpkinseed sunfish (17.39%) was registered in 2010, and of Prussian carp (21.54%) in 2009 (Šipoš et al., 2007). Among sites in Vojvodina where tench was present, the Zasavica Special Nature Reserve should be mentioned, because in 1998, at Valjevac point, tench was present with 7.38% and at Šumareva Ćuprija with 3.15% (Simić et al., 2007). However, it should be stressed that brown bullhead was present with 38.52% and 59.84% respectively (Kostić et al., 2000). Furthermore, the Mrtva Tisa Nature Park should also be mentioned, where the presence of tench has not been registered since 1988 (Kostić and Maletin, 1992), but proportional contribution of pumpkinseed sunfish was 33% and of Prussian carp 20.50% at this site in 1996 (Maletin et al., 1996). In the last several years, the significant tench presence (4.95%) was recorded at Bara Trskovača Natural Monument in 2006, but the proportional contribution of pumpkinseed sunfish was also high (25.79%) (Maletin et al., 2008b). This author stresses that

repopulation with tench broodstock is the only way to increase the number of tench in this watercourse.

CONCLUSION

Based on the ichthyological material that has been collected with electrofishing device and nets of various mesh diameters in the last thirty years, in running and standing waters of Vojvodina, as well as in canal network of the Danube-Tisa-Danube Hydrosystem, it can be concluded that the number of tench-*Tinca tinca* is significantly decreased, and that this species has even disappeared, while proportional contribution of imported species, especially brown bullhead-*Ictalurus nebulosus*, pumkinseed sunfish-*Lepomis gibbosus* and Prussian carp-*Carassius gibelio*, is growing. All allochthonous species from American and Chinese complex, and especially the above mentioned ones, are one of the most prominent disturbing factors for tench.

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PRISUSTVO LINJAKA I ALOHTONIH RIBLJIH VRSTA U VODENIM TOKOVIMA

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Izvod

Na osnovu podataka dobijenih tokom kompleksnog ihtiološkog istraživanja voda Vojvodine u poslednjih trideset godine, posmatrano je prisustvo linjaka, *Tinca tinca*, kao i sledećih alohtonih ribljiv vrsta: terpana, *Ictalurus nebulosus*, sunčanice, *Lepomis gibbosus*, babuške, *Carassius gibelio*, belog amura, *Ctenopharyngodon idella*, belog tolstolobika, *Hypophthalmichthjs molitrix*, sivog tolstolobika, *Aristichthys nobilis* i bezribice *Pseudorasbora parva* koje predstavljaju bitan faktor za nestajanje gore pomenute autohtone riblje vrste. Rezultati su pokazali značajno smanjenje broja, pa čak i nestanak linjaka, dok je istovremeno prisustvo alohtonih vrsta povećano, posebno kada su u pitanju terpan, sunčanica i babuška.

Ključne reči: linjak, alohtone riblje vrste, vodotokovi, Vojvodina.

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INTEGRATED AND ORGANIC APPLE PRODUCTION

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SUMMARY: Implementation of Integrated apple production provides high yields of high quality and health safe fruits. If compared to the conventional fruit production, integrated production is environmentally acceptable because it sets specific approach to plant protection, nutrition and production technology. In order to provide apple export on European market, implementation of integrated production in Serbia is needed on the large scale. The paper presents integrated apple production on the plantation of „Atos Fructum"company, located in Mala Remeta on Fruska Gora. Integrated fruit production allows strictly controlled application of synthetic chemicals, which are listed in Guidelines for integrated pome production (AGRIOS). On contrary, application of any synthetic chemicals in organic apple production is not allowed. Such approach eliminates any potential risk of chemicals application to the environment. Organic production principles point out the importance of apple cultivars with genetic resistance to pests and diseases. Since organic apple production in Serbia is very limited, the purpose of the paper is to point out the importance of organic concept and review its basic principles.

Key words: *apple trees protection, fertilization, fruit quality, resistant apple cultivars.*

INTRODUCTION

According to the quantity of production, it is apples that stand as the most important continental fruit. The world's annual apple fruit production in 2009 was 71.286.000 tons. Major apple producers are China (31.684 million tons), the United States (4,514 million tons), Turkey (2.782 million tons), Poland (2.626 million tons), Iran (2.432 million tons). The leading producers of apples in Europe are Poland (2.626 million tons),

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Italy (2.313 million tons), France (1,953,600 tons), Russia (1.596 million tons) and Germany (1.07 million tons), making apple production in Europe comprise 22.6% of the world's apple production. However, there is no clear statistic evidence on the production of apples in Serbia, but according to FAO, the annual apple production in this country is around 281,800 tones (FAO, 2009).

