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RISK MANAGEMENT IN PLANT PRODUCTION WITH WEATHER DERIVATIVES

TODOR MARKOVIĆ, MILENKO JOVANOVIĆ[1]

SUMARRY: It has been long known that weather conditions are the main factor of uncertainty in plant production. For this reason, an integrated system of risk management in plant production is necessary today in order to somewhat compensate for the loss caused by weather risks. In the past, farmers have bought insurance for protection against fluctuations in crop yields caused by weather risks. Relatively new tools for risk management in plant production are weather derivatives. Although weather derivatives show many advantages over traditional insurance the market for these products is still relatively limited. Therefore, it is necessary to quantify the effect of risk reduction that can be achieved by using weather derivatives on the example of selected farm in Germany. If the field of production is close to the meteorological station, and if a high correlation between weather indices and yield is assumed, then the effect of risk reduction is significant (up to 40 %).

Key words: *risk management, crop insurance, weather derivatives, hedging effectiveness.*

INTRODUCTION

In the recent years various weather disasters have caused serious damage to agricultural areas. Crop insurance is the most frequently used instrument for risk management in plant production.

The research of crop insurance in Europe has been long actualized, while in Serbia only a small number of papers are devoted to this subject. The fact is that always after a flood, drought or a strong storm there are intensive discussions about crop insurance which can compensate for the loss in production (Breustedt, 2003). Economic attractiveness of different instruments for risk management, such as insurance, depends on the farmers' exposure to different risks (Berg, 2005). In addition to traditional yield insurance, some authors suggest the need for expansion of the multi-risk crop insurance which is mainly present in the developed countries of Europe and North America (Berg, 2002). Today, even the actual index-based insurance considers the possibility of

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Original scientific paper / Originalni naučni rad

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using weather derivatives in agriculture (Turvey, 2001; Berg et al., 2005; Mußhoff et al., 2005). It is significant that the most important aspects of the insurance market in the developed countries can also be applied to the countries in transition.

The aim of this paper is to provide the basic theoretical assumptions about the weather derivatives as a new financial instrument in cropweather put option.

MATERIAL AND METHODS

The basic source of data is the documentation of average yields and selling prices of winter barley from the selected farm in Oberfranken (Germany), as well as the information on monthly amounts of rainfall from the nearby meteorological stations for the period from 1999 to 2008.

The successful application of weather derivatives depends on the strength of rainfall influences on the yield of winter barley (Vedenov and Barnett, 2004). During the construction of weather put option the key issue is to determine fair premium, which the buyer is willing to pay for the transfer of risk. To determine the premium, the burn-rate-method is used, where the fair premium is the expected discounted value of payoff from weather derivatives (Berg et al., 2005). Following the same methodology the other parameters necessary for the construction of weather derivatives (weather index, payoff function, payoff limit) are determined.

Using quantitative methods for risk estimation, it is determined whether the weather risk reduction (hedging) is more successful with or without weather derivatives. This paper applies the methods of stochastic simulation and value-at-risk. The concept of stochastic simulation compares the distribution function (cumulative density function) of the winter barley revenue with and without weather derivatives (Brandes et al., 1992). On the other hand, the standard deviation as a measure of dispersion in statistics and percentile in the revenue distribution is being considered, and based on that, the possibility of down side risk reducing with and without weather derivatives is determined (Berg et al., 2005). All necessary calculations are performed using computer software (@ Risk), which was especially developed for the risk management.

RESULTS AND DISCUSSION

Weather derivatives are new financial instruments for transfer of risk, which occurred in mid 90s of the last century. They are the financial instruments that do not consider the base value such as the price of traded goods or any other financial category, but instead they take into account weather variables, such as rainfall (Berg, 2005).

During the construction of weather derivatives it is necessary to determine the following parameters: weather index, the type of derivatives, meteorological station, accumulation period, fair price, strike level, payoff function and payoff limit. The strike level represents the value of index from which the payoff is made, while the amount of payoff is determined with the tick size, which indicates the paid amount per unit index or change of unit index. Weather derivatives can be traded in stock exchange or over the counter - OTC. On the market of weather derivatives option trading is dominant (Becker and Bracht, 1999).

The options belong to the group of forward conditional operations and the customer gains the right, but assumes no obligation to buy or sell a contract to expire on a certain day in the future, and in return, he pays a premium (Berg, 2005). So the buyer of a rainfall option is required to pay the optional premium, but he has the right to a payoff, based on the difference between the weather index and strike level. On the other hand, the seller takes the obligation and receives a premium.

There are call option and put option. Call option gives the holder the right to buy, and put option the right to sell contract and it is frequently used in the crop insurance. From the buyer side payoff of weather put option (I_p) arising from differences between the strike level (R) and realized weather index (x), multiplied with tick size (O) (Berg et al., 2005):

$$I_p = O \cdot Max\left[0,(R-x)\right] \quad (1)$$

In the event that the weather index is above the strike level, it does not come to the payment. The buyer of weather put option would this way be protected from too low index level. If the premium (P) is taken away from the payoff, it comes to the profit (D), which the buyer from the put option gets (Berg, 2005)

$$D_P^{DP} = O \cdot Max\left[0,(x-R)\right] - P \quad (2)$$

Based on the above, the seller profit from the weather put option is calculated in the opposite way from the buyer's gain, that is, the payoff is taken away from the premium (Berg, 2005):

$$D_P^{KP} = P - O \cdot Max\left[0,(x-R)\right] \quad (3)$$

For option pricing the burn-rate-method is used. Fair price (P_f) for the put option can be calculated in the following form (Berg et al., 2005):

$$Pf = \left[(R - E(x|x<R))\varpi(x<R)\cdot O\right] e^{-r\cdot n} \quad (4)$$

In this example, the expression $E(x|x<R)$ represents the expected value assuming that the weather index is below the strike level. The expression $\varpi(x<R)$ is the probability that the weather index is below the strike level, while (e) is the discount factor.

In the example of the winter barley production the hedging effectiveness of weather derivatives is estimated. The basic data are taken from an individual farm in Oberfranken (Germany). This agricultural farm has an area of 75 ha, and 22 ha are under barley. The average yield in the selected time amounted to 8 t/ha (standard deviation 1 t/ha). The average price was 100 €/t, and the revenue 800 €/ha (standard deviation 100 €/ha).

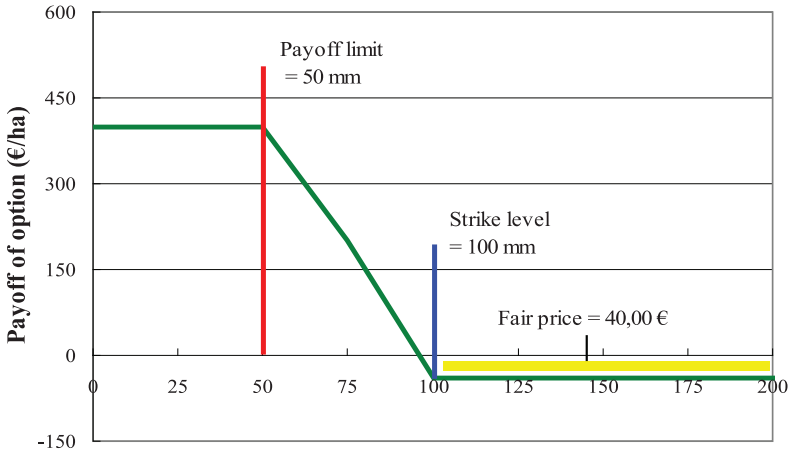

Chart 1: Fair premium and payoff of put option in winter barley production
Grafikon 1: Fer premija i isplata iz prodajne opcije u proizvodnji ozimog ječma

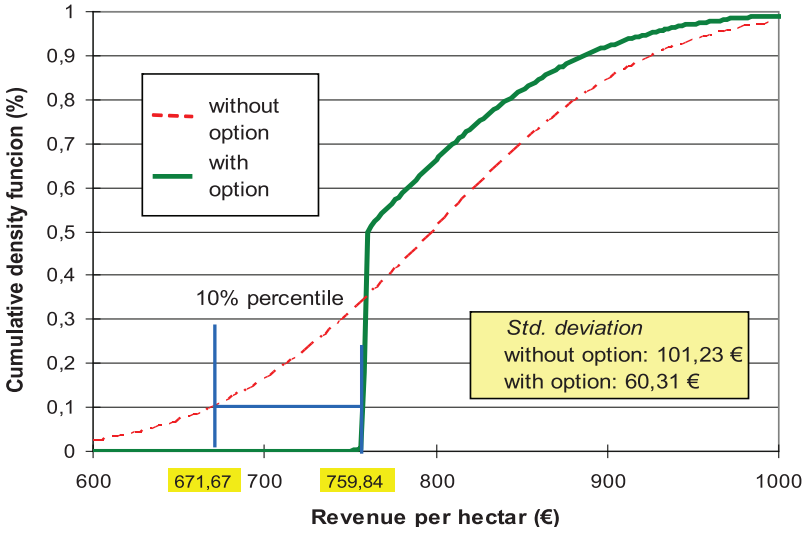

Chart 2: Revenue distribution of winter barley with and without option
Grafikon 2: Raspodela vrednosti proizvodnje ozimog ječma, sa i bez opcije

The analysis was conducted on the farm and it was determined that the amount of rainfall during the period April-May had a decisive influence on the height of winter barley yield. In order to prevent yield variability farmer decides to buy rainfall put option on OTC market. The data on rainfall were taken from a reference meteorological station close to the place of production. The weather index based on the monthly amounts of rainfall in April and May is at the level of 100 mm, which is the strike level, while the tick size is 8 €/mm. The payoff is limited to 50 mm, which means that if the rainfall is below this level, it is not going to be paid more, but the payoff remains the same. The weather contract is valid for two months and the payoff is possible if meas-

ured rainfall is below the strike level (Chart 1). If in the formula (5) the discount factor ($e^{-r \cdot n}$) includes the interest rate of 5% and as the weather period the two months (April-May) are taken, you get the option price of 40 € (Chart 1).

In order to determine the revenue with and without weather derivatives the method of stochastic simulation is used. In case without weather put option the total revenue is equal to the market revenue, while in case with option, the farmer adopts profit from the option according to the formula (1). Rainfall and yield are stochastic size and since there is a strong positive correlation between them, the revenue below 800 €/ha is compensated from the payoff of the put option. When the fair premium of 40 €/ha is paid, it can be noted that the revenue under 760 €/ha is completely "cut off" and the lower revenue cannot happen (Chart 2). The standard deviation is reduced from 101.23 €/ha (without option) to 60.31 €/ha (with option). This way the down side risk is significantly reduced (40.42 %).

If the percentile is taken as the measure of risk reduction, then the lower part of the distribution, where the lowest revenue is, is taken into account. In the case without option in the 10% years, the expected revenue is under 671.67 €/ha, while in the case with option it is increased to 759.64 €/ha. In case of option revenue of 759.84 €/ha instead in 5% it will be lower in almost 35% of years (Chart 2). In this way with put option the down side risk is being reduced.

CONCLUSION

The presented example of using weather derivatives clearly shows that they still indicate the useful tools for weather risk reducing. Special emphasis is placed on reducing the oscillation of economic indicators (for example, revenue), caused by the weather factor. If the place of production is close to the meteorological station, and there is a strong correlation between weather index and yield of winter barley, the effectiveness of risk reduction is significant (up to 40%). But if they are in remote locations and there is a lower correlation coefficient, that significantly reduces the effect of protection. In practice, it is reasonable that a fair premium is increased with transaction costs and risk premium, which also reduces the positive effect of these instruments. However, preliminary calculations show significant potential of weather derivatives in reduction of production risks, and therefore they can be a supplement to the existing instruments for risk management in plant production.

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UPRAVLJANJE RIZIKOM U BILJNOJ PROIZVODNJI PRIMENOM VREMENSKIH DERIVATA

TODOR MARKOVIĆ, MILENKO JOVANOVIĆ

Izvod

Odavno je poznato da vremenske prilike predstavljaju glavni faktor nesigurnosti u biljnoj proizvodnji. Iz tog razloga jedan integrisani sistem upravljanja rizikom danas predstavlja neminovnost, kako bi se donekle kompenzovale posledice vremenskih neprilika. U prošlosti su poljoprivrednici kupovinom osiguranja pokušali da se zaštite od kolebanja prinosa useva i plodova uslovljenih vremenskim rizicima. Relativno nov instrument za upravljanje rizikom u biljnoj proizvodnji predstavljaju vremenski derivati. Iako vremenski derivati pokazuju brojne prednosti u odnosu na klasično osiguranje, tržište ovih proizvoda još uvek je relativno malo. Zbog toga je potrebno na primeru ozimog ječma na izabranom individualnom gazdinstvu u Nemačkoj kvantifikovati efekat smanjenja rizika koji se postiže upotrebom vremenskih derivata. Ukoliko je mesto proizvodnje u neposrednoj blizini meteorološke stanice i ako je pretpostavljena visoka korelacija između količine padavina i prinosa ozimog ječma onda je značajna efikasnost smanjenja rizika (do 40 %).

Ključne reči: upravljanje rizikom, osiguranje useva i plodova, vremenski derivati, efikasnost smanjivanja rizika.

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RECURSIVE FORMULA FOR NUMBER OF COWS IN MILK PRODUCTION*

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SUMMARY: We study the dependence of maximum number of cows K_n after ***n*** *years, involved in milk production, on following parameters: an initial number of pregnant heifers K, average percentage of successful calving, calving of female calves and twin calving, marked as p_1, p_2 and p_3, respectively. We have established a recursive formula for maximum number of cows K_n. If* ***n****=4, then* $K_4=(1+\mathbf{p})\cdot(1+3\mathbf{p})\cdot K$, *where* ***p*** *stands for average percentage increase in number of cows after two years;* ***p*** *is 88% of* $\mathbf{p_1}$, *where* $\mathbf{p_1}$ *stands for average annual percentage increase in number of female calves. It has been performed an analysis for percentage increase in number of cows K_n in milk productions, if the percentage of calving is between 80% and 90%, for* ***n****=2,3,4,5,6. Proposed formulae can be used for numerous additional financial analyses in milk production.*

Key words: *closed household, mathematical model, economical analysis, milk production, pregnant heifers*

INTRODUCTION

In introductory motivational part of the textbook Economy and Financial Mathematics (Matić-Kekić, 2006a), there is a simplified problem we would like to engage in milk production: How many heifers should be obtained per livestock unit so that after the expiry of 8 years we can acquire 500 cows, if its known that 95 % of cows calve every year and that 50% of that number make female calves? The book does not contain the solution of this problem, so the task was given (for the last 5 years) to the students (about 600 – 700 students) of the Faculty of Agriculture, Novi Sad for individual work with explanatory note that cows from the flock are already pregnant and that in the year of their research we can expect the first offspring. After all, three students, managed to get the right solution applying foot-by-foot calculation.

Original scientific paper / *Originalni naučni rad*

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MATERIAL AND METHODS

In this paper, we have applied one mathematical method of modeling - recursive formula (Acketa and Matić-Kekić, 2000) with initial data and one statistic method (nonlinear regression), which has been applied in (Matić-Kekić, 2006; Matić-Kekić et al., 2007; Dedović et al., 2009, 2010). Program packages *Mathematica 5.0* (Wolfram Research, Inc., 2003) and *Statistica 8.0* (StatSoft, Inc., 2009) facilitated appliance of this method and those packages were successfully used in the papers related to agricultural problems (Matić-Kekić and Mudrinski, 2004, 2009; Matić-Kekić et al., 2008; Babić et al., 2011a and 2011b).

RESULTS AND DISCUSSION

Under a closed household we imply a household with starting fund of inseminated heifers which in the next period doesn't obtain new heifers or buy new female or male calves, or sells the ones obtained with reproduction of livestock unit. In this paper we are going to derive mathematical formulae for a number of dairy cows in closed household. First, let us denote with p_1 percentage of calving, p_2 percentage of female offspring and p_3 percentage of twin offspring. Then, we can calculate an average annual percentage increase in number of female calves $\mathbf{p}$ by

(1) $$\mathbf{p} = p_1 \cdot p_2 + p_1 \cdot p_2 \cdot p_3$$

For example, if percentage of calving is $p_1 = 80\%$, percentage of female offspring calving is $p_2 = 47\%$ and percentage of twin offspring calving is $p_3 = 3\%$, then average annual percentage increase in number of female calves is $\mathbf{p} = 38.73\%$. However, since 12% of female calves do not reach age of two (Etgen and Reaves, 1978), percentage of heifers in closed household after 2 years is equal to

(2) $$\mathbf{p} = 0.88 \cdot \mathbf{p}.$$

CORRELATION BETWEEN PERCENTAGE INCREASES IN NUMBER OF COWS AND P_1, P_2 AND P_3

Since UK is a region where cattle-breeding is extremely developed, we present the data from NUTS1 (Nomenclature for Units of Territorial Statistics level 1). Holstein influence appearing in 51% of all 3.47 million dairy cattle in the UK:

- Holstein Friesian (Friesian with more than 12.5% and less than 87.5% of Holstein blood): 1 765 000 (51%)
- Friesian (more than 87.5% Friesian blood): 1 079 000 (31%)
- Holstein (more than 87.5% of Holstein blood): 254 000 (7%)
- Holstein Friesian Cross (any of the above crossed with other breeds): 101 000 (3%)
- Other dairy breeds: 278 000 (8%).

In Serbia, Domestic Spotted cattle of Simmental type and Simmental cattle have an important place in cattle breeding (Bošnjak and Rodić, 2008). Out of the total number of cattle (1 153 000 head) about 40-50% falls into Simmental type, 30-40% into Domestic Spotted and about 20% into Black and white cattle and other breeds and crosses.

Analyzing data in Table 1, we can conclude that average annual percentage increase in number of female calves $\mathbf{p}$ is increased for about:

- 2.662% upon increase of calving for 5% (p_1);
- 2.637% upon decrease of female offspring calving for 3% (p_2);
- 0.863% upon increase of twin calving for 2% (p_3).

Therefore, the most influential parameter on average annual percentage of increasing the number of female calves $\mathbf{\bar{p}}$ is the decrease of female offspring calves p_2 ($\mathbf{\bar{p}} : p_2 = 1:1$), then the percentage of calving p_1 ($\mathbf{\bar{p}} : p_1 = 0.5:1$) and finally, the percentage of twin calving p_3 ($\mathbf{\bar{p}} : p_3 = 0.4:1$). Because of direct proportion between $\mathbf{p}$ and $\mathbf{\bar{p}}$, influence of parameters p_1, p_2 and p_3 is:

(3) $\mathbf{p} : p_2 = 0.88 : 1$, $\mathbf{p} : p_1 = 0.44 : 1$ and $\mathbf{p} : p_3 = 0.352 : 1$

However, large influence of twin calves should be taken examined carefully, because of widely known negative consequences of twin calving.

Table 1. Correlation between p and p_1, p_2, p_3 for different breeds of dairy cows
Tabela 1. Korelacija između $\mathbf{p}$ *i* p_1, p_2, p_3 *za različite rase mlečnih krava*

Breed Rasa	p_1	p_2	p_3	$\mathbf{\bar{p}}$	$\mathbf{p}$
HF	0.8	0.47	0.03	0.3873	0.3408
S	0.8	0.5	0.05	0.42	0.3696
HxNCB HxR DŠ	0.8	0.5	0.03	0.412	0.3626
	0.85	0.5	0.03	0.4378	0.3852
	0.9	0.5	0.03	0.4635	0.4079
S	0.9	0.5	0.05	0.4725	0.4158
S	0.85	0.5	0.05	0.4463	0.3927
HF	0.9	0.47	0.03	0.4357	0.3834
HF	0.85	0.47	0.03	0.4115	0.3621
S	0.95	0.5	0.05	0.4988	0.4389

According to Čobić et all. (1995), genotype Simmental (S) (the total number of cows 710) has p_2=49.83%, and genotypes Holstein x rotbunt (HxR) (the total number of cows 719) and Holstein x German black-white HxNCB) (the total number of cows 714) have p_2=50.47%, while according to Nenadović et all. (1981) genotype Domestic spotted (DŠ) has p_2=49.33%. Genotype Holstein-Friesian (HF) breed has p_2= 46.7%. Average percentage of twin calving p_3, is between 2.93% (Holstein-Friesian breed) to 4.97% (Simmental breed).

If veterinarian control is performed correctly during twin calving process of Simmental breed and if insemination of heifers is 90% successful, then Simmental breed is the leading breed by all parameters for main fund genotype selection, because of the largest increase of number of cows.