Apples are the leading fruit species in Vojvodina, and have recently had a significant part in the total export of agricultural products. Due to increasing market demands and the inclusion in the European market, fruit growers should accept new production technologies such as integrated and organic production which will ensure obtaining high quality yields (Keserović et al., 2011).

The consumption of apples in the European Union is relatively low (19 kg per capita), but it does vary, making the consumption in Denmark 45 kg per capita and in Romania 5 kg per capita (EUROSTAT, 2011). The annual consumption of apples in Serbia is 14.33 kg per capita (FAO, 2001-2003). Low consumption of apples means that there is a longer period of time between instances of eating apples. Thus, the memory of quality can play an important role throughout the next purchase (Hacker, 2001). In order to keep consumers opt for apples during the purchase of fruits, it is necessary to maintain a constant quality of production, as it is certain that the quality of the fruits has a significant effect on consumers' decision when purchasing (Keserović et al, 2003). Integrated and organic apple production can provide a constant quality in production which may provide apple growers with safe placement of fruit on the market. Integrated fruit production allows strictly controlled application of synthetic chemicals, which are listed in Guidelines for Integrated Apple Production (AGRIOS, 2011). On the contrary, application of any synthetic chemicals in organic apple production is not allowed. (Law on Organic Production, Službeni glasnik no. 30/2010). The aim of organic production is to meet the demands of preserving natural resources (Keserović et al., 2008). Due to the goals that organic agriculture is based on, the limited choice and mode of action of pesticides allowed in organic production, the fight against diseases and pests has its advantages over integral and conventional production.

INTEGRATED APPLE PRODUCTION IN ORCHARD OF "ATOS FRUCTUM" COMPANY IN MALA REMETA AND GROWING SYSTEM IN ORGANIC FRUIT PRODUCTION

PLANTING SYSTEM

Integrated production of apples in ''Atos Fructum" company in Mala Remeta is located on the northern slope of Fruška gora mountain. The orchard is established on monocultivar principle at a distance of 3.20 x 0.65 to 0.80 m. The monocultivar apple orchard means that there is only one cultivar in each plot in the orchard, whereas every 25th tree stands for a pollinator. When it comes to the orchard in Mala Remeta, its pollinators are either certain apple or wild species, resistant to two economically important diseases - powdery mildew and apple scab. Orchards like this provide lower production costs and therefore make the protection of apples and chemical thinning easier and more efficient, as well as fruit picking much faster.

In organic fruit production, the planting density has also increased during past several years, but on average, it is still lower than in integrated production. Organic production suggests greater distances between and within the rows (3.0 – 3.5 m x 1.0 – 1.3 m) than in the integral production (Lind et al., 2003), all of which in order to achieve better ventilation and exposure to light and easier and better protection from diseases. This sort of a high density planting system implies using M9 rootstock for standard cultivars, M26 rootstock for spur types and slender spindle canopy system.

CULTIVARS AND ROOTSTOCKS

The orchard in Mala Remeta consists of the following cultivars: Golden Delicious clone B and Reinders®, clones of Jonagold DeCosta and Wilton's Red Jonaprince, Granny Smith clone Challenger® Dalivar, Red Delicious clones Camspur, Sandidge, Top Red and Early Red One, Breaburn Mariri Red, Fuji Kiku 8 and Gala Schniga. The rootstock is a clone of the M-9 337 for standard cultivars and M-26 for spur type Red Delicious.

Similar to integrated apple production, the main rootstock in organic production is M9 (Lind, 2003). However, there are significant differences in choosing cultivars in integrated and organic apple production. Due to the principles it is based on, the organic production requires the planting of tolerant and resistant apple cultivars. The most acceptable way to achieve the aims of organic production is breeding and introduction of new cultivars with genetic resistance to pathogens. This means that the metabolic products of plants prevent infection and pathogen development. Such an approach reduces the treatment of plants with chemicals to minimum, makes the production cheaper, nutritive values of fruits higher and provides a healthy environment.

Breeders around the world mostly use *Malus floribunda* 821 germplasm (Siebold ex. Van Houtte) (V_f gene) in order to make apple cultivars resistant to apple scab. The gene encoding this resistance is dominant (vertical resistance), which means that there is a danger of pathogen mutations and loss of resistance. The resistance provided by the V_f gene was considered permanent for years, as it could not have been overcome at that time. However, new cultivars that carry the V_f gene have not been cultivated in large areas. Parisi and Lespinasse (1993) suggest the existance of another cultivar 6 which causes the apple scab and has brought to question the stability and resistance provided by the V_f gene. On the other hand, the researches conducted by Parisi and Lespinasse (1999) showed that cultivar 6 *V. inaequalis* has caused russeting on most of the cultivars containing the V_f gene. The researches of Bénaouf and Parisi (2000), however, imply that *M. floribunda* has another dominant gene apart from the V_f gene, and that it was marked as V_{fh} gene. The same authors suggest the existence of V_g dominant gene which is held responsible for resistance of the Golden Delicious to cultivar 7 which causes the apple scab.