FORMULAE FOR THE NUMBER OF COWS

Let K be a number for inseminated heifers at the beginning (zero year) which will calve by the end of the year. With K_n we denote a number of dairy cows after **n** years, where at in that **n** year we don't count starting (zero) year. With $\mathbf{\bar{p}}$ we denote an average annual percentage increase in number of female calves and all female calves are kept at the household and inseminated at the age of 14 -15 months, and then successfully calved at the age of 2 (there is $\mathbf{p}$ percentage of K). We have obtained recursive formula

(4) $K_n = K_{n-1} + \mathbf{p} \cdot K_{n-2}$.

For initial data $K_0 = K_1 = K$, using **(4)** we derive number of cows in the following years:

$$K_2 = (1 + \mathbf{p})\, K = k_2 \cdot K$$
$$K_3 = (1 + 2\mathbf{p})\, K = k_3 \cdot K$$
(5) $$K_4 = (1 + 3\mathbf{p} + \mathbf{p}^2)\, K = k_4 \cdot K$$
$$K_5 = (1 + \mathbf{p})(1 + 3\mathbf{p})\, K = k_5 \cdot K$$
$$K_6 = (1 + 5\mathbf{p} + 6\mathbf{p}^2 + \mathbf{p}^3)\, K = k_6 \cdot K$$

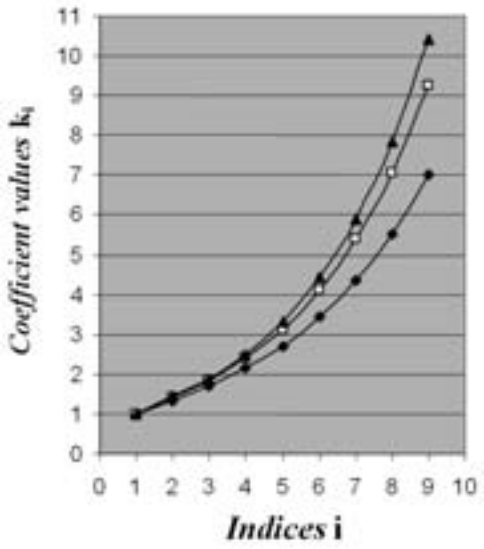
With k_n we have denoted the coefficient which multiply K in formulas for K_n, i.e. $k_1 = 1$, $k_2 = (1 + \mathbf{p})$, $k_3 = (1 + 2\mathbf{p})$, ... , $k_6 = (1 + 5\mathbf{p} + 6\mathbf{p}^2 + \mathbf{p}^3)$. For average percentage increase in number of cows **p** = 34.08%, which was derived with presumption that: percentage of calving p_1 = 80%, percentage of female offspring p_2 = 47% and percentage of twin offspring p_3 = 3% values of coefficient k_n are calculated (Table 1, 2nd row) and graphically presented on Figure 1. In 3rd and 4th row of Table 2, there has been given the coefficients k_i, **i** = 1,2,...,6, which are stand for increase in number of dairy cows after **i** years, in comparison to starting number of inseminated cows K, for an average annual increase in number of cows **p** = 40.79% (when p_1 = 90% , p_2 = 47% and p_3 = 3%), i.e. **p** = 43.89% (when p_1 = 95%, p_2 = 50% and p_3 = 5%). Coefficients of cows by year k_i, **i** = 1,2,...,9 from 2nd, 3rd and 4th row of Table 2 are graphically presented on Figure 1. and marked with symbols ♦, □ and ▲, respectively.

Table 2. Coefficient k_i *,* **i** *= 1,2,...,6, average percentage increase in number of cows* **p** *in two years, and number of cows* K_i, **i***=1,2,...,6, in closed household at the end of the year* **i** *for initial number of inseminated heifers K*

Tabela 2. Koeficijent k_i *,* **i** *= 1,2, ..., 6 za prosečan procenat dvogodišnjeg uvećanja broja prvotelki* **p***, i broj krava K***i***,* **i***=1,2,...,6 u zatvorenom gazdinstvu na kraju* **i***-te godine za početni broj osemenjenih junica K*

p	k_1	k_2	k_3	k_4	k_5	k_6
0.3408	1	1.34	1.68	2.14	2.71	3.44
0.4079	1	1.41	1.82	2.4	3.13	4.11
0.4389	1	1.44	1.88	2.5	3.33	4.43
30	30	40	50	64	81	103
30	30	42	55	72	94	123
30	30	43	56	75	100	133
K	K_1	K_2	K_3	K_4	K_5	K_6

In 5th, 6th and 7th row of Table 2, if the starting number of inseminated cows is K=30, then number of dairy cows is K_i, **i**=1,2,...,6 in closed household at the end of year **i**, consecutive for average percentage increase in number of cows after two years **p** = 34.08%, (5th row), **p** = 40,79% (6th row), i.e. **p** = 43,89% (7th row).



Graph. 1. Graph of coefficients values of increasing number of cows ki, i=1,2,...,9 for average percentage increase in number of cows (34.08%, 40.79% and 43.89%)
Grafikon 1. Prikaz vrednosti koeficijenata uvećanja broja muznih krava ki, i = 1,2, ..., 9 za prosečan procenat dvogodišnjeg uvećanja broja prvotelki p (34,08%, 40,79% i 43,89%).

Using the formula **(2),** we have calculated percentage **p** (Table 3, 1st column) which depends on **p**, p_1, p_2 and p_3 (Table 3, last three columns). Now, using the recursive formula **(4)** we have calculated coefficients k_i at year **i**, **i**=1,2,...,9 (Table 3, columns 2-9).

*Table 3. Coefficients k_i for **i** = 1,2,...,9, for different percentage of calving p_1, calving of female calves percentage p_2, and twin calving percentage p_3.*
*Tabela 3. Koeficijenti godišnjeg uvećanja broja muznih krava k_i, **i** = 1,2, ..., 9 za razne vrednosti procenta teljenja p_1, procenta teljenja ženskih teladi p_2 i procenta teljenja blizanaca p_3.*

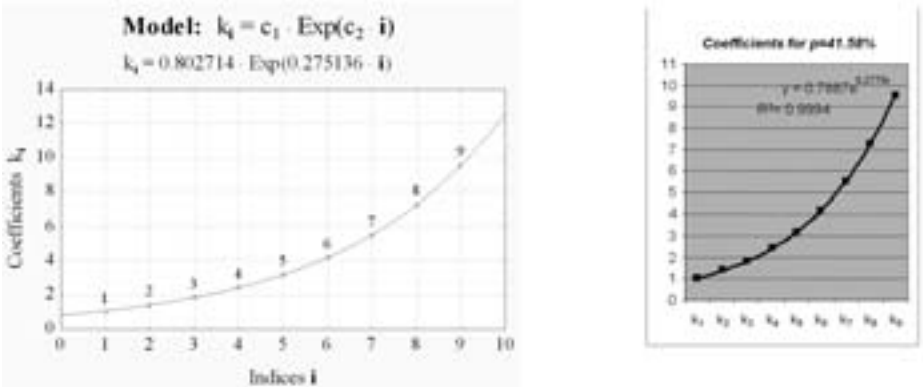
p	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	p_1	p_2	p_3
0.3626	1	1.36	1.73	2.22	2.84	3.65	4.68	6	7.7	0.8	0.5	0.03
0.3347	1	1.33	1.67	2.12	2.68	3.39	4.28	5.41	6.84	0.8	0.47	0.05
0.3834	1	1.38	1.77	2.3	2.98	3.86	5	6.47	8.39	0.9	0.47	0.03
0.3909	1	1.39	1.78	2.33	3.02	3.94	5.11	6.65	8.65	0.9	0.47	0.05
0.4158	1	1.42	1.83	2.42	3.18	4.19	5.51	7.25	9.54	0.9	0.5	0.05
0.3927	1	1.39	1.78	2.33	3.03	3.95	5.14	6.69	8.71	0.85	0.5	0.05
0.3852	1	1.38	1.77	2.30	2.99	3.87	5.02	6.52	8.45	0.85	0.5	0.03
0.3621	1	1.36	1.72	2.22	2.84	3.64	4.67	5.99	7.69	0.85	0.47	0.03

Using program package *Statistica 8.0*, it has been determined a strong correlation between k_i at year **i** of calving (R^2=99.86%). Exponential dependence has been derived and created a mathematical model in order to derive the coefficients c_1 and c_2 using least square method

(6) $k_i = c_1 \cdot \mathrm{Exp}(c_2 \cdot \mathbf{i})$ for **i** = 1,2,...,9

for typical coefficient values of k_i in Table 3, with **p**=41.58%. Function in **(6)** is shown in Graph 2.

As it was shown (Matić-Kekić 2006b), program package Excel is unreliable since the parameter c_1 does not belong to its 95% confidence interval calculated using the program package *Statistica 8.0*. So we relied on program package *Statistica 8.0*.



Graph 2. Correlation between coefficients ki and year i of calving – mathematical model at the program package Statistica 8.0 (left) and the program package Excel (right)
Grafikon 2. Korelacija između koeficijenata k_i i godine i teljenja – matematički model rađen u prgramima Statistika 8.0 (levo) i Excel (desno)

CONCLUSIONS

We have derived the formula **(2)** $\mathbf{p}=0.88\cdot\mathbf{\bar{p}}=0.88\cdot(p_1\cdot p_2 + p_1\cdot p_2\cdot p_3)$, where **p** stands for average percentage increase in number of cows after two years, $\mathbf{\bar{p}}$ stands for average annual percentage increase in number of female calves an initial number of inseminated heifers K, p_1 average percentages of calving, p_2 calving of female calves percentage and p_3 twin calving percentage. Simmental breed is leading breed by all parameters (**p**, $\mathbf{\bar{p}}$, p_2 and p_3) for main fund genotype selection because of the largest increase of number of cows. The most influential parameter on $\mathbf{\bar{p}}$ is the decrease of percentage of female offspring calves p_2. Parameters p_1, p_2 and p_3 influence on percentage of new cows is: $\mathbf{p} : p_2 = 0.88 : 1$, $\mathbf{p} : p_1 = 0.44 : 1$ and $\mathbf{p} : p_3 = 0.352 : 1$. It has been derived formula **(4)** $K_n = K_{n-1} + \mathbf{p} \cdot K_{n-2}$ with initial data $K_0 = K_1 = K$ for number of dairy cows K_n at the end of year **n** at closed household. K_n depends on K and **p**, where K represents the number of successfully inseminated heifers in the zero year, which we can expect their cows until zero year is finished. It has been calculated the coefficients k_i of cows number increase per year **i** and number of cows K_i, for **i**=1,2,…,6 for initial fund K=30 inseminated heifers for average percentage increase in number of cows after two years **p**=34.08% (with $p_1 = 80\%$, $p_2 = 47\%$ and $p_3 = 3\%$ for Holstein-Friesian breed), **p** = 40.79% (with $p_1 = 90\%$, $p_2 = 50\%$ and $p_3 = 3\%$ for Domestic spotted and Holstein breeds) and **p** = 43.89% (with $p_1 = 95\%$, $p_2 = 50\%$ and $p_3 = 5\%$ for Simmental breed) in Table 1, and Figure 1. Changing the percentage of calving p_1, we have obtained coefficients k_i, which are presented in Table 2.

The proposed formulae can be used for numerous additional financial analyses in milk production. For example, if the grace period for the term of the credit repayment of

5 years, it is easy to determine what starting fund K is required in order to have a dairy cattle fund of 50 cows after 5 years.

Since k_4=2.51 (Table 2, 4th row), it means that initial fund must contain 20 inseminated calves of Simmental breed. In case of Dutch-Friesian, if the percentage of calving is 80%, then initial fund must contain 24 inseminated calves.

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REKURZIVNA FORMULA ZA BROJ KRAVA U OKVIRU PROIZVODNJE MLEKA

SNEŽANA MATIĆ-KEKIĆ, NEBOJŠA DEDOVIĆ, SNEŽANA TRIVUNOVIĆ

Izvod

Neka je početni broj osemenjenih junica označen sa K, dok su sa p_1, p_2 i p_3 označeni redom prosečni procenti teljenja, teljenja ženskog teleta i teljenja blizanaca. Uz fiksiranje vremena uspešnog osemenjavanja na 14-15 meseci starosti junica izvedena je formula za maksimalan broj mlečnih krava K_n u proizvodnji mleka na zatvorenom gazdinstvu, dobijen od polaznog fonda od K steonih junica, nakon **n** godina. Na primer, za **n** = 4 je

$K_4 = (1 + \mathbf{p}) \cdot (1 + 3\mathbf{p}) \cdot K$,

gde je **p** 88% od $\bar{\mathbf{p}}$, a sa $\bar{\mathbf{p}}$ je označen godišnji prosečni procenat uvećanja broja ženske teladi $\bar{\mathbf{p}} = p_1 \cdot p_2 + p_1 \cdot p_2 \cdot p_3$. Izvršena je analiza za koliko procenata se povećava broj grla u proizvodnji mleka K_n, ako procenat teljenja varira od 80% do 90%, za **n**= 2, 3, 4, 5, 6. Predložene formule mogu da posluže za mnogobrojne dodatne finansijske analize u proizvodnji mleka. Tako, na primer, ako je "grace" period za otplatu kredita 5 godina, lako može da se utvrdi koliki početni fond K je potrebno zasnovati pa da se nakon 5 godina ima muzni fond od 50 grla.

Ključne reči: zatvoreno gazdinstvo, model uvećanja stada, finansijska analiza, proizvodnja mleka, steone junice.

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THE INFLUENCE OF NITROGEN AND SUPLHUR FERTILIZATION ON THE YIELD FORMATION AND QUALITY OF BROCCOLI

MIROSLAV ŠLOSÁR, ANTON UHER, JÁN KÓŇA, TOMÁŠ PECHOČIAK[1]

SUMMARY: In the field experiment with broccoli, we investigated an effect of four different variants of nitrogen and sulphur fertilization on the yield formation and broccoli quality in the term of acid ascorbic content and nitrate accumulation in broccoli florets. The influence of fertilization on the selected parameters of broccoli yield was similar in different years. The broccoli yield was statistically significantly increased at all fertilization variants compared to the control variant. The highest yield of broccoli was reached at the variant 4 when it was fertilized on the level of nutrients N:S = 200:60 kg.ha^{-1}. The increase of yield compared with the control variant was 26.8 %. The applied fertilization positively affected the accumulation of acid ascorbic in the broccoli florets. The highest content of ascorbic acid was determined at the variant 2 (N = 200 kg.ha^{-1}). In comparison with control variant, the ascorbic acid content was increased about 12.9 %. On the other hand, the applied nutrition resulted in increased accumulation of nitrates in the broccoli. The greatest increase of nitrates content, in comparison with the control variant, was ascertained at the variant 4 (about 31.0 %).

Key words: *broccoli, fertilization, yield, acid ascorbic, nitrates.*

INTRODUCTION

Vegetables have a very important role in human nutrition and their health benefit is undoubted. Nevertheless, we can observe a deficient level of annual vegetable consumption today. According to human nutrition specialists, the recommended consumption of vegetables is on the level of 128 kilograms. But in Slovakia, we are deeply below this value - 85 kilograms (Valšíková and Uher, 2009). This fact was also confirmed in previous research by Dudrikova, et al. (2009) and Kubicová and Fatrcová-Šramková (2010), who point to very low consumption of vegetables, especially by young people.

Original scientific paper / *Originalni naučni rad*

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In comparison with other agricultural crops, vegetables require relatively high rates of nutrients for cultivation. It is well known that nitrogen nutrition markedly affects the quantity and quality of grown crops. But it is also very important not to forget to apply other macroelements (Fecenko and Ložek, 2000). Nitrogen and sulphur, as well as phosphorus and potassium, are determining nutrients for growing of broccoli and other cruciferous vegetables in relation to quality, but also quantity of harvested phytomass (Goodlass et al., 1997).

The vitamin C (ascorbic acid) is one of most important nutritional compounds in broccoli. It must be ingested in food form because human body is not able to form ascorbic acid (Lee and Kader, 2000). Byers and Perry (1992) indicate that acid ascorbic prevents cancer by inhibiting creation of nitroso-compounds in stomach and stimulation of immune system. The content of acid ascorbic in broccoli can be influenced by various factors such as genotypic differences (Singh et al., 2007), climatic conditions (Vallejo, F. et al., 2003), farming technology – for example fertilization (Kováčik and Takáč, 2009), maturity and harvesting methods (Lee and Kader, 2000), and postharvest handling procedures (Serrano et al., 2006).

Nitrates are present naturally in soils, water, plants – particularly in vegetables as a consequence of nitrogen fixation (Mor et al., 2010). The natural levels of nitrate in vegetables generally are high in comparison with other food groups. It is estimated that 75-80% of the total daily intake comes from vegetables (Dennis and Wilson, 2003). Nitrate content varies considerably according to species (Mor et al., 2010). Nitrate contamination in vegetables occurs when crops absorb more than they require for their sustainable growth. Spinach, lettuce, broccoli, radish, etc., have the tendency to accumulate nitrates (Greenwood and Hunt, 2006).

Nitrate is largely unreactive but can be reduced to nitrite. Nitrite ion can react with secondary or tertiary amines to form nitrosocompounds – some of them being implicated in the etiology of cancers (Sebecic and Dragojevic, 1999). Another important concern is that vegetables are an important part of most babies' diets (Huarte-Mendicoa et al., 1997). Young babies with low stomach acidity may suffer from infantile methemoglobinemia due to excessive nitrates in their diet where nitrite is substituted for oxygen in hemoglobin and death may occur (Ezeagu, 1996).

MATERIAL AND METHODS

The small-plot field trial with broccoli was established in the area of the Botanical Garden of Slovak Agricultural University in Nitra in 2008 and 2009. In this trial, we used a middle-late broccoli cultivar CORONADO F1 which forms compact and finely-granular florets of dark green colour.

In the field trial, we monitored the effect of four different fertilization variants on the quantity and quality of broccoli yield:

1) variant – control (without application of fertilizers),
2) variant – application of nitrogen at the level N = 200 $kg.ha^{-1}$,
3) variant – application of nitrogen and sulphur at the level N:S = 200:50 $kg.ha^{-1}$,
4) variant – application of nitrogen and sulphur at the level N:S = 200:60 $kg.ha^{-1}$.

Table 1. Agrochemical soil characteristics before the establishment of the trial

Year	Content of nutrients in mg.kg^{-1} of soil							Humus (%)
	pH/KCl	N_{an}	P	K	S	Ca	Mg	
2008	7.13	16.8	130	570	67.5	6300	695	3.42
2009	7.24	10.5	200	580	34.0	6700	660	4.38

Broccoli seedlings were planted out at the experimental area on 24th June 2008 and 22nd June 2009. Each variant had four repeats. In every repeat, we planted nine seedlings into the plating space 0.5 x 0.5 m.

At the fertilization variants 2, 3 and 4, we used the following fertilizers: LAD 27 (27% of N) and DASA 26/13 (26% of N and 13% of S). The fertilizer DASA 26/13 was applied three weeks before outplanting. The calculated dose of LAD 27 was applied in two terms - three (50%) and six weeks (50%) after planting.

The harvest of broccoli was realized in four terms from 15th August to 9th September 2008 and from 20th August to 3rd September 2009. We harvested broccoli florets included the edible part of stump which was 10 centimetres long. The content of the monitored qualitative compounds was determined in average sample which was prepared from broccoli florets harvested on 3rd September 2008 and 31st August 2009. The content of acid ascorbic was analyzed by titration method. The nitrates content was determined by nitrate electrode screening method. The obtained results were evaluated by analysis of variance (ANOVA). The tests were performed on confidence level 95%.

RESULTS AND DISCUSSION

The influence of fertilization on the selected quantitative and qualitative yield parameters of broccoli yield was similar in different years. In addition, there were no significant differences in the experimental years. Therefore, the results are presented as the means of two years of the experiment (Table 2).

Table 2. Quantitative and qualitative parameters of broccoli yield

Variant	Yield			AW[1] (g)	Acid ascorbic			Nitrates		
	t.ha^{-1}	Rel. %[2]			mg.kg^{-1}	Rel. %		mg.kg^{-1}	Rel. %	
1	15.32	100	-	382.9	405.70	100	-	703.9	100	-
2	19.91	129.9	100	497.7	467.50	115.2	100	833.7	118.4	100
3	20.40	133.2	102.5	509.8	440.45	108.6	94.2	897.1	127.5	107.6
4	20.70	135.1	104.0	517.6	448.35	110.5	95.9	922.1	131.0	110.6

[1]Average weight of broccoli florets, [2]Relatively in %

On the basis of the obtained results, it can be concluded that a statistically significant increase of broccoli yield was observed at all variants with applied fertilization in comparison with unfertilized control variant in both years of the experiment (Table 3). The highest yield of broccoli was attained at the variant 4 – 20.70 t.ha^{-1}. This value, in comparison with the control variant, introduced an increase of broccoli yield about 35.1% (Table 2). If we compare nitrogen and combined nitrogen-sulphur variants with each other, it can be seen that the significant yield of broccoli florets was showed only in 2009 (Table 3).

The average weight of broccoli florets ranged from 382.9 to 517.6 g and it increased in the following variants order: 1 (control) < 2 < 3 < 4 (Table 2). At the variant 4, we achieved the increase of average florets weight about 134.7 g compared to the control variant.

Thus, the obtained results of our research confirmed the fact that applied nitrogen nutrition has fundamental affect on the yield of grown vegetable as it was demonstrated in the previous research by Babik and Elkner (2002) and Varga, et al. (2004).

The applied nitrogen fertilization also affected the quality of the grown broccoli or content of monitored qualitative parameters of broccoli florets. We observed statistically significant increase of ascorbic acid content at all fertilization variants compared to the control variant in both experimental years, besides variant 3 in 2009. Differences between variant 2 (N) and variants 3 and 4 (N+S) were not statistically significant.

The average content of ascorbic acid in broccoli ranged from 405.70 to 467.50 $mg.kg^{-1}$ of fresh matter (Table 2). The highest vitamin C content was determined at the variant 2 where nitrogen only was applied. At this variant, we achieved the increase of ascorbic acid content about 15.2% in comparison with the control variant. At variants with nitrogen-sulphur fertilization, we observed decrease of vitamin C content compared to the variant where only nitrogen was applied.

Table 3. P-value for dependence of several fertilization variants

Compared variants	Yield		Acid ascorbic		Nitrates	
	2008	2009	2008	2009	2008	2009
1-2	0.000*	0.000*	0.002*	0.004*	0.000*	0.000*
1-3	0.000*	0.000*	0.011*	0.053	0.000*	0.000*
1-4	0.000*	0.000*	0.005*	0.006*	0.000*	0.000*
2-3	0.440	0.049*	0.059	0.154	0.006*	0.009*
2-4	0.081	0.026*	0.072	0.373	0.004*	0.002*
3-4	0.036*	0.460	0.709	0.400	0.458	0.043*

*P-value is statistically significant (P<0.05)

Opinions regarding the influence of nitrogen fertilization on the acid ascorbic content are very different. Xu, et al. (2010) found that applied nitrogen fertilization tended to decrease the acid ascorbic content in broccoli florets. On the other hand, our results confirmed the fact that we can reach the higher content of acid ascorbic in grown vegetable by optimization of nutrient doses. This fact was presented by Ducsay and Varga (2001) in the trial with Chinese cabbage. Vallejo, et al. (2003) observed the positive effect of sulphur fertilization on the accumulation of vitamin C content in selected broccoli cultivars. On the basis of the gained results, we can also state increased accumulation of ascorbic acid at the variants where sulphur was applied.

Table 4. Correlation coefficient (*R*) between yield and qualitative parameters of broccoli

Variant	Correlation coefficient					
	A x B		A x C		B x C	
	2008	2009	2008	2009	2008	2009
1	0.997***	0.144*	0.784***	0.777***	0.732***	0.293*
2	0.374**	0.257*	0.438**	0.315*	0.573**	0.622**
3	0.456**	0.257*	0.686***	0.362**	0.143*	0.339**
4	0.892***	0.054*	0.788***	0.705***	0.942***	0.125*
Total	0.747***	0.670***	0.894***	0.934***	0.520**	0.601**

A–yield; B–acid ascorbic; C-nitrates
*weak dependence–R (0;0.33); **middle dependence–R (0.33;0.66).
***strong dependence–R (0.66;1.0)

Broccoli belongs to vegetables species which tend to accumulate nitrates in its edible parts. We observed statistically significant differences between unfertilized control and variants with nitrogen fertilization in both experimental years. There were also statistically significant differences detected between nitrogen and nitrogen-sulphur variants (Table 3).

The average nitrate content ranged from 703.90 to 922.1 mg.kg^{-1} of fresh matter (Table 2). The highest nitrate content was determined at the variant 4 where only nitrogen was applied. At this variant, the increase of nitrate content about 31.0% was detected in comparison with the control variant. We also observed the cumulative effect of nitrates in broccoli florets at variants 2 and 3 where the nitrates content was increased compared to the control variant about 18.4 and 27.5%. Thus, the obtained experimental results confirm the fact that nitrate fertilization has statistically significant cumulative effect on nitrate contents in edible part of the vegetable (Table 3). This fact was showed in previous research by Babik and Elkner (2002), Rop (2000), Wang and Li (2004) and Ahmadil, et al. (2010), who studied the effect of nitrogen fertilization on the nitrates content in broccoli or other vegetable species (spinach, cabbage, Peking cabbage et al.).

CONCLUSION

In our research, we studied the effect of four different variants of nitrogen fertilization on the quantity and quality of broccoli yield. On the basis of the obtained results, we can conclude that the statistically significant increase of broccoli yield was reached at all variants with applied fertilization compared to the control variant. The highest broccoli yield was reached at the combined nitrogen-sulphur variant (N:S=200:60 kg.ha^{-1}). In the term of broccoli quality**,** we observed the positive effect of applied fertilization on the amount of ascorbic acid in broccoli florets. Its highest content was determined at the variant where nitrogen only was applied (fertilized at the level N=200 kg.ha^{-1}). The applied nitrogen fertilization tended towards increased accumulation of nitrates in broccoli. The greatest increase of nitrates content was determined at the combined nitrogen-sulphur variant (N:S=200:60 kg.ha^{-1}).

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UTICAJ ĐUBRENJA AZOTOM I SUMPOROM NA FORMIRANJE PRINOSA I KVALITET BROKOLE

MIROSLAV ŠLOSÁR, ANTON UHER, JÁN KÓŇA, TOMÁŠ PECHOČIAK

Izvod

U ogledu sa brokolom ispitivan je uticaj četiri različite varijante đubrenja azotom i sumporom na formiranje prinosa i kvalitet brokole (sadržaj vitamina C i nakupljanje nitrata u cvasti brokole). Uticaj đubrenja na odabrane komponente prinosa brokole, je bio sličan u obe godine ispitivanja. Kod svih ispitivanih varijanti đubrenja uočeno je statistički značajno povećanje prinosa u odnosu na kontrolu. Najveći prinos brokole je postignut na varijanti 4 (đubrenje u dozi N : S = 200 : 60), gde je u poređenju sa kontrolom uočeno povećanje prinosa od 26,8%. Primenjena đubriva su imala uticaj na akumulaciju vitamina C u cvasti brokole. Najveći sadržaj vitamina C je ostvaren na varijanti 2 (200 kg N/ha). Na ovoj varijanti uočeno je povećanje sadržaja vitamina C u odnosu na kontrolu za 12,9 %. Nasuprot tome, primenjena đubriva su dovela do povećanja sadržaja nitrata u konzumnim cvastima brokole. Najveći porast sadržaja nitrata u poređenju sa kontrolnom varijantom je ostvaren na varijanti 4 (za 31%).

Ključne reči: brokola, đubrenje, prinos, vitamin C, nitrati.

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PLANT COVER OF NATURAL PASTURES LOCATED IN THE VICINITY OF THE TOWN OF NOVI KNEŽEVAC*

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SUMMARY: Specific features of the plant cover of the natural pastures located in the vicinity of the town of Novi Kneževac (the Vojvodina Province, Serbia) result from their floristic, ecological, plant geographic, florogenetic and phytocoenological characteristics. Floristic characteristics result from 205 taxa. Of the 205 taxa, 191 taxa (177 species, six subspecies, three varieties and five forms) were listed on account of their distinctive floristic characteristics. Ecological characteristics result from 55 halophyte taxa with the ecological index $\boldsymbol{S}_{+}$*. Plant geographic characteristics result from one endemic species of the Pannonian Plain Statice gmelini subsp. hungaricum and subendemic species in the Pannonian Plain Achillea asplenifolia, Puccinellia limosa and Roripa kerneri. Florogenetic characteristics result from postglacial relic Scilla autumnalis. The registered taxa form stands of 12 plant communities that belong to the vegetation classes Phragmitetea, Thero-Salicornietea, Festuco-Puccinellietea and Festuco-Brometea. Taking in consideration the considerable presence of halophyte taxa, the presence of Pannonian and sub-Pannonian floristic elements, and domination of phytocoenoses of the class Festuco-Puccinellietea it was concluded that the studied plant cover is part of the halobiome of the Pannonian Plain.*

Key words*: Novi Kneževac (the Vojvodina Province – Serbia), natural pasture, flora, vegetation.*

INTRODUCTION

Novi Kneževac is a town on the Tisza River, located in northern Banat (the Vojvodina Province, Serbia). A climate diagram after Walter made on the basis of the

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Original scientific paper / *Originalni naučni rad*

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data from the meteorological station in Kikinda shows that the studied location has the average annual rainfall of 555 mm and the average annual temperature of 11.1^{0}C (Ljevnaić-Mašić, 2010). The beginning of the vegetation period (April) is characterized by a substantial increase in precipitation and a high but gradual increase in temperature. After reaching maximum levels in June, the rainfall drops considerably. In contrast to precipitation, the temperature, that had gradually increased at the beginning of the vegetation period, remains steady and high in the middle of the vegetation period, and, only after a considerable dry spell, starts to go down perceptibly in October. Because of the above precipitation and temperature patterns, the studied area passes through a semi-arid period from mid-July to late September, which negatively affects the present vegetation cover.

East of the town of Novi Kneževac, there are large areas of the solonetz and the solonchakic solonetz soils (Benka and Salvai, 2005), which are not cultivated due to low contents of organic matter. These areas, which are overgrown with their natural vegetation, are used by local farmers mostly for cattle grazing, less frequently for mowing.

The objective of this study was to establish the status of the extant flora and vegetation cover of the natural pastures located in the vicinity of the town of Novi Kneževac.

MATERIAL AND METHODS OF THE STUDY

The data on the natural plant cover of the pastures located in the vicinity of Novi Kneževac combine a part of the results of a previous study of plant cover of saline soils of Banat (Knežević et al., 2008) and the results of a subsequent study conducted in the location mentioned above.

The observed plants were determined and their names identified in accordance with the nomenclature in the publications 'Flora of SR Serbia' (Josifović, M. ed. IX, 1970-1976), Flora Europaea (Tutin et al., 1960-1980).

Of the 205 taxa found in the flora of the natural pastures surrounding the town of Novi Kneževac, 191 were listed as distinct species.

The latter taxa comprised of 177 species, six subspecies, three varieties and five forms. The varieties *Aster tripolium* L. var. *pannonicus* (Jacq.) Beck, *Chenopodium rubrum* L. subsp. *botryoides* Sm. var. *crassifolium* (Hornem) Kov. i *Sonchus arvensis* L. var. *uliginosus* (M.B.) Grec. and forms *Aster sedifolius* L. f. *subsquamosus* Soó, *Bromus commutatus* Schrad. f. *violaceus* Podp., *Mentha aquatica* L. f. *erromera* Top., *Poa bulbosa* L. f. *vivipara* Koel. i *Scleranthus annus* L. f. *minimus* Schur. were put on the list because their higher taxonomic categories were not found in the studied flora.

The listed taxa were divided according to floristic elements on the basis of publications of Soó (1964-1985).

Values of the salinity index (S) of the observed taxa were estimated according to the criterion of Landolt (Landolt, 1977; Knežević, 1994).

The syntaxonomic position of the observed plant communities at the natural pastures located in the vicinity of Novi Kneževac was defined according to the publication 'Halophytic Vegetation of the Yugoslav Part of the Banat Region' (Knežević et al. 1998).

RESULTS AND DISCUSSION

Flora of saline habitats in the vicinity of Novi Kneževac:

1. *Achillea asplenifolia* Venet. /**S$_+$**/
2. *A. millefolium* L. /**S$_-$**/
3. *A. millefolium* L. subsp. *pannonica* (Scheele) Hayek /**S$_-$**/
4. *A. setacea* W. et K. /**S$_-$**/
5. *Acorellus pannonicus* (Jacq.) Palla /**S$_+$**/
6. *Agropyrum repens* (L.) Beauv. /**S$_+$**/
7. *Agrostis alba* L. /**S$_-$**/
   A. alba L. f. *coarctata* Rchb.
8. *Alisma plantago-aquatica* L. /**S$_-$**/
9. *Allium scorodoprasum* L. /**S$_-$**/
10. *Alopecurus geniculatus* L. /**S$_+$**/
11. *A. pratensis* L. /**S$_-$**/
12. *Andropogon ischaemum* L. /**S$_-$**/
13. *Artemisia austriaca* Jacq. /**S$_-$**/
14. *A. maritima* L. subsp. *monogyna* (W. et K.) Gams. /**S$_+$**/
15. *A. maritima* L. subsp. *salina* (Willd.) Gams. /**S$_+$**/
16. *Aster sedifolius* L. f. *subsquamosus* Soó /**S$_+$**/
17. *A. tripolium* L. var. *pannonicus* (Jacq.) Beck /**S$_+$**/
18. *Astragalus cicer* L. /**S$_-$**/
19. *Atriplex hastata* L. /**S$_+$**/
20. *A. litoralis* L. /**S$_+$**/
21. *A. tatarica* L. /**S$_+$**/
    A. tatarica L. var. *diffusa* (Ten. Gürke
22. *Ballota nigra* L. /**S$_-$**/
23. *Berteroa incana* (L.) DC. /**S$_-$**/
24. *Bidens tripartitus* L. /**S$_-$**/
25. *Bolboschoenus maritimus* (L.) Palla /**S$_+$**/
    B. maritimus (L.) Palla var. *compactus* (Hoffm.) Jáv.
26. *Bromus commutatus* Schrad. f. *violaceus* Podp. /**S$_-$**/
27. *B. erectus* Huds. /**S$_-$**/
28. *B. mollis* L. /**S$_-$**/
29. *Bupleurum pachnospermum* Pančić /**S$_-$**/
30. *B. tenuissimum* L. /**S$_+$**/
31. *Calystegia sepium* (L.) R. Br. /**S$_-$**/
32. *Camphorosma annua* Pall. /**S$_+$**/
33. *Capsella bursa pastoris* (L.) Medik. /**S$_-$**/
34. *Carduus acanthoides* L. /**S$_-$**/
35. *Carex hirta* L. /**S$_-$**/
36. *C. praecox* Schreb. /**S$_-$**/
37. *C. pseudocyperus* L. /**S$_-$**/
38. *C. spicata* Huds. /**S$_-$**/
39. *C.. stenophylla* Wahlbg. /**S$_-$**/
40. *C. vulpina* L. /**S$_-$**/
41. *Carthamus lanatus* L. /**S$_-$**/
42. *Centaurea cyanus* L. /**S$_-$**/
43. *Cerastium caespitosum* Gilib. /**S$_-$**/
44. *C. semidecandrum* L. /**S$_-$**/
45. *Chenopodium album* L. /**S$_-$**/
46. *Ch. glaucum* L. /**S$_+$**/
47. *Chenopodium polyspermum* L. /**S$_-$**/
48. *Ch. rubrum* L. subsp. *botryoides* Sm. var. *crassifolium* (Hornem) Kov. /**S$_+$**/
49. *Chrysopogon gryllus* (L.) Trin. /**S$_-$**/
50. *Cichorium intybus* L. /**S$_-$**/
51. *Cirsium arvense* (L.) Scop. /**S$_-$**/
52. *C. lanceolatum* (L.) Scop. /**S$_-$**/
53. *Convolvulus arvensis* L. /**S$_-$**/
54. *Crepis capillaris* (L.) Wallr. /**S$_-$**
55. *C. setosa* Hall. /**S$_-$**/
56. *Crypsis aculeata* (L.) Aitt. /**S$_+$**
57. *Cynodon dactylon* (L.) Pers. /**S$_-$**/
58. *Epilobium adnatum* Griseb. /**S$_-$**/
59. *Erodium cicutarium* (L.) L. Hérit./**S$_-$**/
60. *Erophila verna* (L.) Schevall. /**S$_-$**/
61. *Eryngium campestre* L. /**S$_-$**/
62. *Euclidium syriaca* (L.) R. Br. /**S$_-$**/
63. *Euphorbia cyparisias* L. /**S$_-$**/
64. *E. platyphyllos* L. /**S$_-$**/
65. *Festuca vallesiaca* Sch. /**S$_-$**/
66. *F. vallesiaca* Sch. subsp. *pseudovina* (Hack.) A. et G. /**S$_+$**/
    F. vallesiaca Sch. subsp. *pseudovina* (Hack.) A. et G. f. *rutila* Hack.
67. *Filago germanica* L. /**S$_-$**/
68. *Filipendula hexapetala* Gilib. /**S$_-$**/
69. *Fragaria viridis* Duchense /**S$_-$**/
70. *Gagea pratensis* (Pers.) Dumort. /**S$_-$**/
71. *Galium aparine* L. /**S$_-$**/
72. *G. pedemontanum* All. /**S$_-$**/
73. *G. verum* L. /**S$_-$**/
74. *Geranium columbinum* L. /**S$_-$**/
75. *Glyceria maxima* (Hartm.) Holombg. /**S$_-$**/
76. *Glycyrrhiza echinata* L. /**S$_-$**/
77. *Gratiola officinalis* L. /**S$_+$**/
78. *Gypsophila muralis* L. /**S$_-$**/
79. *Heleocharis palustris*(L.)R.Br./**S$_-$**/
80. *Heleochloa alopecuroides* (Pill. et Mitterp.) Host /**S$_-$**/