There are a few more major genes used worldwide as donors of resistance to apple scab beside the V_f gene, and they are: V_r, V_a, V_b, V_{bj} i V_m from another *Malus* species (Gessler et al., 2006). These genes have been used in a breeding program for decades in order to create resistant apple cultivars. In time, however, new pathogen types have overcome the monogenic resistance (Parisi and Lespinasse, 1993, 1999). Durable disease resistance is one of the main objectives of apple breeding worldwide. In order to achieve durable resistance to apple scab in the breeding of new apple cultivars for or-

ganic production, it is necessary to combine polygenic and monogenic resistance (Kellerhals and Duffy, 2006).

Even though the principles of organic agriculture propose usage of resistant and less sensitive cultivars, most organic fruit growers opt for cultivars that do not belong to these groups, as well as the ones commonly used when we talk about conventional and integral production. It is because the resistant cultivars are of a poorer quality and storage capability (Weibel et al., 2007). Apart from Topaz, no other leading apple cultivars in the system of organic fruit production in Europe (Golden Delicious, Jonagold, Elstar, Gala, Braeburn) belongs to the group of resistant cultivars (Trapman, 2008). Also, there are leading cultivars in the field of organic production in South Tyrol, commonly used in conventional and integral production. They are: Gala, Braeburn, Golden delicious, Crips pink, Fuji. Topaz, Red Topaz and Santana represent cultivars potentially good for organic production because they contain the V_f gene, have solid storage capability and are of equal or even better quality than cultivars frequently used in conventional production (Trapman and Jansonius , 2006).

PRUNING AND THINNING

The integrated production in apple orchards in Mala Remeta has changed the ways of traditional pruning. As opposed to short pruning, the long pruning suggests that a two-year old branch – the carrier of the native tree – is not shortened during the second year. Instead, only growth competitors at its end get removed. Such pruning reduces the vigour of fruit trees, provides better floral buds formation, easier chemical thinning, better colour of the fruits and reduction of fruit drops. Appropriate pruning enables moderate vigor of trees which is desirable in organic farming as it makes controlling apple scab easier (Leser and Treutter, 2005; Rühmann et al., 2002).

Along with pruning, thinning apples is a pomotechnical measure which is used to control the crop load and to prevent biennial bearing (Keserović et al., 2007). A heavy crop load reduces flower bud initiation, resulting in low yields the following year (Keserović et al. 2005). Following the guidelines of integrated concept, two or three thinning bioregulators are applied and manual correction must also be provided in order to achieve the objectives in terms of size and fruit quality (Stopar, 2002; Link, 2000), thus increasing the proportion of extra and first-class fruits. Protection against codling moth is performed easily with sparse trees, where the fruits are not clustered. The usual bioregulators applied are naphthalene acetamide (NAD), naphthyl acetic acid (NAA) and benzyladenine (BA) (Keserović et al., 2011).

Ammonium (ATS) and potassium thiosulfate (PTS) are experimentally used in apple orchards in Mala Remeta as flower thinners since they are considered user-, environment- and consumer-safe (Milić et al, 2011). Flower thinning with ATS and PTS significantly decreases the number of fruits, while at the same time increases the average fruit weight, although the highest chemical rates (3% ATS, 1.5% PTS) retards fruit growth. ATS and PTS do not affect fruit shape and substance, but they definitely do increase starch degradation, total soluble solids content and titratable acidity. Based on the research of Milić et al. (2011), ATS and KTS may be recommended as the first step in a chemical thinning program.

In organic apple production, the usage of chemical-synthetic thinning agents for crop regulation is not allowed. There are several different ways of performing the thin-

ning operation in organic fruit production: manual thinning, mechanical thinning and chemical thinning with organic pesticides, as well as some other organic compounds. Given that manual thinning demands a huge number of working hours, this type of thinning is basically used as a correction measure after mechanical or chemical thinning only. Lime sulphur is used in organic production as a fungicide against apple scab on one hand (Kunz et al, 2008), while on the other hand it can also cause the thinning effect (Clever, 2006). In Switzerland, however, neither of the mentioned products is allowed. In a nutrition experiment in organic apple production, using the N-vinasse (a by-product of molasses) as a foliar fertilizer, Weibel et al. (2008) have accidentally discovered that N-Vinasse has a considerable thinning effect when applied during flowering period.

RUSSETING

Russeting is the formation of cork cells in the apple (*Malus* × *domestica* Borkh.) skin. For most apple cultivars appearance of russet is unfavourable as it results in a poorer fruit quality and substantial economic losses to growers. Golden Delicious, a very important apple cultivar grown in Serbia, is susceptible to russeting. In apple orchards in Mala Remeta, the influence of commercial agents based on gibberellins (GA4+7) and gibberellic acid and cytokinins (GA4+7 + BA) was examined to prevent the russeting on Golden Delicious clone B. Using these products is permitted in integrated production since their use is a practical method for prevention or reduction of the russeting incidence (Taylor and Knight, 1986) caused by climatic factors (Yuri and Castelli, 1998). In addition to preventing the russeting occurrence using phytohormones, it is possible to get bigger and elongated fruits (Barandoozi and Talaie, 2009) along with a better market price.

The biggest problem for organic apple growing, however, is the appearance and, in particular, the amount of russet on the fruit. While the usage of phytohormones is not allowed in organic fruit production, using copper for apple protection is probably the most important factor in russeting. When it comes to apple cultivar protection, it has been noticed that better results are obtained when copper gets replaced with sulphur, lime sulphur (Jong and Mass, 2008) or potassium bicarbonate (Mitre and Mitre, 2009). In addition to this, a product for the suppression of *Erwinia amilovora* based on yeast *Aureobasidium pullulans* has been registered in organic fruit production. This product is prone to lead to the formation of russet on fruits (Matteson Heidenreich et al., 1997).

FERTILIZATION

The system of fertilization in the apple orchard in Mala Remeta is established in accordance with the integral concept of production, particularly with the content analysis of mineral elements in plants and soil. Every three years one soil analysis and every year two foliar analyses are conducted to determine the amount of nutrients which should be added. Fertilization is done through the irrigation system whilst any correction is done by a foliar fertilizer. Given the fact that an irrigation system is installed in this apple orchard, the soil between rows is covered with grass and the soil in rows is treated with herbicides. To maintain the soil, a rotary mower is used, leaving grass mowed and in-rows treated with herbicides simultaneously.

It is not allowed to use readily soluble mineral fertilizers in organic production. Instead, it is possible to use farm-produced natural fertilizers (manure, compost), green manure, mulches, varied crop rotations and careful tilling of the soil. For new orchards it is necessary to choose sites with quality soil, especially in organic production, which must be of good quality with adequate organic matter content (Granatstein, 2000).

The biggest problem in organic production is fertilization during fruit bearing due to the fact that the amount of nutrients in organic fertilizer is not precisely dosed as in synthetic fertilizers, and the nutrients are released longer. Nitrogen is easily leaching from the rhizosphere and in the organic production nitrogen deficiency can be a problem (Rosen and Allen, 2007; Nagy et al., 2009). The low content of nitrogen in organic apple production is associated with weed competition and inadequate accessibility of nitrogen from manure and compost (Nagy et al., 2010).

Nevertheless, in an organic orchard, nutrients can be added to the soil though manure, compost, green manure as well as any other organic substances that are allowed in organic production. Nmin method should be used to determine precisely the annual amount of required nitrogen. The basic principle of this method is fertilizing in accordance with the real needs of fruit trees for nitrogen, thus achieving a good quality of fruits while avoiding the risk of using excessively high doses of nitrogen and preventing pollution.

APPLE TREES PROTECTION

Integrated fruit production allows strictly controlled application of synthetic chemicals, which are listed in Guidelines for integrated pome production (AGRIOS, 2011). The introduction of integrated apple production in the intensive growing system (mono cultivar blocks, antihail nets, irrigation, nutrition based on the analysis of soil and leaves, grass belts, dense planting, etc.) is changing the conditions for the appearance of diseases and pests. Being familiar with some useful entomoacarophauna as well as predator/prey relationship is a prerequisite for the introduction of integrated pest management in apples as means of protection are chosen according to these relations. In order to determine these relationships, several methods are used in integrated pest management: a visual overview of 50 branches, 50-branch shakings in entomological catcher, examination of 25 leaves on each variety, determining the number of insects on pheromone traps and determination of the economic damage threshold, the use of corrugated hunting traps to monitor development of *C. pomonella* and monitoring conditions of leaf wetness. The need for spraying is determined by the thresholds for spraying, set for the integrated protection as they are adjusted to predator/prey ratio. Thus, the threshold for treatment against phytophagous mites is 50-60% of leaves with mite presence, 15% of the peak shoots with colonies of *A. pomi*, 6 cumulative caught moths *of C. pomonella* during one week and so on. Each variety is strictly monitored at intervals of 10-14 days and eventually, if the orchard age is uneven, each block is monitored separately. Quantities of protection agents in most cases are determined by a meter of tree height per ha. While apple scab and powdery mildew were significantly suppressed before leaf wetness by combining contact and penetrative fungicide, fire blight (*E. amylovora*) is suppressed by reducing inoculum with agents based on copper. To conclude, this brings us to a total of 11 sprays being conducted with each cultivar on average.