81. *H. schoenoides* (L.) Host /**S**$_{+}$/
82. *Helminthia echioides* (L.) Gärtn. /**S** $_{-}$/
83. *Hordeum maritimum* Stokes subsp. *gussoneanum* (Parl.) A. et G. /**S**$_{+}$/
84. *Inula britannica* L. /**S**$_{+}$/
85. *Juncus articulatus* L. /**S** $_{-}$/
86. *J. atratus* Krock. /**S** $_{-}$/
87. *J. compressus* Jacq. /**S**$_{+}$/
88. *J. gerardi* Lois. /**S**$_{+}$/
89. *Knautia arvensis* (L.) Coult. /**S** $_{-}$/ *K. arvensis* (L.) Coult. f. *pinnatifida* (W. et Gr.) Peterm.
90. *Kochia laniflora* (Gmel.)Borb./**S** $_{-}$/
91. *K. prostrata* (L.) Schrad. /**S**$_{+}$/ *K. prostrata* (L.) Schrad. l. *rubens* Lag.
92. *Koeleria gracilis* Pers. /**S** $_{-}$/
93. *Lactuca saligna* L. /**S**$_{+}$/
94. *L. serriola* L. /**S** $_{-}$/
95. *Lathyrus hirsutus* L. /**S** $_{-}$/
96. *Lemna minor* L. /**S** $_{-}$/
97. *Lepidium draba* L. /**S** $_{-}$/
98. *L. perfoliatum* L. /**S** $_{-}$/
99. *L. ruderale* L. /**S** $_{-}$/
100. *Lolium perenne* L. /**S** $_{-}$/
101. *Lotus tenuis* Kit. /**S** $_{-}$/
102. *Lycopus europaeus* L. /**S** $_{-}$/ *L. europaeus* L. f. *turfosus* Beck
103. *L. exaltatus* L. /**S** $_{-}$/
104. *Lysimachia nummularia* L. /**S** $_{-}$/
105. *Lythrum hyssopifolia* L. /**S**$_{+}$/
106. *L. virgatun* L. /**S** $_{-}$/
107. *Marrubium peregrinum* L. /**S** $_{-}$/
108. *Matricaria chamomilla* L. /**S**$_{+}$/
109. *M. inodora* L. /**S**$_{+}$/
110. *M. suaveolens* (Pursh) Buch. /**S** $_{-}$/
111. *Medicago falcata* L. /**S** $_{-}$/
112. *M. lupulina* L. /**S** $_{-}$/ *M. lupulina* L. f. *canescens* (Menyh.) Soó
113. *Melilotus officinalis* (L.)Pallas /**S** $_{-}$/
114. *Mentha aquatica* L. f. *erromera* Top. /**S** $_{-}$/
115. *M. pulegium* L. /**S**$_{+}$/
116. *Muscari racemosum* (L.) Mill. /**S** $_{-}$/
117. *Myosotis collina* Hoffm. /**S** $_{-}$/
118. *Myosurus minimus* L. /**S** $_{-}$/
119. *Oenanthe silaifolia* M.B. /**S**$_{+}$/
120. *Onopordon acanthium* L. /**S** $_{-}$/,
121. *Ornithogalum gussonei* Ten. /**S** $_{-}$/,
122. *O. umbellatum* L. /**S** $_{-}$/,
123. *Panicum crus-galli* L. /**S** $_{-}$/
124. *Pastinaca sativa* L. /**S** $_{-}$/
125. *Pholiurus pannonicus* (Host) Trin. /**S**$_{+}$/
126. *Phragmites communis* Trin. /**S**$_{+}$/
127. *Picris hieracioides* L. /**S** $_{-}$/
128. *Pimpinella saxifraga* L. /**S** $_{-}$/
129. *Plantago lanceolata* L. (**S** $_{-}$) *P. lanceolata* L. var. *lanceolata*
130. *P. maior* L. /**S**$_{+}$/
131. *P. maritima* L. /**S**$_{+}$/ *P. tenuiflora* W. et K. /**S**$_{+}$/ *P. tenuiflora* W. et K. f. *depauperata* Domin
132. *Poa annua* L. /**S** $_{-}$/
133. *P. bulbosa* L. f. *vivipara* Koel./**S** $_{-}$/
134. *P. pratensis* L. /**S** $_{-}$/
135. *Podospermum canum* C.A.Mey. /**S**$_{+}$/
136. *Polygonum aviculare* L. /**S** $_{-}$/
137. *P. lapathifolium* L. /**S** $_{-}$/ *P. lapathifolium* L. var. *tomentosum* (Schrk.) Bay.
138. *Portulaca oleracea* L. /**S** $_{-}$/
139. *Potentilla argentea* L. /**S** $_{-}$/
140. *P. supina* L. /**S** $_{-}$/
141. *Prunus spinosa* L. /**S** $_{-}$/
142. *Puccinellia limosa* (Schur) Holmb. /**S**$_{+}$/
143. *Pulicaria vulgaris* Gärtn. /**S**$_{+}$/
144. *Ranunculus aquatilis* L. /**S** $_{-}$/
145. *R. paucistamineus* Tsch. /**S** $_{-}$/
146. *R. repens* L. /**S** $_{-}$/
147. *Roripa austriaca* (Cr.) Bess. /**S** $_{-}$/
148. *R. kerneri* Menyh. /**S**$_{+}$/
149. *Rosa canina* L. /**S** $_{-}$/
150. *Rumex obtusifolius* L. /**S** $_{-}$/
151. *R. palustris* Sm. /**S** $_{-}$/
152. *R. patientia* L. /**S** $_{-}$/
153. *R. stenophyllus* Ledeb. /**S**$_{+}$/
154. *Salvia nemorosa* L. /**S** $_{-}$/
155. *Schoenoplectus lacuster* (L.) Palla /**S** $_{-}$/ *S. lacuster* (L.) Palla f. *conglomeratus* (Junge) Soó
156. *Scilla autumnalis* L. /**S** $_{-}$/
157. *Scleranthus annus* L. f. *minimus* Schur. /**S** $_{-}$/
158. *Sclerochloa dura* (L.) Beauv. /**S** $_{-}$/
159. *Sedum caespitosum* (Cav.) D.C. /**S**$_{+}$/
160. *Sinapis arvensis* L. /**S** $_{-}$/
161. *Sonchus arvensis* L. var. *uliginosus* (M.B.) Grec. /**S**$_{+}$/
162. *S. asper* (L.) Hill. /**S** $_{-}$/
163. *Spergularia salina* J. et C. Presl. /**S**$_{+}$/
164. *Stachys germanica* L. /**S** $_{-}$/
165. *Statice gmelini* Willd. subsp. *hungaricum* (Klokov) Soó (**S**$_{+}$)

166. *Taraxacum officinale* Weber /**S**$_+$/
167. *Teucrium scordium* L. /**S**$_+$/,
168. *Thymus glabrescens* Willd. /**S**$_-$/
169. *Th. marschallianus* Willd. /**S**$_-$/
170. *Torilis arvensis* (Huds.) Link. /**S**$_-$/
171. *Trifolium angulatum* W.et K. /**S**$_+$/
172. *T. arvense* L. /**S**$_-$/
173. *T. campestre* Schreb. /**S**$_-$/
T. campestre Schreb. var. *minus* (Koch) Gremli
174. *T. filiforme* L. /**S**$_-$/
175. *T. fragiferum* L. /**S**$_+$/
176. *T. hybridum* L. /**S**$_-$/
177. *T. ornithopodioides* (L.) Sm. /**S**$_+$/
178. *T. parviflorum* Ehrh. /**S**$_+$/
179. *T. pratense* L. /**S**$_-$/
180. *T. repens* L. /**S**$_+$/
T. repens L. f. *microphyllum* Larg.-Fossat
181. *T. striatum* L. /**S**$_+$/
182. *T. strictun* (L.) Jusl. /**S**$_+$/
183. *Typha angustifolia* L. /**S**$_+$/
184. *Ventenata dubia* (Leers.) F.Schultz /**S**$_-$/
185. *Verbena officinalis* L. /**S**$_-$/
186. *Veronica anagalloides* Guss. /**S**$_-$/
187. *V. arvensis* L. /**S**$_-$/
188. *Vicia angustifolia* L. /**S**$_-$/
189. *Xanthium italicum* Moretti /**S**$_-$/
190. *X. spinosum* L. /**S**$_-$/

Of the 205 registered taxa (177 species, six subspecies, eight varieties, 13 forms and one lusus), 191 were listed as separate species. The latter croups comprised 177 species, six subspecies, two varieties and five forms. The varieties *Aster tripolium* L. var. *pannonicus* (Jacq.) Beck, *Chenopodium rubrum* L. subsp. *botryoides* Sm. var. *crassifolium* (Hornem) Kov. i *Sonchus arvensis* L. var. *uliginosus* (M.B.) Grec. and forms *Aster sedifolius* L. f. *subsquamosus* Soó, *Bromus commutatus* Schrad. f. *violaceus* Podp., *Mentha aquatica* L. f. *erromera* Top., *Poa bulbosa* L. f. *vivipara* Koel. i *Scleranthus annus* L. f. *minimus* Schur. were put on the list because their higher taxonomic categories were not found in the studied flora.

The 14 unlisted taxa had a lower taxonomic rank than subspecies, and we registered their higher taxonomic categories in the studied flora.

This group included five varieties (*Atriplex tatarica* L. var. *diffusa* (Ten.) Gürke, *Bolboschoenus maritimus* (L.) Palla var. *compactus* /Hoffm./ Jáv., *Plantago lanceolata* L. var. *lanceolata*, *Polygonum lapathifolium* L. var. *tomentosum* (Schrk.) Bay. i *Trifolium campestre* Schreb. var. *minus* (Koch) Gremli), eight forms (*Agrostis alba* L. f. *coarctata* Rchb., *Festuca vallesiaca* Sch. subsp. *pseudovina* (Hack.) A. et G. f. *rutila* Hack., *Knautia arvensis* (L.) Coult. f. *pinnatifida* (W. et Gr.) Peterm., *Lycopus europaeus* L. f. *turfosus* Beck, *Medicago lupulina* L. f. *canescens* (Menvh.) Soó, *Plantago tenuiflora* W. et K. f. *depauperata* Domin, *Schoenoplectus lacuster* (L.) Palla f. *conglomeratus* (Junge) Soó i *Trifolium repens* L. f. *microphyllum* Larg.-Fossat) and one lusus (*Kochia prostrata* (L.) Schrad. l. *rubens* Lag.).

The group of 191 taxa, listed as present in the flora of natural pastures located in the vicinity of Novi Kneževac, comprised 55 or 28.80% taxa which were labeled with the ecological index S$_+$ and 136 or 71.20% taxa with the ecological index S$_-$.

Thus, in the flora of the investigated natural pastures is less halophytes than in the flora of pastures located in vicinity of the town Melenci-Rusanda (Knežević et al. 2003), Melenci-Ostrovo (Knežević et al. 2005b), Novi Bečej-Slano Kopovo (Knežević et al. 2005a) and Kumane (Knežević et al. 2009a), and more than in the flora of pastures located in vicinity of the town Elemir-Okanj (Knežević et al. 2009b).

In the florogenetic sense, a characteristic feature of the studied pastures was the presence of the sub-Mediterranean species *Scilla autumnalis*, which is a postglacial relic.

In the plant geographic sense, the characteristic feature of the community was the presence of *Statice gmelini* subsp. *Hungaricum*, an endeme of the Pannonian Plain, and *Achillea asplenifolia*, *Puccinellia limosa* and *Roripa kerneri*, subendemes of the Pannonian Plain.

Therefore, based on the presence of the taxa labeled with the ecological index S_+ (55 or 28.80% of the listed taxa) and the presence of Pannonian endemic (*Statice gmelini* subsp. *hungaricum Statice gmelini* subsp. *hungaricum*) and Pannonian subendemic taxa (*Achillea asplenifolia*, *Puccinellia limosa* and *Roripa kerneri*), it was concluded that, in the ecological sense, the natural pastures on the solonetz and solonchakic solonetz soils located in the surroundings of the town of Novi Kneževac are part of the halobiome of the Pannonian Plain.

The above statement is further supported by the fact that the taxa found in the natural pastures on the solonetz and solonchakic solonetz soils located in the vicinity of the town of Novi Kneževac formed a complex mosaic comprised of stands of 12 plant communities, most of which are typical for saline habitats.

Sintaxonomic position of the identified plant communities:

Phragmitetea Tx.et Prsg. 1942
Phragmitetalia W. Koch 1926 emend. Pign. 1953
Phragmition communis W. Koch 1926 emend. Soó 1947
Ass. *Scirpo-Phragmitetum* W. Koch 1926

Bolboschenetalia maritimi Hejný 1967
Bolboschoenion maritimi continentale Soó (1945)1947 emend. Borhidi 1970
Ass. Bolboschoenetum maritimi continentale Soó (1927)1957

Thero-Salicornietea Tx. 1955, Tx. et Oberd. 1958
Thero-Salicornietalia (Br.–Bl. 1931) Tx. 1954 ex Tx. et Oberd. 1958
Thero-Salicornion Br.–Bl. 1931 em Tx.1950
Ass. *Acorelletum pannonici* Soó (1939)1947

Festuco-Puccinellietea Soó 1968
Festuco-Puccinellietalia Soó 1968
Puccinellion limosae (Klika 1937) Wendel. 1943
Ass. *Puccinelletum limosae* (Rapcs. 1927) Soó 1930
Ass. *Pholiuro-Plantaginetum tenuiflorae* (Rapcs. 1927) Wendel. 1943
Ass. *Hordeetum histricis* (Soó 1933) Wendel. 1943
Ass. *Camphorosmetum annuae* (Rapcs. 1916) Soó 1933 corr. Soó 1938

Halo-Agrostion albae pannonicum Knežević 1990
Ass. *Agrostio-Alopecuretum pratensis* Soó (1933) 1947
Ass. *Agrostio-Eleochariti-Alopecuretum geniculati* (Magyar 1928) Soó (1939) 1947

Artemisio-Festucetalia pseudovinae Soó 1968
Festucion pseudovinae Soó 1933.
Halo-Festucenion pseudovinae Vučković 1985
Ass. *Artemisio-Festucetum pseudovinae* (Magyar 1928) Soó 1945
Xero-Festucenion pseudovinae Vučković 1985
Ass. *Achilleo-Festucetum pseudovinae* (Magyar 1928) Soó 1945.

Festuco-Brometea Br.–Bl. et Tx.1943
Festucetalia valesiacae Br.–Bl. et Tx.1943
Festucion rupicolae (*sulcatae*) Soó (1940) 1964
(*Cynodonto-Festucion* /*rupicolae-pseudovinae*/ Soó 1971)
Ass. *Festuco –Andropogonetum ischaemi* Vučk. 1985

Stands of the associations *Scirpo-Phragmitetum* and *Bolboschoenetum maritimi continentale* were found only in fragments and on small areas.

Stands of the associations *Acorelletum pannonici*, *Puccinelletum limosae, Pholiuro-Plantaginetum tenuiflorae*, *Hordeetum histricis*, *Camphorosmetum annuae*, *Agrostio-Alopecuretum pratensis* and *Agrostio-Eleochariti-Alopecuretum geniculati* were floristically clearly differentiated, but they covered modest areas.

Stands of the associations *Artemisio-Festucetum pseudovinae*, *Achilleo-Festucetum pseudovinae* and *Festuco–Andropogonetum ischaemi* were typically developed and they made the dominant plant cover of the studied pastures.

CONCLUSION

Specific features of the plant cover of the natural pastures on the solonetz and solonchakic solonetz soils in the vicinity of the town of Novi Kneževac are created by its floristic, plant geographic, florogenetic and phytocoenological characteristics.

In the floristic sense, the characteristic feature of the studied location was the presence of 205 plant taxa (177 species, six subspecies, eight varieties, 13 forms and one lusus). It is important to mention that out of the 191 taxa found to comprise the flora of the studied natural pastures and listed as such (177 species, six subspecies, three varieties and five forms), 55 or 28.80% of them were labeled with the ecological index S_{+}, and 136 or 71.20% with the ecological index S_{-}.

In the plant geographic sense, the characteristic feature of the community was the presence of *Statice gmelini* subsp. *Hungaricum*, an endeme of the Pannonian Plain, and *Puccinellia limosa* and *Roripa kerneri*, subendemes of the Pannonian Plain.

Because of these characteristics, it was concluded that the studied natural pastures located in the vicinity of the town of Novi Kneževac are, in the ecological sense, part of the halobiome of the Pannonian Plain.

In the florogenetic sense, a characteristic feature of the studied pastures was the presence of *Scilla autumnalis*, a sub-Mediterranean species which is a postglacial relic.

In the phytocoenological sense, a characteristic feature of the studied pastures was the presence of stands of 12 plant communities that belong to the vegetation classes *Phragmitetea* Tx.et Prsg. 1942 (Ass. *Scirpo-Phragmitetum* and Ass. *Bolboschoenetum maritimi continentale*), *Thero-Salicornietea* Tx. 1955, Tx. et Oberd. 1958 (Ass. *Acorelletum pannonici*), *Festuco-Puccinellietea* Soó 1968 (*Puccinelletum limosae, Pholiuro-Plantaginetum tenuiflorae*, *Hordeetum histricis*, *Camphorosmetum annuae*, *Agrostio-Alopecuretum pratensis* and *Agrostio-Eleochariti-Alopecuretum geniculati*, *Artemisio-Festucetum pseudovinae* and *Achilleo-Festucetum pseudovinae*) and *Festuco-Brometea* Br.–Bl. et Tx.1943 (*Festuco–Andropogonetum ischaemi*).

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BILJNI POKRIVAČ PRIRODNIH PAŠNJAKA U OKOLINI NASELJA NOVI KNEŽEVAC

ALEKSA KNEŽEVIĆ, DEJANA DŽIGURSKI, BRANKA LJEVNAIĆ - MAŠIĆ, BRANKO ĆUPINA, DRAGIŠA MILOŠEV

Izvod

Specifičnost biljnog pokrivača prirodnih pašnjaka u okolini naselja Novi Kneževac (Vojvodina – Srbija) su florističke, ekološke, biljnogeografske, florogenetske i fitocenološke karakteristike. Florističke karakteristike čini 205 taksona od kojih je, zbog florističke osobenosti, numeracijom posebno izdvojen 191 takson (177 vrsta, 6 podvrsta, 3 varijeteta i 5 formi). Ekološka karakteristike čini 55 halofitskih taksona okarakterisanih indeksom $\mathbf{S}_{+}$. Biljnogeografske karakteristike čine endem panonske nizije *Statice gmelini* subsp. *hungaricum* i subendemi panonske nizije *Achillea asplenifolia*, *Puccinellia limosa* i *Roripa kerneri*. Florogenetsku karakteristiku čini postglacijalni relikt *Scilla autumnalis*. Konstatovani taksoni formiraju sastojine 12 fitocenoza koje pripadaju klasama *Phragmitetea*, *Thero-Salicornietea*, *Festuco-Puccinellietea* i *Festuco-Brometea*. Na osnovu brojnosti halofitskih taksona, prisustva panonskih i subpanonskih elemenata flore i dominacije fitocenoza klase *Festuco-Puccinellietea* konstatujemo da je istraživani biljni pokrivača deo halobioma Panonske nizije.

Ključne reči: Novi Kneževac (Vojvodina – Srbija), prirodni pašnjak, flora, vegetacija.

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SOME BIOCHEMICAL BLOOD PARAMETERS OF GATACK BREED COWS FROM GACKO REGION

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SUMMARY: In this paper are presented values of biochemical parameters of blood of 11 Gatačko breed cows in different reproductive or production stages and the results of food analysis. Hyperproteinemy and hyperglobulinemy were found in all, and hyperalbuminemy was found in 8 tested animals (72.72%). The concentration of urea below the lower physiological limit was found in 8 animals (72.72%) (mean value 6,06±0,54 mmol/L). The cholesterol concentration in 9 animals (81.81%) was above the upper physiological limit (mean value 4,16±0,42 mmol/L). Hyperbilirubinemy was found in all tested animals (mean value 25,55±2,62 mmol/L). Calcium blood level was below the lower physiological limit in 4 animals (36.36%). Phosphorous blood level was below the lower physiological limit in one animal (9.09%), and above the upper physiological limit in remaining 10 (91.91%). ALT activity was elevated in 10 animals (91.91%) (mean value 71,27±4,64 U/L). The average activity of AST was within physiological values, and the increase was found in 5 animals (45.45%). Analysis of the meal showed that meal was insufficient for animals in crude protein and energy content.

Key words: *Gatack breed cattle, blood, biochemical parameters.*

INTRODUCTION

Analysis of biochemical blood parameters is used as valuable indicator of health and productive potential of the individual. Its task is to reach a conclusion about the health and metabolic status of cows, based on the values and attitudes of certain constituents of blood. Data on the values of blood biochemical parameters for high-yielding milk cows are available in the literature, while such data for the indigenous breeds (Busha, Podolsko and Gatačko cattle) are rare and unsufficient.

Most authors agree that for the evaluation of metabolic status of animals it is necessary to determine the parameters that indicate energy status (concentrations of glucose, urea, triglycerides and cholesterol), functional state of the liver (liver enzyme activity, concentrations of albumins, globulins, total protein and bilirubin) and mineral

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metabolism (calcium and phosphorus blood levels) (Brugere-Picoux and Brugere, 1987; Radojičić et al., 2002; Stojević et al., 2002; Ivanov et al., 2005).

Blood plasma and serum contain about 60-80 g/L of proteins, and albumins comprise 35-50% of that (Kaneko et al., 2008). Albumins are indicators of functional status of liver and nitrogen supply (Roil et al., 1974; Radojičić et al., 2002; Stojević et al., 2002; Ivanov et al., 2005). Hiperalbuminemy occurs most often in relation to dehydration, hemoconcentration and magnesium deficiency, and hipoalbuminemy with impairing of morphological and functional integrity of liver, kidney and gastrointestinal disorders, blood loss, malnutrition and parasitoses, firstly distomatosis (Brugere-Picoux and Brugere, 1987; Ivanov et al., 2005; Kaneko et al., 2008; Stojević et al., 2008). The increasing the globulin concentration comes with infections and tumors appearance, and reducing with the deficiency of cobalt, magnesium, copper and iron, malnutrition and parasitoses (Brugere-Picoux and Brugere, 1987; Ivanov et al., 2005). The concentration of total protein in blood serum can be reduced by anemia, malnutrition and morphological and functional disorders of liver, and increased by dehydration and chronic infections (Brugere-Picoux and Brugere, 1987; Ivanov et al., 2005, Kaneko et al., 2008).

The activity of liver enzymes in blood serum is an important diagnostic parameter of morphological and functional state of liver. According to many authors (Radojičić et al., 2002; Stojević et al., 2008; Kaneko et al., 2008), levels of aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) are commonly used as an indicator of morphological and functional state of liver. In dairy cows, increase of AST and GGT (gamma-glutamyl transpherasis) is usually associated with fatty liver, reduced appetite and the appearance of ketosis in early lactation (Cebra et al., 1997; Steen, 2001; Stojević et al., 2002; Stojević et al., 2008). The increased concentrations of cholesterol may also indicate hepatocyte damage. The concentration of bilirubin is a very important factor to evaluate detoxificative function of liver, and its increase comes as a result of massive hemolysis or liver damage (Stojević et al., 2002; Ivanov et al., 2005; Horvat et al., 2007; Stojević et al., 2008).

The concentration of urea in the blood is an important indicator of nitrogen and energy supply. Its decrease comes with protein deficiency, especially in the combined energy and protein deficiency, and its increase comes with the surplus of protein, with an absolute or relative lack of energy (Stamatović and Jovanović, 1990; Stojević et al. 2002; Horvat et al., 2007). The concentration of calcium and phosphorus in the blood is an indicator of mineral metabolism. Disturbances in their metabolis; Ivanov et al. 2005; Horvat et al., 2007).