Due to the principles on which the organic production is based, as well as the lim-

ited choice and modes of action of pesticides whose usage is allowed in organic production (all in favour of quality protection), it is necessary to:

1) obey the basic principles of planting organic orchard,
2) apply appropriate cultural measures,
3) preserve and introduce population of natural predators,
4) perform monitoring in the orchard, and
5) establish protection by using products allowed in organic production.

In order to prevent major apple diseases (*Venturia inaequalis*, *Podosphaera leucotricha*, *Erwinia amylowora*), it is possible to use products based on copper and sulphur in organic production. In addition, organic producers use lime sulphur at the moment when the fungus needs to germinate on the leave surface (Jamar, 2008; Kunz et al., 2008). On the other hand, a wide range of viruses (Granulosisvirus CpGV), bacteria (*Bacillus thuringiensis*), plant extracts (azadirachtin, pyrethrin, quassia, rotenone), and other substances permitted in organic production can also be used so that the most important pests are repelled. In this matter, it is also important to introduce and preserve beneficial organisms such as earwig (*Forficula auricularia*), predatory mites (*Amblyselius andersoni*, *Typhlodorus pyri*), parasitoid wasp (*Aphelinus mali*), blue-tits and coal-tits (*Cyanistes caeruleus*, *Periparus ater*), as well as birds of prey.

CONCLUSION

The implementation of integral apple production would have an enormous impact on fruit production in Serbia as this modern type of technology enables reaching a high level of quality yields by using a minimal amount of pesticides. Given the various experiences throughout the years in Mala Remeta, integral production can be suggested as the best way of apple production in Serbia. However, the most acceptable way of achieving goals in organic production is creation and implementation of new cultivars that have genetic resistance to pathogens. Nevertheless, new cultivars which would be resistant or even more tolerant to some of the most important diseases and pests, but yet of the same quality as the leading ones in conventional production, are yet to be created. Together with this fact, it is the range and the mode of action of products used in organic production that make this type of production still less worth than conventional and integral production. Still, according to the demands of the European market and the advantages of agroecological conditions for apple breeding, more attention to apple organic production should be paid. It is inevitable for thorough research to be conducted before implementing new technology. Finally, to conclude with, it should be emphasized that different experiences from developed countries where organic production has been represented for years can undoubtedly prove handy in this matter.

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INTEGRALNA I ORGANSKA PROIZVODNJA JABUKE

ZORAN KESEROVIĆ, MARKO INJAC, NENAD MAGAZIN,
BISERKA MILIĆ, MARKO DORIĆ

Izvod

Integralni koncept proizvodnje voća omogućava dobijanje visokih prinosa kvalitetnih i zdravstveno ispravnih plodova. U odnosu na konvencionalnu, integralna proizvodnja je ekološki prihvatljiva jer nalaže poseban pristup zaštiti, ishrani i tehnologiji proizvodnje voća. Da bi se obezbedio plasman na Evropsko tržište neophodno je intenzivirati uvođenje integralnog koncepta proizvodnje voća u Srbiji. U radu je predstavljen integralni koncept proizvodnje jabuke u zasadu „Atos Fructum" u Maloj Remeti na Fruškoj gori. U integralnoj proizvodnji dozvoljena je strogo kontrolisana upotreba sintetičkih hemijskih sredstava, navedenih u Uputstvu Radne grupe za integralnu proizvodnju jabuke Južnog Tirola (AGRIOS). U organskoj proizvodnji, primena sintetičkih hemijskih sredstava nije dozvoljena. Ovakvim pristupom eliminiše se svaka potencijalna opasnost od primene hemijskih sintetičkih sredstava. Principi organske proizvodnje nalažu uvođenje sorti jabuke koje poseduju genetsku otpornost prema parazitima. Ovakav način proizvodnje u Srbiji je veoma slabo zastupljen, te se u radu razmatra značaj organske proizvodnje i dat je pregled osnovnih elemenata organske proizvodnje jabuke.

Ključne reči: zaštita voćaka, ishrana voćaka, kvalitet plodova, otporne sorte jabuke.

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