This paper presents the values of some biochemical parameters of blood of Gatačko cattle cows at various stages of production and the reproductive cycle (early lactation, late lactation and dry period) as well as their association with health status and results of feed analysis.

MATERIALS AND METHODS

The study included a total of 11 Gatačko cattle cows from individual farming, from Gacko Municipality. Cows were examined during early lactation (15-60 days after calving, n = 7), about 150th day of lactation (n = 2) and about 15 days before dry period (n = 2). Data on the daily milk production were not collected regularly, but, according to data obtained from the owner, the average daily milk yield at the peak of lactation

ranged from 12-18 liters. Age of animals ranged from 2 to 11 years, and body weight between 400 and 500 kilograms. Blood and feed samples were taken during January and February 2010. when the animals stayed indoors (the period of winter feeding).

All cows were kept in usual environmental conditions for the region (indoors in winter and on pasture in summer). The barns are modest in terms of environmental conditions, buried in the ground, with no light and manure cleaning system. Bearings are the long type, with the use of bedding. In winter, cows are feed only with hay, usually four times a day (about 12 kg of hay per day). Animal get bran only a couple of days after calving, usually blurred in the warm water. Except allready mentioned, cows do not get other feed. Cows are given salt twice a week (hand of salt per cow). They usually drink water once a day, and the amount of water drunk ranges from 30-40 liters. In two barns there were automatic drinkers. Animals were clinically healthy, and, according to data obtained from the owner, had regular reproductive cycle.

Blood samples were taken from all tested animals, by puncture of v. coccigea, in sterile vacutainers without anticoagulant. The samples were left at room temperature for spontaneous coagulation. Blood serum was separated by centrifugation and frozen until analysis. Along with taking blood samples, hay samples were taken for chemical analysis.

Analysis of blood biochemical parameters was performed in The Veterinary Institute of Republic of Srpska "Dr Vaso Butozan" in Banja Luka, on a biochemical analyzer VetTest Chemistry Analyzer, IDEXX Laboratories, UK. In the blood samples we determined following biochemical parameters: concentrations of albumin, globulin, total protein, total bilirubin, urea and cholesterol, activity of AST and ALT, calcemy and phospahatemy. Feed analysis was carried out at The Agricultural Institute of Republic of Srpska, by using standard methodology. The hay samples were analyzed for the following parameters: dry matter content, ash, crude protein, fiber, calcium and phosphorus. On that basis, the energy and nutrient value of ration was calculated.

The results were statistically analyzed and presented in tables.

RESULTS AND DISCUSSION

The results of blood biochemical parameters of tested animals are shown bellow in Table 1.

Table 1. Values of biochemical blood parameters of observed animals (n=11)
Tabela 1. Vrijednosti biohemijskih parametara krvi ispitanih životinja (n= 11)

Parameter/*Parametar*	**M**	**SD**	**SE**	**CV**	**IV**
Total protein / *Ukupni proteini* (g/L)	94.36	9.86	2.97	10.45	79-110
Albumines / *Albumini* (g/L)	40.55	4.50	1.36	11.10	35-47
Globulines / *Globulini* (g/L)	53.91	7.87	2.37	14.59	43-69
ALT /*ALT* (U/L)	71.27	15.39	4.64	21.59	38-86
AST / *AST* (U/L)	129.45	23.39	7.05	18.07	88-182
Total bilirubin / *Ukupni bilirubin* (μmol/L)	25.55	8.70	2.62	34.05	13-37
Cholesterole / *Holesterol* (mmol/L)	4.16	1.40	0.42	33.63	2.74-7.96
Urea / *Urea* (mmol/L)	6.06	1.79	0.54	29.60	3.70-8.60
Calcium / *Kalcijum* (mmol/L)	2.50	0.22	0.06	8.68	2.20-2.96
Phosporus / *Fosfor* (mmol/L)	2.38	0.39	0.12	16.55	1.55-2.89
Calcium-phosporus ratio / *Odnos kalcijuma i fosfora*	1.08	0.20	0.06	18.64	0.79-1.58

According to our knowledge, research for determination of physiological values of blood biochemical parameters for this breed was not conducted so far, so we took data from the relevant literature as reference values *(Kaneko et al., 2008).* The average concentration of albumin was 40.55 ± 1.36 g / L, which is significantly higher compared to the reference values (30.3 to 35.5 g / L). A similar situation is with the concentrations of globulin (53.91 ± 2.37 g / L) and total protein (94.36 ± 2.37 g / L). Bearing in mind that hyperproteinemy was present in all tested animals, lack of drinking water (according to data obtained by Grubić and Adamović, 1998, daily needs of cattle weighted 450 kilograms amount to about 55 liters of water), and the fact that tested animals were fed only with dry forages (hay), elevated concentrations of proteins can be considered as a consequence of hemoconcentration due to unfavorable balance of water. The fact that there was no significant change of albumin to globulin ratio, as well as the relatively large loss of water through the milk confirms this claim. As a potential cause of hyperproteinemy we should not neglect the small liver fluke larvae, which had resulted in the development of chronic cholangitis and hepatitis, which prompted the synthesis of antibodies and increase the concentration of total protein.

ALT activity in one tested cow (9,09%) was within the reference interval, while in the others it was above the upper reference limit (average 71.27 ± 4.64 U / L). Similar to ALT, AST activity was also elevated, but the average value remained within the reference values (129.45 ± 7.05 U / L). At the same time in 5 animals (54.54%) had values above the upper physiological limit. Elevated liver enzyme activity indicates presence of hepatocyte damage, probably due to mechanical damage of liver by parasites. In addition to that, a high concentration of total bilirubin was found in all tested animals (average 25.55 ± 2.62 mmol / L), and it was almost three times higher than the upper reference limit (0.17 to 8.55 mmol / L). Having in mind relatively low milk yield of tested cows, it can be said that in these cows lipomobilisation is not developed, so that it is not the main reason for increased bilirubin concentrations. In interpreting the results we should not neglect the fact that the samples came from animals who live at an altitude of 1000 to 1300m, and therefore it can be expected that their red blood cells number and hemoglobin (and consequently bilirubin) concentration would be at the upper physiological limit. Concentrations of total cholesterol values were within the reference interval in only two animals (18.18%), while in others the value was above the upper physiological limit. The average concentration of cholesterol (4.16 ± 0.42 mmol / L) was above the upper physiological limit, which indicates the presence of damage of liver by small liver fluke larvae, so the damaged hepatocytes can not accept and use the blood cholesterol that comes from fat tissue.

The concentration of urea in the blood of three tested animals (27.27%) was within reference values (7.14 to 10.70 mmol / L), while in the others it was below lower range. The same situation is with the average value, which amounted to 6.06 ± 0.54 mmol / L. From this finding it can be concluded that the meal did not contain enough digestible crude protein and energy. Chemical analysis showed that the protein content in the ration was reduced by about 50%, and energy content by 42% compared to the norms (Adamović and Grubić, 1998), which prevents the binding of ammonia and its incorporation into microbial protein. High cellulose content (35.6%) indicates that hay was not stored at the optimal time, but considerably later, when hay stalk was indurated with celulose-lignine complex, which reduces digestibility and energy value of ration.

Calcium concentration value below the lower physiological limit was registered at 4 animals (45.45%). One animal (9.09%) had a phosporus concentration value below lower, while the others had values above the upper physiological limit. Average values of calcium and phosporus concentration were within and above the reference value, respectively, which indicates that the disorder of mineral metabolism is present. This claim is confirmed by the calcium-phosphorus ratio, that ranged from 0,79:1 to 1,58:1 (average 1.08 ± 0,06:1), which can be considered as unfavorable ratio (based on physiological values, the optimum ratio of calcium and phosphorus in the blood serum is approximately 1,25:1). The ratio of calcium and phosphorus in the ration was disturbed in favor of calcium (2,4:1), and in blood serum in favor of phosphorus which can be attributed to higher digestibility of phosphorus relative to calcium, as well as the significant excretion of calcium through milk in relation to phosphorus.

CONCLUSION

Previous researches for determining reference values of biochemical parameters of blood were mainly focused on the high-productive breeds, while the data on indigenous breeds are unavailable.

Based on the presented data it was found that tested animals had damaged liver, most probably as a result of parasitic invasion of the small fluke larvae, which was manifested by a reduced ability of bilirubin excretion and increased liver enzyme activity. The facts that most of the year animals spend on pasture, and that the owners do not conduct regular dehelmintisation was confirming the dicroceliosis as one of the main causes of liver damage. However, despite the liver damage, its functional capacity was significantly preserved, which was indicated by physiological or even slightly elevated concentrations of albumin. Mineral status of tested cows indicates disturbed ratio of calcium and phosphorus in blood and the ration, and low concentration of urea indicates the deficit of energy and protein in ration, all confirmed by the results of chemical analysis. Drinking water supply of tested animals was insufficient, which results in hemoconcentration and hyperproteinemy.

Bearing in mind the age of tested cows, length of their productive life and relatively high production of milk in rather unfavorable conditions, and generally good health, we come to the conclusion that the Gatačko breed cattle is a very interesting genetic resource, whose development and advancement in the future is needed.

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NEKI BIOHEMIJSKI PARAMETRI KRVI KRAVA GATAČKE RASE IZ REGIJE GACKO

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Izvod

U radu su date vrijednosti biohemijskih parametara krvi 11 krava gatačke rase u različitim proizvodno-reproduktivnim fazama i rezultati analize obroka. Hiperproteinemija i hiperglobulinemija ustanovljene su kod svih, a hiperalbuminemija kod 8 ispitanih životinja (72,72%). Koncentracija uree ispod donje fiziološke granice ustanovljena je kod 8 životinja (72,72%) (prosječno 6,06±0,54 mmol/L). Koncentracija holesterola kod 9 životinja (81,81%) bila je iznad gornje fiziološke granice (prosječno 4,16±0,42 mmol/L). Hiperbilirubinemija je ustanovljena kod svih ispitanih životinja (prosječno 25,55±2,62 mmol/L). Kod 4 životinje (36,36%) ustanovljena je vrijednost kalcemije ispod donje fiziološke granice. Kod jedne životinje (9,09%) fosfatemija je bila ispod donje, a kod ostalih 10 (91,91%) iznad gornje fiziološke granice. Povišena aktivnost ALT ustanovljena je kod 10 životinja (91,91%), (prosječno 71,27±4,64 U/L). Prosječna aktivnost AST bila je unutar fizioloških vrijednosti, a povišenje je ustanovljeno kod 5 životinja (45,45%). Analiza obroka pokazala je da obrok nije bio usklađen sa potrebama životinja u pogledu sadržaja sirovih proteina i energije.

Ključne riječi: gatačko goveče, krv, biohemijski parametri.

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IMPORTANCE AND CURRENT ISSUES IN AGRICULTURAL INSURANCE IN SERBIA

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SUMMARY: Plant production as well as livestock are exposed to numerous production risks that if transfered to insurance can improve agricultural results. We have determined the need for research of importance and issues in agricultural insurance in Serbia as the aim of the research. Research results suggest that agricultural insurance is undeveloped, because of uninterested farmers and insurers, although the increase in premiums and losses is marked during the observed period (2004-2009). Crop insurance has dominant share in premiums and losses. In future we expect that agricultural insurance premium subsidies will generate increased interest of farmers for insurance and consequently greater profitability and private insurance industry interest, resulting with multiple positive effects on favourable insurance conditions and better farmers' protection against production risks.

***Key words**: risk, risk management, agriculture, insurance, microinsurance, Serbia.*

INTRODUCTION

Agricultural production is endangered by numeriosu risks such as hail, floods, droughts, price changes, fires, thefts and the likes. Climate changes, trade liberalisation, geneticaly modified and organic food production, which obligatory element is agroeconomic analysis (Pejanović et.al, 2009), are the cause of new risk types that imperil not only individual farmers but all participants of agrobusiness value chain, including input suppliers, processors and consumers. The most papers that focus on risks in crops production have weather conditions and input and output prices as research topic while those that focus on risks in livestock are mainly oriented towards desease risk (see, Gramig et. At., 2006 and Shaik et al. 2006). All agricultural risks can be devided into regulatory or institutional risks, trade or price risks and production risks. In order to minimise potentially negative influence of risk realisations, farmers can apply various measures at their farms or can apply risk dispersion measures (EC, 2001), one of which is insurance (Njegomir, 2008).

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According to American Risk and Insurance Association, insurance is the pooling of fortuitous losses by transfer of such risks to insurers, who agree to indemnify insurers for such losses, to provide other pecuniary benefits on their occurrence, or to render services connected with the risk (Rejda, 2005, 20). Observed from the perspective of farmers, in exchange for determined small amount of fixed expense, insurance as a risk management method provides protection against much larger loss, which appearance is uncertain but anticipated by contract conditions. Insurance provides pooling of agricultural risks such as losses from hail or fire, theft of farms' property, death or illness of animals as well as death or illness of farmers. Understanding the state and problems that appear in agricultural insurance is important having considered the importance of agricultural production as well as the fact that healthy agricultural sector can lessen negative implications of economic crises (FAO, 2009).

MATERIAL AND METHOD

Analysing available documentation on crop and livestock insurance provided by National bank of Serbia, we have acquired neccessary information for research and economic analysis of the state and tendencies of premiums and losses of agricultural insurance in Serbia, with emphasis on the analysis of performance of agricultural insurance, separately for crop insurance and livestock insurance. We observed the data of all insurance companies operated in Serbia during the period from 2004 and 2009. The reason for the observed period is the fact that this period was influenced by many legal, political, social and economic developments that have influenced the development of domestic insurance market. We apply analytical method combined with model "desk research".

RESULTS AND DISCUSSION

The crucial role of insurance in agricultural production and society is indirect economic protection of property and life from the negative effects of natural forces and unfortunate events, thus making enterpreneur activities of farmers more stable and certain. The research done by World bank indicate that inadequate access to insurance is one of ten most crucial factors in food crisis problem solving and represents serious barrier to agricultural productivity, investments and marketing systems efficiency (World Bank, 2008, 40). Additionally, the importance of agricultural insurance is evident through the World trade organisation's elimination of state subsidies for insurance premium from free trade agreement in 1994, subject to condition that insurance provides indemnification for climatic and natural catastrophes (Baez and Wong, 2007).

Contrary to enormous importance that insurance has different studies demonstrate conflicting opinions regarding factors that positively affects farmers to sign insurance contracts. For example, Velandia *et al.* (2009) have determined that insurance contract acceptance is positively influenced by the level of risks faced by farmers while it is negatively affected by proportion of owned acres, off-farm income and education. However, Sherrick *et al.* (2004) have demonstrated that farmers are more willing to sign insurance contract if their perceived risk is greater and if they undertake production at properties that are larger, older and not in lease. Also, by analysing agricultural insurace

in France Enjolras and Sentis (2008) have determined that size and financial strenght of the farm, more diversified production and catastrophic climate events positively affects farmers decisions for insurance protecion.

Agricultural insurance is special type of insurance that belong to property insurance group. The separation of agricultural insurance as a special type of insurance is the result of its characteristics, mainly the reduced ability or complete inability for diversification application and great correlation among risks. For example, crops in same geographic areas are under threat of the same kind of risks, which translates into greater possiblitiy for loss generation either by one large or many smaller losses caused by the one event. Miranda and Glauber (1997) have determined that exposure to losses of risks portfolios of agricultural insurers is ten times greater than that of auto hull or fire insurers. Also, they have found that portfolios of crop insurers in the USA are twenty to fifty times riskier than the total exposure to risk would be if yields of individual farmers were stohasticaly indpendent. The high correlation among individual risks of agricultural production as well as the need to determine risk exposures of each individual farm, often geographicaly disperesed, causes high operational and administrative expences, much higher than in other types of insurance.

The development of insurance industry in Serbia has followed the economic development (Njegomir and Stojic, 2010). Although the insurance existed before the World War II, it has gained in importance only after 1990 thanks to the law changes that facilitated business development based on market principles. However, regulation and supervison of insurers have not been adequate. This is confirmed with the fact that almost all of reserves have been eliminated from balance sheets of insurance companies during the hiperinflation of 1993 and the fact that many insurers have been established, often with inadequate management practices, such as incompletness of business books, insurance reserves transfers to affiliated non-insurance companies and irregular payments of obligations to insureds. Such developments have generated loss of public trust in insurance, including agricultural insurance. The turning point in the development of Serbian insurance industry has been the introduction of the new insurance law in 2004 and assigning supervisory role to National bank of Serbia (NBS). This changes have contributed to the establishment of the rule of law and market discipline. These developments positively affected public trust in insurance industry (see Table 1).

Gross insurance industry premium has increased by around 230% during the observed period. The growth of agricultural insurance premium was somewhat slower, if the observed period is till 2008 the growth was 192% a year while if the whole period is taken into consideration the growth was approximately 134%. However, while gross premium of the whole insurance industry grew continiously, agricultural insurance premium movements were more variable. The economic crisis has dramaticaly affected agricultural insurance and premium growth declined for both crop and livestock insurance. During the observed period the share of agricultural insurance in non-life insurance has moved in a range between 4% and 2.4% in area of premiums and in a range between 7.8% and 4.2% in an area of losses. These data emphasise the fact that agricultural insurance in Serbia is in a unenviable state as they show that it negatively affects productivity of insurance industry as a whole and indicate insufficient development of this type of insurance. During the observed period loss ratio, the index that is calculated by deviding losses with premiums, vary in the case of crop insurance but is relatively stable in the case of livestock insurance. However, the excesses of premiums

over paid losses are rarely above 10%, which indicates low profitability. The existence of low profitability is emphasised with the fact that because of unattainable data administrative expenditures have not been taken into consideration. Also, when compared average loss ratio of 78.38% with European average loss ratios in agricultural insurance that ranges from 60% to 70% it is obvious that insurers in Serbia have achieved much poorer results than average European insurer.

Traditionally only products that provide indemnification for losses on crops or livestock are offered in Serbian agricultural insurance market. The dominant type of agricultural insurance is crop insurance, which share in total agricultural insurance premium has been in a range between 60% and 69%, the rest being livestock insurance. These two types of agricultural insurance is offered by eight of total 22 insurers in Serbia (Globos, Milenijum insurance, Sava insurance, Takovo insurance, Triglav Kopaonik insurance, DDOR Novi Sad, Dunav insurance and Delta Generali insurance) which total insurance market share is 80%.

Table 1. Agricultural insurance in Serbia during the period 2004-2009 (in 000 dinars)
Tabela 1. Osiguranje poljoprivrede u Srbiji u periodu od 2004 do 2009 (u 000 dinara)

			2004	2005	2006	2007	2008	2009
Premium	Crop insurance	Premium	578262	611733	611691	751461	1105208	746736
		Yearly growth	1	5.79%	-0.01%	22.85%	47.07%	-32.43%
		Number of policies	10396	9418	9351	10305	15186	10165
	Livestock insurance	Premium	259748	328554	409737	516619	511247	377500
		Yearly growth	1	26.49%	24.71%	26.09%	-1.04%	-26.16%
		Number of policies	2299	2396	2278	2582	2250	1807
	The share of crop insurance in total agricultural insurance		69.00%	65.06%	59.89%	59.26%	68.37%	66.42%
	The share of agricultural insurance in non-life insurance		4.00%	3.00%	2.98%	3.18%	3.53%	2.46%
	Gross insurance industry premium		22636133	34689787	38328614	44780018	52186631	53534646
	The share of agricultural insurance in gross insurance industry premium		3.70%	2.71%	2.66%	2.83%	3.10%	2.10%
Losses (accounted)	Crop insurance	Amont	411888	349786	542323	702677	569745	536290
		Yearly growth	1	-15.08%	55.04%	29.57%	-18.92%	-5.87%
		Number of losses	2198	2449	2429	3575	2358	3228
	Livestock insurance	Amount	253970	285655	376068	470998	451801	336123
		Yearly growth	1	12.48%	31.65%	25.24%	-4.08%	-25.60%
		Number of losses	6387	6886	8691	10573	9407	6022
	The share of crop insurance in total agricultural insurance		61.86%	55.05%	59.05%	59.87%	55.77%	61.47%
	The share of agricultural insurance in non-life insurance		7.80%	5.62%	6.27%	7.03%	5.21%	4.24%
	Total insurance industry losses		8794061	11724710	15423471	17675831	20936826	22436002
	The share of agricultural insurance in total insurance industry losses		7.57%	5.42%	5.95%	6.64%	4.88%	3.89%
	Loss ratio in crop insurance		71.23%	57.18%	88.66%	93.51%	51.55%	71.82%
	Loss ratio in livestock insurance		97.78%	86.94%	91.78%	91.17%	88.37%	89.04%
	Loss ratio in agricultural insurance		79.46%	67.58%	89.91%	92.56%	63.20%	77.60%

Source: author calculations, National bank of Serbia
Izvor: kalkulacije autora, Narodna banka Srbije

Serbian agricultural insurance market has passed through continious changes. New types of insurance coverage has been introduced recently. One of new product offerings are insurance packages for village, which combine crop and livestock insurance with other property and libability insurance types in order to provide „umbrella" coverage for farmers. It is especially worth to mention insurance based on index, innovation in agricultural insurance done in 2010 by Delta Generali. This type of insurance provide cover against drought, the risk that has previously been excluded from insurance. It is now possible to obtain coverage for mercantile and corn seed but more crops will be possible to insure in the future. The reference index used is determined by meteorological reports of Republic Hydrometeorological Service of Serbia and represents reduction in yields based on reduction in rainfall during reference time period in comparison with perennial average.

Agricultural insurance market in Serbia is still undeveloped when compared with other countries. For example, in 2008 the insurance premium per hectare of land has been US$ 60 in USA, US$ 13.38 in China and only US$ 3.45 in Serbia. We consider that the crucial causes for such undevelopment has been low purchasing power, insufficient consciousness of the need and possibilities of risk management as well as exaggerated orientation to everyday risks, that is risks characterised by relatively low loss intensity but great probability of realisation. The existence of government subsidies in majority of countries have been confirmed as a positive mean of agricultural insurance promotion. They positively influenced farmers interest in agricultural insurance protection. With increased interest of farmers as insureds for insurance protection it is possible to expect positive developments in loss ratios, which will then motivate more insurers to offer insurance protection to farmers. Althought the government subsidies in Serbia are 40% of insurance premium, which might be considered as high government involvement, they are still much behind of those in the region (for example, government subsidies amount to 75% of agricultural insurance premium in Croatia and 60% in FYR Macedonia). We assume that their existance and expansion of the amount in the forthcoming years can be critical in agricultural development promotion in future.

CONCLUSION

Insurance is the most important and the type of farmers production risk management technique that is most widespreadly and most often used. Insurance protection against risks that imperil agricultural production should be primarily provided by private insurance industry. We consider that the role of government should be limited to the creation of favorable regulatory and institutional environment for the development of private initiative. Direct government intervention should be limited to catastrophic events and situations when private initiative fails. However, in the early stages of the agricultural insurance development, as is the curent situation with Serbian agricultural insurance, when insurance companies achieve low profitability that are often even negative, the government intervention through subsidies is necessary. As government subsidies for insurance premiums are exampted from the rules that forbid direct government intervention set out by World trade organisation, they are the only form by which government can influence the development of domestic agricultural insurance.

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ZNAČAJ I PROBLEMI OSIGURANJA POLJOPRIVREDE

VLADIMIR NJEGOMIR, RADOVAN PEJANOVIĆ

Izvod

Biljna proizvodnja i stočni fond izloženi su brojnim rizicima čijim transferom u osiguranje se mogu unaprediti rezultati poljoprivrede. Za cilj rada smo odredili potrebu istraživanja značaja i problema u osiguranju poljoprivrede Srbije. Rezultati istraživanja sugerišu da osiguranje poljoprivrede nije dovoljno razvijeno, zbog nezainteresovanosti poljoprivrednih proizvođača i osiguravača, iako je tokom posmatranog perioda (2004-2009) došlo do značajnog rasta premija ali i šteta. Osiguranje useva ima dominantno učešće i u premijama i u štetama. U budućem periodu očekujemo da subvencionisanje osiguranja poljoprivrednih proizvođača generiše veću zainteresovanost poljoprivrednika za osiguranjem i posledično veću rentabilnost i veću zainteresovanost privatnog osiguravajućeg sektora, što će se multiplikativno pozitivno odraziti na povoljnije uslove osiguranja i bolju zaštitu poljoprivrednika od proizvodnih rizika.

Ključne reči: rizik, upravljanje rizikom, poljoprivreda, osiguranje, mikroosiguranje, Srbija.

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RISK ANALYSIS AND DECISION MAKING PROCESS IN AGRICULTURAL ENTERPRISE OF THE REPUBLIC OF SERBIA

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GORAN KRAJINOVIĆ, BAHRIJA KAČAR[1]

SUMMARY: Profit is the most important aim of the agricultural enterprise. It is in connection with creation of the Value on the market and with the process of its confirming on the financial market. So in the paper is discussing the logic of financing the agricultural enterprises growth in the transition country and making the comparisons with the level of risk acceptable, nature of the offered financing and the decision making process. Such analysis is representing additional guidance to the managers and its investment decisions under the conditions of extreme instability and risk.

Key words: *Agriculture, Enterprises, Risk analysis, Decision Making.*

INTRODUCTION

The essence of the corporate philosophy is based on treating the enterprise as a widely opened system (Djuričin, 4). It presumes creation of the mechanism and providing conditions for the functioning of the simulative macroeconomic and market oriented system created by the government, on one side, and on the other side, declaration of business risk and all the relevant consequences to the enterprise or to its owners. Understanding of those preposition is one of the main issues for the agricultural enterprises in the countries under transition.

Privatization of the state owned an so called social owned agricultural enterprises is one of the basic goals in the process of creating the corporative structure of the enterprises and in orientation toward market economy. Non defined ownership is one of the main sources of the business risk in the enterprises and limitation for the defining the financial requirements and creating the profit. It is the source for the instability of the enterprise, its inefficiency and low competitiveness. On the other side, in such condi-

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Original scientific paper / *Originalni naučni rad*

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tions, raises the number of sponsors of the enterprise who are bearing the consequences of the inefficient business orientation.

As it is stated, uncertainty and the sources of uncertainty in the agricultural enterprises could be expressed through description of phenomenon, but the quantitative data very often are insufficient to be incorporated into the analysis and to help the management to govern an agricultural enterprise in a profit manner. The result of this statement is that risk analysis must provide to the owners of the enterprise quantitative elements to judge the risk of any business decision or project implementation. So we can define the risk of the project as the probable variability of its future returns. For the variable such as sales from which future returns are derived, sales risk can be defined as the variability of expected sales. In practice, during project analysis, there are usually several variables for which doubts exist as to their best estimates. The purpose of risk analysis is to help management to isolate the risks and provide a means by which the various project outcomes can be reduced to a format from which a decision can be made. So, the final consequence of risk analysis is opinion or judgments regarding the possible range of future returns on investments in agricultural enterprises, as well as the possibility of each value to be created within this range.

But besides the possibility of evaluation the business risk of one project, often there is a need for judgment of all expected risks in the agricultural enterprise, portfolio risk. The agricultural enterprises portfolio risk can be analyzed also by considering the relationship between the proposed project and the existing investments and operations of the company. It must be stressed that in spite of judging the project as a risk one, it could be accepted for implementation on the basis of portfolio risk analysis. But this type of analysis is out of our interest in this paper.

METHODS FOR QUANTIFYING RISK

In the particular project or in the companies portfolio risk could be usually analyzed by running the three common methods of risk analysis: method of probability distribution; method of simulation; and method of sensitivity analysis (Krasulja, 6).

Method of probability distribution: Risk is associated with variability of returns expected. So, the more variable are the expected future returns, the riskier is the investment or project, an vice versa. However, it is useful to define risk more precisely. In the case of any investment decision or any kind of business decision, it has to be done forecast of future events. Probability expectation could be defined or measured from the point of view of a project analyst, or as an expectation of each possibly outcome. In its simplest form, a probability distribution could consists of an optimistic, pessimistic and most likely estimate or alternatively, high (boom), low (recession) and a “best guess” (normal market conditions) estimates.

Method of Simulation: When cash flows are correlated over time, the calculation of standard deviation will not give a correct result of the Net Present Value (NPV) variation of projects or / and Internal rate of return (IRR). The mathematic explication of NPV and equation for calculating the IRR is:

$$NPV = \sum_{n-1}^{n} C F_n (1 + r)^n - C$$

$$C_o = CF_1 / (1 + r)^1 + CF_2 / (1 + r)^2 + \ldots + CF_n / (1 + r)^n \text{ where:}$$

CF_n – cash flow in any period n (year 1,2,3, …),
r - rate of return or interest rate:
C – value of the project;
C_o – investments

Also, in practice, it may be necessary to produce separate probabilities for alternative sales revenue, different items of costs, and different possible life spans. These problems can be solved by using Monte Carlo simulation analysis, by the computer, which allows the consideration of many variables and their probability distributions as a result taking in account in the risk analysis as a part of the process of decision making in the agricultural enterprises.

Method of Sensitivity Analysis: The purpose of sensitivity analysis is to define the variables to which the NPV of the project in agricultural enterprise is most sensitive, and it is normally used to measure the extent to which these variables can change before the investment results in a negative NPV. Sensitivity analysis is useful for determining consequences of specified changes in variables such as product price, sales volume, input costs and investment life span. However, only risk analysis can provide any indication of the likelihood that such events will actually occur. Also, break-even analysis can be viewed as a form of sensitivity analysis. It enables the analyst to determine the impact of changes in cost, production volume and price on profits of the agricultural enterprise. The standard mathematical expression of the method is:

P - S (p - mc - vc) - Fc, where:
P = profit;
mc = material costs;
S = Sales
vc = variable costs,
p = selling price;
Fc = fixed costs

Sensitivity analysis is carried out by replacing the input variables that determine profitability with their expected values given in feasibility studies in order to determine profitability in quantitative terms.

RESULTS

As a graphical method of risk analysis the *Method of probability distribution* is showing the connection between actual investments in agricultural project and returns expected.

Such relation is shown in the next examples illustrated as it is shown below:

Example 1. Pay-off matrix for Project X and Project Y

	Annual cash flow	
State of the Market	Projekat X	Projekat Y
	RSD – Currency (dinars)	
Boom (high)	1.200	2.000
Normal (most likely)	1.000	1.000
Recession (low)	800	0

Source: Authors

The probability expectation in the example is: in case of boom is 0.2, in normal conditions is 0.6, and probability of recession is 0.2. Given the annual cash flows under the three possible states of the future market and their probability of occurring, weighted average projected cash flows can be calculated by multiplying each cash flow by its probability of occurrence (see below).

Example 2. Calculation of expected values

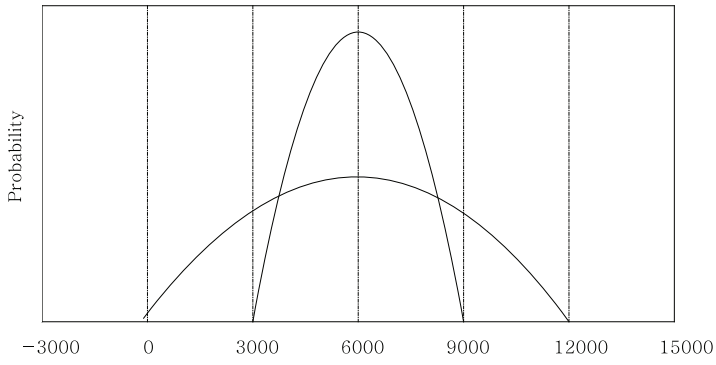
Appraisal	Cash flow	Probability	Weighted average or Expected Value
Project X			
Boom	1.200	0.2	240
Normal	1.000	0.6	600
Recession	800	0.2	160
Project Y			
Boom	2.000	0.2	400
Normal	1.000	0.6	600
Recession	0	0.2	0

Source: Authors

Calculated average value is defined as expected value of cash flow in particular investment or any business operation of the agricultural enterprise. Also, it could be stated as a basic result in comparison with the values created through alternative business decisions.

Business risk always exists due to the particular investment and could be presented in graphical form to obtain a clear picture of variability of actual outcomes (Graph 1).

Assume two mutually exclusive projects P_x and P_y which are with the same net cash flows of USD 4.000. In the same time, it could be two following cases: investment project P_x has probability of cash inflows in the range of USD 3.000 - USD 9.000 while investment project P_y has probability in the range of USD 0 - USD 12.000. Looking at the net cash flow (which is equal in both cases), one is temped to rank both investments equally. But a look at the graph above shows that the variability of cash flows for project P_y is greater than of project P_x and therefore the project P_y is riskier project.



Source: Authors

Graph 1. Probability of Cash Flow

The variability of distribution is normally measured by standard deviation. The earlier example is used to calculate the standard deviation as presented below (example 3).

Example 3. Calculation of Standard Deviation

Probability	Calculation of Standard Deviation		RSD		
	Net Cash Flow	Expected value (1 x 2)	Deviation	Standard deviation	Variance (1 x 5)
1	*2*	*3*	*4*	*5*	*6*
Project X					
0.2	1,200	240	200	40,000	8,000
0.6	1,000	600	0	0	0
0.2	800	160	(200)	40,000	8,000
expected value	1.000			variance	16.000
				Standard deviation	126.49
Project Y					
0.2	2,000	400	1,000	1,000,000	200,000
0.6	1,000	600	0	0	0
0.2	0	0	(1,000)	1,000,000	200,000
expected value	1.000			variance	400.000
				standard deviation	632.45

Source: Authors

Columns 1, 2 and 3 are taken directly from previous example. In column 4, the expected value from each possible outcome is subtracted to derive the deviations about the expected value. In column 5, deviations are squared. In column 6, squared deviation is multiplied by associated probability and the products are summed to obtain the variance of the probability distribution.

The calculation provide outcomes for P_x = USD 126.49 and P_y = 632.45. So, it could be stated that the project P_y is bringing a higher risk than project P_x.

Method of Sensitivity Analysis: In the implementation of this method management of the agricultural enterprise should pay special attention and control those variables to which NPV is particularly sensitive. In our example, it can be used the simplest type of sensitivity analysis with variables that are influencing profitability as follows:

Example 4. Sensitivity analysis

Variable	Best estimate	Increasing variable by 10%	Resulting profit RSD	Change in profit %	Rank order of variables
Sales (unit)	10,000	11,000	120,000	20	2
Price (USD/unit)	50	55	150,000	50	1
Variable Costs (USD/unit)					
– raw material	10	11	90,000	(10)	3
– other variable costs	20	22	80,000	(20)	2
Fixed costs (USD)	100,000	110,000	90,000	(10)	3
Pofit (USD)	100,000				

Source: Authors

The profit is calculated on the basis of 10% increase in variables, taking each variable at a time.

The sensitivity analysis based on a discounted cash flow model is presented in the next example:

Example 5. Implementation of the method of Sensitivity analysis based on DNT

Cash flow	Year 0 RSD	Year 1 RSD	Year 2 RSD	Year 3 RSD
Cost of equipment	(100,000)	0	0	0
50.000 kom USD 2/ kom	0	100,000	100,000	100,000
Variable costs	0	50,000	50,000	50,000
Net cash flow	(100,000)	50,000	50,000	50,000
The cost of capital is 12% and the NPV is RSD 20.095				

Source: Authors

The calculated variables are:

1) *Sales volume*. The net cash flow will have to fall to RSD 41.634 (RSD 100.000/2.4019 discount factor) for the NPV to be zero, because it will be zero when the present value of the future cash flow is RSD 100.000. The discount factors for 12% and Years 1,2 and 3 are 0.8929; 0.7972; and 0.7118 respectively.
   As the likely net cash flow is + RSD 50.000, the net cash flow may decline by about RSD 8.366 each year before NPV becomes zero.
   Total sales revenue may therefore drop by RSD 16.732 (net cash flow = 50% of sales). At selling price of RSD 2 per product, this represents 8.366 units. Alternatively, the sales volume may decline by 16.7% before the NPV becomes negative.
2) *Product price*. When the sales volume is RSD 50.000 products yearly, total sales revenue can drop to RSD 91.634 (100.000 - 8.366) before the NPV becomes negative. This represents a selling price of RSD 1.83 or a reduction of approximately RSD 0.17 per product which represents about 8.4% reduction in selling price.
3) *Variable costs*. The total variable costs can increase by RSD 8.366 or RSD 0.17 per product. This represent increase of 17%.
4) *Initial outlay*. The total outlay can increase by the NPV before the investment breaks even. The initial outlay may therefore increase by RSD 20.095 or 20%.
5) *Cost of capital*. IRR for the project is nearly 30%. Consequently, the cost of capital can increase by 250% before the NPV becomes zero.

The above analysis can be summarized as follows:

Variable	Switching value (%)	Variable	Switching value (%)
Sales Volume	16.7	Initial outlay	20.0
Selling price	8.4	Cost of capital	200.0
Variable costs	17.0		

Source: Authors

The element to which the NPV appears to be most sensitive is the price followed by sales volume and variable cost.

DISCUSSION

The implementation of the *Method of probability distribution* could erase certain problems in the use of standard deviation as a measure of risk. Taking in account an example which shows the probability distributions for investments P_{x1} and P_{y1}. Assume that P_{x1} has an expected return of RSD 2.000 and standard deviation of RSD 600, and investment P_{y1} also with the same standard deviation of RSD 600 has higher expected return of RSD 8.000. Investment P_{y1} has more risk per RSD of the return than P_{x1}. On this basis, it is reasonable to assign a higher degree of risk to investment P_{y1} than to investment P_{x1}, event though both have identical standard deviations. So, it must be derived the standard deviation by the expected value of net cash flows to obtain the coefficient of variation, to handle this problem. For investment P_{y1}, RSD 600 is divided by RSD 2.000 (expected value), obtaining 0.30 as the coefficient of variation and 0.075 for project P_{x1} which is much lower coefficient of variation. So the later project has been less risky than previous.

In the implementation of the *Method of Simulation* the scope of simulation analysis is connected with providing the probability distribution for Cash Flows (CF), internal rate of return (IRR) and net present value (NPV). This method is using often and the interpretation is so common that we decided not to present this method particular.

Method of Sensitivity Analysis: In the case of the sensitivity calculation which is based on the increase in variables of 10% and parallel taking of each variable at a time, it could be pointed out that if the change of variables is equal by percentage, profit is most sensitive to changes in price. It is followed by sales volumes and other variable costs with similar changes, and at last with the same level of changes in case of raw material and fixed costs ranking. Although this simple analysis has pointed the most sensitive variables, no insight is provided into the probability of these changes taking place. For example, the average of 10% increase in row material cost may be very high while the probability of a 10% increase in product price may be low. In the case of sensitivity analysis based on a discounted cash flow model, it could be shown that it is important for the project sponsor to pay particular attention to the items like sales volume, selling price and variable costs so that can be fully monitored. Sensitivity analysis has a number of shortages. In particular, the method requires that changes in each key variable are isolated, but the management is also interested in the combined effect of changes of two or more variables. In addition, the method is giving no indication to the likely probability of changes in key variables or of a combination of variables occurring.

CONCLUSION

The transition process involves new relations in running the agricultural enterprises. Privatization and clear ownership are forcing the owners of the firm and managers to restructure the enterprise and make it more efficient. But, in the process of judging the business possibilities, they are faced with the higher risk than earlier. Business risk is caused by the internal and external variables and with its occurring. So, besides running the profit of the firm, owners and the managers are faced with certainty of its making.

When we talk about possible methods discussed it could be concluded that in connection between actual investments in agricultural project and returns expected the

method of probability distribution could efficiently help in determination of probability distributions for investments (P_{x1} and P_{y1}). Parallel, for the probability distribution for Cash Flows (CF), internal rate of return (IRR) and net present value (NPV) the best solution could be using the method of simulation. From the calculation, certain conclusions can be derived such as: "the likelihood is 80% that IRR will equal 12% "or" there is a 10% chance that the project will result in loss of RSD 35.000", etc. Finally, in the case of sensitivity analysis method implementation it is possible to take care of particular items like sales volume, selling price and variable costs and on that basis monitor the possible risks in spite of a number of possible shortages.

Implementation of those methods for quantifying the risk could bring more stabile conditions for agribusiness enterprises that are operating even at higher risk than other enterprises. This is an important and relevant aspect of the problem of creating the profit and enabling the agricultural enterprise growth. Such methods are providing necessary tools for the managers and owners of the firms for its better management and better decision making. In that process, one can identify the level of risk but also the certainty and possibility of creating the cash flow in the agricultural enterprise.

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ANALIZA RIZIKA I PROCES DONOŠENJA ODLUKA U POLJOPRIVREDNOM PREDUZEĆU U REPUBLICI SRBIJI

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Izvod

Profit je najznačajniji cilj poljoprivrednog preduzeća. On je povezan sa stvaranjem vrednosti i njenog potvrdjivanja na tržištu. Stoga se u radu diskutuje logika finansiranja rasta poljoprivrednog preduzeća u zemlji koja se nalazi u tranziciji i pravi komparacija sa nivoom prihvatljivog rizika, prirodom finansiranja i procesom donošenja odluka. Ovakva analiza predstavlja dodatni vodič za menadžere i njihove investicione odluke u uslovima ekstremne nestabilnosti i rizika.

Ključne reči: poljoprivreda, preduzeća, analiza rizika, donošenje odluka.

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ANALYSIS OF SOMATIC CELL COUNT IN MILK OF COWS CHRONICALLY INFECTED WITH COXIELLA BURNETII

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SUMMARY: Various infectious agents who are present in the animals can directly- existence of changes in the mammary gland, or indirectly-increased number of leukocytes in the systemic circulation, leading to an increase in the number of somatic cells in milk. Q fever is a rickettsial disease that is most common in home and abroad. Infected cows usually suffer asymptomatic, and clinically observable changes can be abortions, reduced fertility, low vital offspring, mastitis, ceratoconuctivitis, bronchopneumonia. The influence of infection with Coxiella burnetii on the movement of somatic cells in the infected cow was tested on a group of Holstein-Friesian cows. For most cows we observed an increase in the number of somatic cells. Somatic cells in milk samples ranged from 103 000 to 2 000 000 per ml of milk. Average somatic cell count in all milk samples was 853 000.

***Key words**: Coxiella burnetii, milk, somatic cell.*

INTRODUCTION

The number of somatic cells in milk is an indicator of the health status of mammary gland. Disorders of secretion caused by the influence of different causes lead to an increase in the number of somatic cells, and their increase with high confidence indicates the effect of unfavorable factors on the mammary gland. What is the significance of the increased number of somatic cells to assess the general health of animals when we know that somatic cells in the highest percentage are leukocytes. General health disorders caused by the action of agents that are not connected to the mammary gland may be the reason for the increase in the number of somatic cells. Infection with Coxiella burnetii in cows often runs innaparent, and the animals remain in production, not exhibiting any clinical symptoms. Clinical manifestations of the disease is mainly related to disturbances in reproduction, but symptoms can occur in other organ systems (Rodolakis et al., 2007). Q fever is manifested only in cattle abortions, and in case of chronic diseases cause is primarily excreted in milk and can be isolated from mammary

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gland. In these animals secretion disorder is present to a greater or lesser extent (Guateo et al., 2006, Vidic et al., 2008).

MATERIALS AND METHODS

On the dairy farm of Holstein-Friesian cattle in ten animals were serologically confirmed Q fever using the micro-CF test, antigen Coxiella burnetii phase II was used, strain Nine Mile. Positive finding was considered a finding of specific antibodies in the serum dilution 1:4 and more. From infected cows cumulative milk samples were taken for the control of somatic cells. Cows were free of clinical symptoms including abortions, in good body condition and milk production was below the average herd production. The milk samples were taken at intervals of 42 days during the morning milking of cows. Milk was sampled by Vaikato attaches to the milking units during sampling, to obtain the average and homogeneous sample. After sampling, in each sample was added sodium azide as a preservative, and then homogenize the sample by mixing so the preservative is equally distributed, to prevent the collapse of somatic cells. After sampling the samples were transported to the laboratory at the temperature of the refrigerator. Laboratory analysis of samples was performed in the laboratory for the control of raw milk dairies "Subotica" on fosomatic device by flow citometry.

RESULTS AND DISCUSSION

By summary analysis of cumulative milk samples for somatic cells, we found in the majority of cows increased number of somatic cells above the hygienic standards of milk. Table 1 shows the results of somatic cells count for three consecutive milk sampling.

Table 1. Somatic cell count in cumulative milk samples
Tabela 1. Broj somatskih ćelija u zbirnim uzrocima mleka

	first sampling *prvo uzorkovanje*	second sampling *drugo uzorkovanje*	third sampling *treće uzorkovanje*
SCC x 10^3	526	1024	147
	1795	103	361
	900	2000	2277
	588	1193	1004
	169	836	419
	685	236	1092
	743	1236	113
	1485	1048	
	130	981	
	1240		
average / *prosek*	826	961	773

In the first sampling of ten cows that were in control, in only two the number of somatic cells was in the range of milk hygienic standards. In the second sampling of the nine cows in the control in two somatic cell count was within the limits of hygiene standards, and the third sampling of seven cows in the three somatic cell count was within the limits of hygiene. Of the total number of samples in 40% somatic cell count was over one million, which is a very high value. In 25% of samples somatic cell count was in the range of hygienic standards. In the same period the number of somatic cells in herd milk sample did not exceed 300,000 per ml. Infected animals accounted 5% of the herd. Infection of cows with Coxiella burnetii affects the appearance of disorders of milk secretion (Vidic et al., 1999), and mastitis was one of the possible symptoms of this infection (Šeguljev et al., 1994). In chronically infected cows the mammary gland and associated lymph nodes are predilective place for Coxiella burnetii and it can be isolated primarily from the udder and milk (Vidic et al., 2005). Number of somatic cells in milk varied in cows in the experiment from 103 000 to 2 000 000 per ml, and the average number of somatic cells in all samples was 853 000 per ml. The increase in the number of somatic cells in infected cows indicated Vidic et al., 1990, where most of the cows had somatic cells in milk above the limits of hygiene. On the association between infection with Coxiella burnetii and the existence of subclinical mastitis in cows indicated Barlow et al. 2008 who found in infected cattle movement in the number of somatic cells from 229 000 to 762 000 per ml. Along with changes in number of somatic cells changes in share of different cell types also occur. So share of polymorphonuclear cells is increasing up to 85% of all somatic cells in case of subclinical mastitis (Boboš and Vidić, 2005).

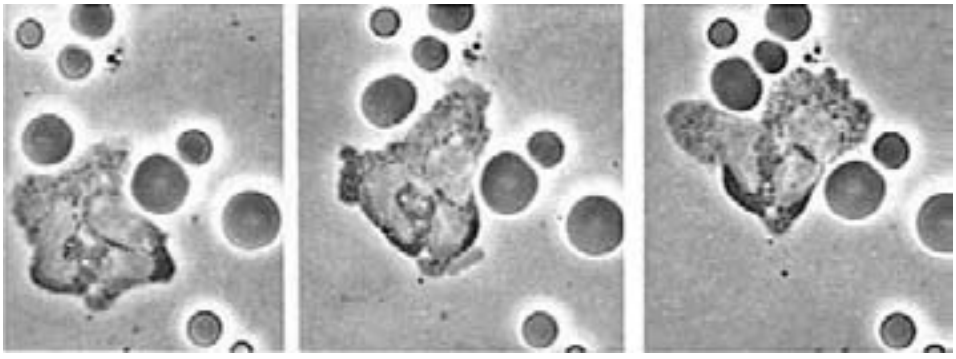

Figure 1. Wiew of milk cells with microscope (12000:1)
Active movement of cells among the fatty drops during time. First picture shows beging of watcing and every other is two minutes after.
Slika 1. Prikaz ćelija mleka pomoću mikroskopa (12000:1)
Aktivno kretanje ćelija medju masnim kapljicama. Prva slika prikazuje početak posmatranja a svaka sledeća je u razmaku od dva minuta.

CONCLUSION

Chronically infected animals used in production can produce milk with changed chemical composition and hygienic quality. Permanent increase in the number of somatic cells in cows with no clinical symptoms related to the mammary gland or other

organs needs to emphasize the need for further research in order to exclude chronic infection. Infection of cows with Coxiella burnetii may cause the increase of somatic cells in milk and this possibility deserves special attention of veterinarians who deal with medical conditions dairy herds.

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ANALIZA BROJA SOMATSKIH ĆELIJA U MLEKU KRAVA HRONIČNO INFICIRANIH SA COXIELLA BURNETII

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IVANA DAVIDOV, MARIJA PAJIĆ

Izvod

Različiti infektivni agensi prisutni u organizmu životinje mogu na direktan-postojanje promena na mlečnoj žlezdi, ili indirektan način-porast broja leukocita u sistemskoj cirkulaciji, dovesti do porasta broja somatskih ćelija u mleku. Q groznica je bolest koja se od svih koksieloza najčešće javlja u svetu i kod nas. Inficirane krave najčešće boluju

asimptomatski a od klinički uočljivih promena mogu postojati pobačaji, smanjena plodnost, slabo vitalan podmladak, mastitisi, keratokonjuktivitis, bronhopneumonije. Uticaj infekcije sa Coxiella burbetii na kretanje broja somatskih ćelija kod inficiranih krava ispitan je na grupi krava holštajn-frizijske rase. Kod većine krava zapaženo je povećanje broja somatskih ćelija. Broj somatskih ćelija u uzorcima mleka kretao se od 103 000 do 2 000 000 u ml mleka. Prosečan broj somatskih ćelija u svim uzorcima mleka bio je 853 000.

Ključne reči: Coxiella burnetii, mleko, somatske ćelije.

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THE LIPIDS CONTENT IN BLOOD AND LIVER OF DAIRY COWS DURING THE TRANSITIONAL PERIOD

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SUMMARY: The objective of the present investigation was to determine the correlation in the blood concentrations of lipids and content in the liver in dairy cows (n=40) in transitional period. The cows were divided into four groups: the first group included late pregnant cows (n=10) from the 10 th to 4 th day before calving, the second group included late pregnant cows (n=10) from the 4 th to 1 th day before calving, the third group included puerperal healthy cows (n=10) whereas the fourth group included puerperal ketotic cows (n=10). Samples of liver and blood tissues were taken from all cows. Pathohistological examination of liver samples showed statistically significant higher lipid infiltration (P<0,01) in ketotic cows compared to healthy cows. Biochemical examination of blood serum showed significantly higher values (P<0,01) of free fatty acids in ketotic cows such as significantly lower concentrations of glucose (P<0,01), triacylglycerols (P<0,01) and total cholesterol (P<0,05) compared to the groups of healthy cows. Significant increase of the concentrations of free fatty acids, such as positive correlation (r=0.51, P <0.05) between the free fatty acids in the blood and the content of lipids in liver in ketotic cows compared to healthy ones, suggests that during intensive lipomobilization newly synthetized triacylglycerols accumulated in the hepatocytes.

***Key words** : cows, fatty liver, ketosis, lipids, glucose.*

INTRODUCTION

The transitional period in dairy cows included 3 weeks before and 3 weeks after calving when metabolic processes were adapted to provide energy and precursors required for synthesis of milk compounds (Grummer 1995; Overton and Waldron, 2004). Consequently, such a state caused negative energy balance, a high mobilization of lipids from bodily fat reserves as well as hypoglycaemia (Veenhuizen et al.1991; Gaál 1993;

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Vazquez-Anon et al. 1994). Lipomobilization, characterized by high concentrated free fatty acids in the blood, starts in late pregnancy, reaching its maximum in the early lactation. Free fatty acids are re-esterified and accumulated in the form of triacylglycerols in the liver, primarily due to a decrease capacity of hepatocytes for transport lipids by very low density lipoproteins (VLDL). As a result, lipomobilization intense ketogenosis and lipogenesis in the liver and as consequence lower concentrations of glucose, triacylglycerols and total cholesterol in the blood was manifested (Herdt et al. 1983; Holtenius 1989; Veenhuizen et al.1991; Vazguez-Anon et al. 1994; Sevinc et al. 2003; Đoković et al 2007, 2009, 2010). Primary homeoretic adaptation of glucose metabolism in the early lactation leads to an increased gluconeogenesis in the liver to direct glucose to the mammary gland for lactose synthesis. Metabolic conditions of negative energy balance (fasting, parturition and lactation) lead to an increased uncontrolled rate of mobilization of body fat and its increased accumulation in liver cells, resulting in disturbance of the morphological and physiological integrity of the liver (Grummer et al. 1995; Vazguez-Anon et al. 1994; Bobe et al. 2004). Mild fatty infiltration of liver cells in dairy cows during transition and maximum lactation is considered to be almost physiological. Liver can be categorized into normal liver or mild (0-20% of lipids), moderate (20-40% of lipids) and severe fatty liver (more than 40% of lipids) as dependent on the degree of pathology (Gaal,1993). The objective of the present investigation was to determine the correlation in the blood concentration of lipids and content lipids in the liver in dairy cows in transitional period.

MATERIAL AND METHODS

The late pregnant and calved cows (n=40) were chosen from a Holstein dairy herd (PIK-Bečej) and divided into four groups: in the first group (A) included late pregnant cows (n=10) from the 10^{th} to 4^{th} day before calving; the second group (B) included late pregnant cows (n=10) from the 4^{th} to 1^{th} day before calving; third group (C) included puerperal healthy cows (n=10) whereas the fourth group (D) included puerperal ketotic cows (n=10). The liver and blood samples were taken from all the cows.The diagnosis of ketosis was based on the clinical symptoms and determined high concentrations of urinary ketone (Lestradet test). The cows were 650 kg by weigt, had 3 lactations and 7625.2 ± 329.17 l milk of average milk production. The trial cows were kept in tie-up stalls in a barn housing. Diet and feeds were in conformity with purpose and animal utilisation. The blood samples were collected by punction of jugular vein into disposable test tubes from 10 h a.m. to noon or from 4 to 6 h after milking and feeding. The blood samples were allowed to clot spontaneously at room temperature. The serum was than centrifuged at 3000 rot/min.and preserved at -18 °C until analyzed. Glucose concentration was determined from fluoride plasma. The values for blood serum parameters were determined by photometric methods using an automatic analyser Cobas Mira and the following tests: glucose (Cat. No. 11803), triacylglycerols (TAG) (Cat. No. 11828), free fattty acids (FFA) (Cat No.FA 115) and total cholesterol (Cat. No. 11828). Shortly after blood sampling the liver tissue was sampled through liver percutaneus biopsy using a biopsy instrument following a modified method of Gaál after Hojovcava-Kacafirek (1967). The biopsy was performed at the right 11^{th} intercostal region, approximatelly 2 cm below the horizontal line through the tuber coxae, with 3-5 cm long and 3-4 mm wide liver specimen. The liver specimens were fixed in neutral 10% formaldehyde solution. Cryostat sections were stained with hemotoxylin and eosin and Sudan-III. Lipid conteined in the hepatocytes was determined through com-

puter image analysis (Software Q Win) made on aplliance (Leica Q 500 MC).The significance of differences of lipids, and glucose concentrations in the blood serum and the content of lipids in the liver between the animal groups used in experiment were determined by ANOVA procedure. Data are expressed as means ± standard deviation (x ± SD). Correlation coefficients were obtained using linear regression models. Differences with P<0,05 and P<0,01 were considered statistically significant (microsoft STATISTICA ver.5.0, Stat. Soft. Inc.1995).

RESULTS

The results of selected metabolic parameters in the groups of the cows in transitional period are shown in Table 1.

Table 1. Selected metabolic profile parameters in the groups of the cows in transitional period (means ± standard deviation)
Tabela 1. Vrednosti određenih metaboličkih parametara kod ispitivanih grupa krava u tranzicionom period (*srednja vrednost* ± *standardna devijacija*).

Groups / *Grupe*	Late pregnancy / *Visoki graviditet*		Puerperium / *Puerperium*	
	A	B	C	D
n	10	10	10	10
Glucose / *Glukoza* (mmol/l *)*	2.94 ± 0.32^{A}	3.12 ± 0.42^{B}	2.71 ± 0.35^{bc}	1.80 ± 0.43^{ABCD}
FFA / *SMK* (mmol/l)	0.27 ± 0.14^{A}	0.54 ± 0.26^{ABc}	0.46 ± 0.10^{AC}	0.74 ± 0.12^{ACD}
Triacylglycerols *Trigliceridi* (mmol/l)	0.32 ± 0.04^{A}	0.41 ± 0.03^{ABC}	0.35 ± 0.04^{C}	0.27 ± 0.03^{ABCD}
Total cholesterol *Ukupni holesterol* (mmol/l)	1.75 ± 0.20^{a}	1.71 ± 0.30^{b}	1.86 ± 0.62^{c}	1.39 ± 0.29^{acd}
Content of lipids in liver *Sadržaj masti u jetri* (%)	5.30 ± 1.10^{A}	$6.31 \pm 1,18^{B}$	8.37 ± 1.24^{C}	32.91 ± 13.23^{ABCD}

^{a,b,c,d} – Values with different superscripts are significantly different; values market by small superscripts differ significantly (P<0,05); values market by capital superscripts differ high-significantly (P<0,01).
a,b,c,d *– Vrednosti sa različitim superskriptima su statistički različite; vrednosti obeležene malim superskriptima označavaju značajnost (P<0,05); vrednosti obeležene velikim superskriptima označavaju značajnost (P<0,01).*
(A - group of cows from 10 th to 4 th days before calving, B- group of cows from 4 th to 1 st days before calving; C- group of puerperal healthy cows; D- group of puerperal ketotic cows).
(A- grupa visoko gravidnih krava u periodu od 10. do 4.dana pre teljenja, B - grupa visoko gravidnih krava u periodu od 4. do 1. dana pre teljenja, C- grupa klinički zdravih tek oteljenih krava, D- grupa oteljenih krave obolelih od ketoze).

From Table 1 it can be seen that significant changes of the most parameters in blood in group of ketotic i.e. the group of cows with fatty liver. Biochemical examination in the blood serum showed significantly higher values (P<0,01) of free fatty acids in ketotic cows such as significantly lower concentrations of glucose (P<0,01), triacylglycerols (P<0,01) and total cholesterol (P<0,05), compared to values in the blood serum in the groups of healthy cows. The free fatty acids concentration was significantly higher (P<0,01) among groups of cows in late pregnancy. In the group of ketotic cows, the

content of lipids in the liver was higher (P<0,01) compared to groups of healthy cows before and after calving

DISCUSSION

In dairy cows, fatty liver occurs primarily in the first 4 weeks after calving, when up to 50 % of all cows have some accumulation of lipids in liver (Grummer et al. 1995; Bobe et al. 2004). In the group of puerperal ketotic cows the content of lipids in the liver was significantly higher (P<0,01) compared to groups of healthy cows. That ketosis and fatty liver are closely in association were obtained by other authors (Gröhn 1985; Veenhuizen et al.1991; Vazguez-Anon et al. 1994; Đokovic et al. 2007). Glucose is a blood parameter defining the energy metabolism in lactating cows. In the group of ketotic cows has determined hypoglycaemia which was statistically lower (P<0,01) compared with groups of healthy cows. Needs for glucose in early lactation in dairy cows is higher than the amount which the body can provide in the condition of high milk production and that it is a important factor in development of hypoglycaemia and ketosis. In ketotic cows there is a decreased ability of hepatocytes to synthesize glucose by gluconeogenesis which causes fatty liver development (Gröhn 1985; Veenhuizen et al.1991; Vazguez-Anon et al. 1994). Energy metabolism in dairy cows in transitional period is closely linked to lipid metabolism. The best indicator of negative energy balance and the degree of mobilization of lipids from bodily fat reserves in the transitional period is increased of FFA concentrations in the blood. (Veenhuizen et al.1991; Vazquez-Anon et al. 1994; Overton and Waldron 2004). The FFA concentrations have been significantly increasing (P<0,01) (among groups A and B) as the calving day was approaching that shows that lipomobilization begins immediately before calving, respectively from four to one days before calving. Similar results were obtained by *Dyk et al.*(1995). Significantly higher (P<0,01) FFA concentrations have been determined in the blood of ketotic cows than in the groups of healthy cows. In accordance, in this experiment has been determined significant positive correlation (r=0.51. P<0,05) among the FFA in the blood and content of lipids In liver in the group of ketotic cows. This agrees with previous studies (Gröhn 1985: Veenhuizen et al. 1991; Gaál 1993; Vazguez-Anon et al. 1994). At ruminants relatively low TAG concentrations in the blood serum and further decline of their concentrations could be the consequence of lipid infiltration of liver cells. In this experiment has been determined significantly lower (P<0,01) TAG concentrations in the blood in the group of ketotic cows than to the values in groups of healthy cows, such as significant negative correlation (r=-0.55, P<0,05) between the TAG concentrations in blood and amount of lipids in the liver in the group of ketotic cows. The indicated that the blood TAG concentrations decreased and proportionally to that increases their amount in the liver cells in which they accumulated. These results are in accordance with observation (Herdt et al. 1983, Gerloff et al.1986; Holtenius 1989, Sevinc et al. 2003). In this study the were determined significantly lower (P<0,05) level of the total cholesterol in the blood in ketotic cows than of healthy groups of cows, which indicate that at condition of ketosis and fatty infiltration of liver cells in dairy cows, their ability to synthesize and transport cholesterol and TAG are decreased (Gerloff et al.1986; Gaál 1993, Sevinc et al. 2003).

CONCLUSION

Pathohistoligical examination of liver samples shows statistically significant higher lipid infiltration (P<0,01) in ketotic cows compared to groups of healthy cows .

Biochemical examination of blood serum showed significantly higher values (P<0,01) of free fatty acids in ketotic cows such as significantly lower concentrations of glucose (P<0,01), triacylglycerols (P<0,01), total cholesterol (P<0,05) compared to the groups of healthy cows.

Significant increase of the concentrations of free fatty acids, such as positive correlation (r=0.51, P<0,05) between the free fatty acids in the blood and the content of lipids in liver in ketotic cows compared to healthy ones, suggest that during intensive lipomobilization newly synthetized triacylglycerols accumulated in the hepatocytes.

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SADRŽAJ LIPIDA U KRVI I JETRI KOD MLEČNIH KRAVA U TRANZICIONOM PERIODU

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VLADIMIR KURĆUBIĆ, JOVAN STOJKOVIĆ

Izvod

Cilj ovog rada je bio da se odrede korelacije između koncentracija lipida u krvi i sadržaja lipida u jetri kod mlečnih krava (n=40) u tranzicionom periodu. Ispitivane krave su podeljene u četiri grupe. U prvoj grupi (n=10) nalazile su se visoko gravidne krave u periodu od 10. do 4.dana pre teljenja, u drugoj grupi visoko gravidne krave (n=10) u periodu od 4. do 1. dana pre teljenja, u trećoj grupi (n=10**)** su se nalazile klinički zdrave tek oteljene krave, a u četrvroj grupi (n=10) ketozne krave. Od svih ispitivanih krava uzeti su uzorci krvi i jetre. Patohistološka ispitivanja uzoraka tkiva jetre pokazala su statistički značajne veće (P<0,01) vrednosti sadražaja masti u jetri kod grupe ketoznih krava u odnosu na sadržaj masti u jetri kod grupa zdravih krava. Biohemijska ispitivanja krvnog seruma pokazala su statistički veće (P<0,01) koncentracije slobodnih masnih kiseliina, kao i statistički manje vrednosti glukoze (P<0,01) triglicerida (P<0,01) i ukupnog holesterola (P<0,05) kod grupe ketoznih krava u odnosu na vrednosti kod zdravih krava. Značajno veće vrednosti (P<0,01) slobodnih masnih kiselina, kao i pozitivna koleracija (r=0.51, P<0,05) između koncentaracijai slobodnih masnih kiselina u krvi i sadržaja masti u jetri kod grupe ketoznih krava, ukazuje da za vreme intenzivne lipomobilizacije novosintetisani trigliceridi se nakupljaju u ćelijama jetre.

Ključne reči : krave, masna jetra, ketoza, lipidi, glukoza.

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ULTRASOUND EXAMINATION OF HORSE TENDONS - COMPARING ULTRASOUND AND ANATOMY FINDINGS

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SUMMARY: The aim of this research was to investigate a procedure of ultrasound examination of horse tendons in the region of metcarpus and metatarsus. The investigation was caried out using abattoir samples with performing ultrasound during a clinical examination of horses. The anatomy studies consisted of cutting a frozen samples in order to establish analogy between macroscopic picture of transverse section at standard zones of the metacarpus/metatarsus region and ultrasound image at the same position. The superfitial digital flexor tendon, deep digital flexor tendon, inferior chek ligament and suspensory ligament were the structures of interes in this research. The shapes, echogenity, structure of health horses were described.

Key words: *horses, tendons, ultrasound examination.*

INTRODUCTION

Tendon and ligament injuries are relatively common among equine athletes. Horses engaged in strenuous work such as racing, jumping, endurance or barrel racing are more likely to sustain a tendon or ligament injury. However, injuries can and do occur in horses of all breeds and uses, including the backyard or light use horse. Tendon lesions are a dominant in equine locomotor system pathology. The palmar tendons of the horse's hand are the most often affected, and among them, the superficial digital flexor tendon is the most frequently injured, especially in its metacarpal part. Early development of diagnostic ultrasound in veterinary medicine focused on cardiology, theriogenology and examination of abdomen and thorax. Ultrasound examination of the soft-tissue structures of the limbs of horses developed more slowly. In 1982, Rantanen et al., introduced diagnostic ultrasonography as a potential tool for examining tendons and ligaments in horses. Since than has greatly improved veterinarians' capacity to accurately diagnose the presence and extent of soft-tissue injury and to monitor the progress

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Original scientific paper / *Originalan anučni rad*

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of healing, using measurable criteria such as the size of the lesion, the echogenicity of the lesion, and the quality of fiber pattern (Van Shie and Bakker, 2000). The ultrasonographic appearance of normal and injured tendons has been described (Denoix et al., 1994; Gillis et al., 1993; Gillis et al., 1995; Henry et al., 1986; Rantanen et al., 1985; providing a improvement of patient rehabilitaion program to become more practic, an to base on the evidence of direct visualization of tendon architecture using a mentioned criteria rather than clinical signs based on pain, heat, sweling and lameness. So diagnostic ultrasound is considered the gold standard in the evaluation of tendons and ligaments in horses. The most important criteria for the ultrasonographic assessment of tendon or ligament injuries over time are changes in echogenicity, size, contour, and definition of the margins (Smith et al., 1994).

MATERIAL AND METHODS

The investigation was carried out using abattoir samples, and live animals. At abattoir samples, collected from 14 animals, the visual postmortal examination was performed in order to determine the fore-hind and left-right limbs. Then the samples were marked and left hind and left fore limb were frozen at -37ºC, and tranfersaly cut at several zones (as describe below) with electric saw. The right fore and right hind limb from one of the animals were prepared for ultrasound examination. The preparation for ultrasound examination consists of washing, dehairing and shaving to the level of the skin. The ultrasound examination was performed using the ultrasound device Falcovet (Esaote Pie Medical) and linear probe 6-8 Mhz of general purpose, using B mod, real time ultrasonography. The linear probe has an advantage over the convex because it provides better visualization of tissue closer to probes. The examination procedure, started from the proximal part of the metacarpus/metatarsus on the palmar/plantar site. The three zones marked as (FZ1, FZ2, FZ3), (fig.1a) were used for three standard transfersal positioned probe at fore limbs, and four zones marked as (HZ1,HZ2,HZ3,HZ4), (fig. 1b) were used with analogy at hind limbs. This method divides metacarpus/metatarsus in three/four zones. Zone 1 of the matacarpus (fig 1a) includes the origin of the suspensory ligament and inferior chek ligament and the deep and superficial flexor tendon emerge ring from the carpal sheath. Zone 2 is the middle one third of the metacarpus and includes structures distal to the bifuriction of the suspensory ligament. Zone 3 is the distal one third of the matacarpus from the bifurication of the (SL) to the fetlock joint. The metatarsus is anatomically longer and it is separated in four equal zones (fig 1b). This method is only because of the need for recording the parameters at standard position for further control examination. Ultrasound examination means continuous examination over all parts of tendons, not just at places of described. In this investigation comparing the anatomy and ultrasound image the conclusion about quality, possibilities, analogy, sharpness of ultrasound image were done. With live animals the ultrasound examination were executed on six horses with no clinically obviously lameness, comparing the image between the two same limbs. The ultrasound examination consist of identification of superficial digital flexor tendon (SD), deep digital flexor tendon (DD), inferior chek ligament (ICL), suspensory ligament (SL), bone (Mc3), (fig. 1c). The longitudinal scaning of tendons were performed too in order to determine a direction and structure of collagen fibers.

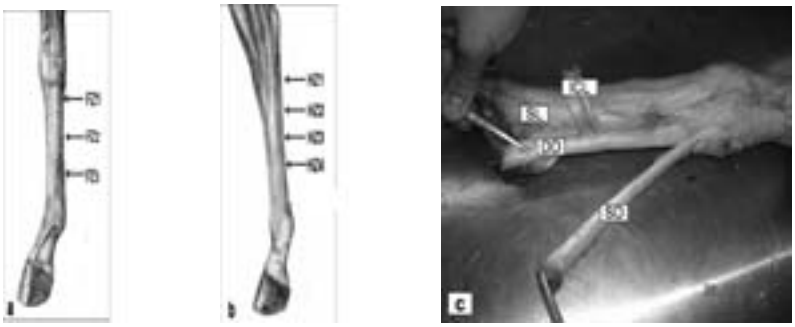

Figure 1. The position of probe during tendon examination at **a** - metacarpus, **b** - metatarsus. **c** - section of fore limb, **SD**-superfitial digital flexor tendon, **DD** - deep digital flexor tendon, **ICL** - inferior chek ligament, **SL**-suspensory ligament.

*Slika 1. Položaj sonde prilikom pregleda tetiva, **a** - na metakarpusu, **b** - na metatarzusu. c - preparat prednje noge, **SD** - površinska sagibačka tetiva, **DD** - duboka sagibačka tetiva, **ICL** - akcesorni ligament duboke sagibačke tetive, **SL** - suspenzorni ligament.*

RESULTS

The *musculus flexor digitorum superficialis* begining from the medial epicondyle of the humerus in the front limb. Near the carpal joint, this muscule becomes hhe **superficial digital flexor tendon (SD)**. The tendons of the SD and deep digitalis flexor tendon (DD) continue distaly through carpal canal. Below the carpus the tendon are positioned subcutanoesly and it's easy to palpate. The SD is ellipsioid shape just below the carpus, and distaly throught zone 1, she become to flattening (fig. 3). The flattening are continuous throught zone 2 (next 8 cm), end transverse section of the tendon are in "comma" shape with sharp edge orientated lateraly. This shape are wraps DD. When SD become the wraping around DD it become difficul or imposibile to "catch" a complete transverse section of SD in one probe position. So its critical to change probe position over the SD. Emerging from the carpal canal in zone 1, the **deep digital flexor tendon** (DD) has a triangular shape on transverse image. The near zone 2 the DD becomes narower and round. In the begining of this zone the DD merges with ICL. ICL has its origin from the palmar side of carpal joint's ligaments and joint capsule. From its begining ICL has s rectangular shape. The ICL has the most echogenic structure compared to the other structures. In zone 2 ICL merge with DD, and DD becomes a largr and rounder than a SD. Through Zone 3 the DD becomes elliptical as its surounded with SD and compressed in to sesamoidean growe. The distaly DD are passing through branches of SD. Distal to this region, a ultrasound examination is difficult to achieve, becouse of problem to adjust a probe in right position, to get a optimal angle of reflection. On the hind limb the DD has its origin from large muscle in regio cruris. The simmilar orientation, shape, and echogenity can be viwed over metatarsal region, but becouse of longitivity of metatarsus this region is divided in four zones. The inferior chek ligament (ICL) is not significant in the hind limb and merges DD in the zone 3.

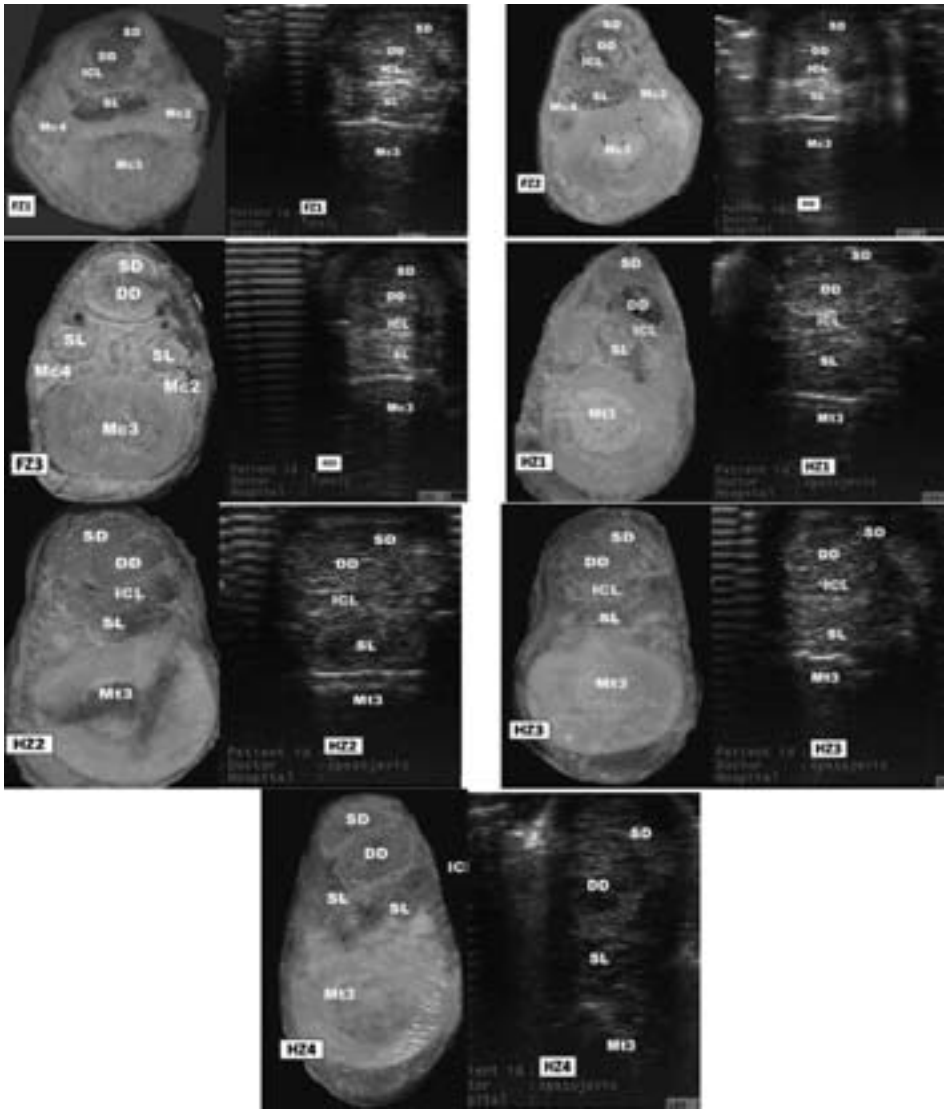

Figure 2. Transverse section and transverse and longitudinal ultrasound image of fore and hind limb. **FZ1, FZ2, FZ3, FZ4** - fore limb zone 1,2,3,4. **HZ1, HZ2,HZ3, HZ4** - hind limb zone 1,2,3,4,5; FL-longitudinal ultrasound image of fore limb, **HL**- longitudinal ultrasound image of hind limb. **SD**-superfitial digital flexor tendon, **DD** - deep digital flexor tendon, **ICL** - inferior chek ligament, **SL**-suspensory ligament, **Mc2,3,4**-metacarpal bone 2,3,4.

Slika 2. Transferzalni i longitudinalni ultrazvučni sken prednje i zadnje noge

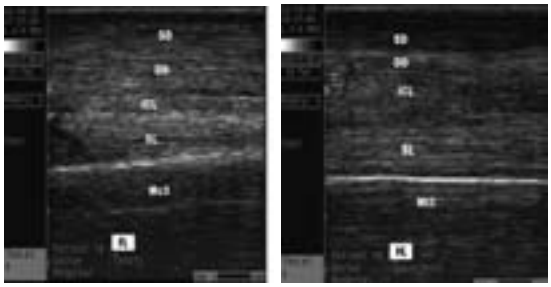

Figure 3. Ultrasound image from the longitudinal scaning. **FL**-longitudinal ultrasound image of fore limb, **HL**- longitudinal ultrasound image of hind limb. **SD**-superfitial digital flexor tendon, **DD** - deep digital flexor tendon, **ICL** - inferior chek ligament, **SL**-suspensory ligament, **Mc3**-metacarpal bone 3, **Mt3** - metatarsal bone 3.
Slika 3. Ultrazvučni longitudinalni sken FL - prednje noge, HL - zadnje noge.

Suspspensory ligament (SD) has its origin from palmar/plantar side of carpal/ tarsal joint. In young foals its exist as *m.interoseus* with three branches. Later this muscular tissue becomes a tendineuous structure, but commonly with the remaining of muscular tissue depending of the age, breed and individual characteristic. The SL is hyperechoic, compared to DD and SD and the least proximal part of ICL. Becouse of remaining muscular fibers in proximal part of SL anechoic or hypoechoic areas may be a function of hypertrophy of the muscle fibers in well trained horses and its commonly bilateral finding. Similar area in the distal portion of SL or branches should be considered as abnormal. In zone 1 SL can be recognized as rectangular shape in transversal section closlly positioned on the surface of Mc/Mt3. In the zone 2 the SL can be more easily recognized becouse of increased quantity of hypoechoic connective tissue separating SD from DD. In the proximal part of zone 3 the SL divides into diverging medial and lateral branches. They are obliquely possitioned to the proximal sesamoid bones. It usaully quit difficult to image both branches in one single ultrasound image. So its nessesary to change position of the transducer. The difficulity can be and to indentificate branches, unless one palpate the branch and place probe while watching a horse leg and not the ultrasound image. Near the branch the ligament become a more triangular as it is nears to proximal sesamoids. The sasamoid contains essentially no musscle tissue and have a homogenous hyperechoic structure. Both branches are equal in the diameter and should be less than 1 cm in the normal adult race horse. The majority part of SL branches has insertion of the palmar/plantar side of the proximal sesamoidean bones, but smal part are passing dorsaly and joint with extensor tendon.

DISCUSSION

Diagnostic ultrasound imaging of the equine soft tissues began after the development of gray-scale real time scanner in the early 1980s. As the equipment become more portable and less expensive, ultrasound imaging of the equine reproductive tract was in widespread use by mid-1980-s. Imaging of the equine tendons and ligaments become more practical with availability of high-frequency near-focused ultrasound transducers and high resolution real-time display system. The field of use of ultrasound later became

ultrasound guided surgical procedures as desmotomy of the inferior chek ligament (accessory ligament of deep digital tendon) (Aziz, 2010).

Echogenicity depends on the amount of reflection and scattering of the ultrasound beam as it crosses the animal's tissues. In tendons, for a given ultrasound frequency and settings of the ultrasound machine, maximal echogenicity is obtained when the collagen fibre bundles are aligned parallel to the tendon axis and perpendicular to the ultrasound beam (Crevier-Denoix et al., 2005).

In this research a shape, *in situ* localization, and echogenity of tendous and ligaments in regio metatasus/metacarpus were assessed. The ultrasound image reveals quit real picture of composition and topography beneath transducer. The suspensory ligament was the most echogenic structure. This also were conclusion by other authors(Wood et al., 1993; Nicoll et al., 1993; Micklethwaite et al., 2001). In the proximal part of SL the echogenity were variable due to different amount of muscular fibers, as remains of *m.interoseus*. This can lead to misdiagnosis, as pronounce of normal findings as stretch or area of inflammation.

The SL is predominantly a strong tendinous band containing variable amounts of muscular tissue and fat (Dyson, 1998). Hence, the variable amount of muscular tissue could contribute to hypoechogenic areas in the proximal part (Dyson, 1998) The SD was the least echogenic which is similar to findings (Woodet al., 1993).

CONCLUSION

The ultrasound examination of horses tendons is usefull tool for visualisation of tendons and their structures. Shape, echogenity, fiber patterns are the basic parameters to make further decision about pathological changes.

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ULTRAZVUČNI PREGLED TETIVA KOD KONJA - UPOREĐIVANJE ULTRAZVUČNOG NALAZA I ANATOMSKE STRUKTURE

BOJAN TOHOLJ, JOVAN SPASOJEVIĆ, MILENKO STEVANČEVIĆ, MILE SAVOVIĆ, IGOR APIĆ, MARKO CINCOVIĆ, STOJANAC NENAD

Izvod

Cilj ovoga istraživanja je bio da se ispita procedura ultrazvučnog pregleda tetiva kod konja u regiji metatakpusa i metatarzusa. Istraživanje je obavljeno pregledom konja kao i koristeći preparate ekstremiteta sa klanice. Anatomski deo studije se sastojao u uočavanju struktura koje su bile predmet interesovanja ultrazvučnog pregleda a to su površinska sagibačka tetiva, duboka sagibačka tetiva, akcesorni ligament duboke sagibačke tetive i suspenzorni ligament. Preparati su zamrzavani na -37ºC a zatim su sečeni u transverzalnoj ravni po tačno određenim zonama koje su deo standardizovane procedure kod ultrazvučnog pregleda tetiva. Ultrazvučni pregled podrazumeva analizu svih pomenutih struktura u svim njihovim delovima, ali je u specifičnim zonama, izvršeno snimanje fotografija ultrazvučnog nalaza. Poređene je vršeno između desne

noge koja je zamrznuta i secirana i leve noge koja je pripremljena za ultrazvučni pregled. Opisivan je oblik, ehogenost i struktura pomenutih tetiva kod konja bez klinički prisutne hromosti.

Ključne reči: konji, tetive, ulrazvučni pregled .

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EMERGENT VEGETATION IN THE MAIN CANALS OF THE HS DTD IN THE REGION OF BAČKA

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SUMMARY: *During many years of phytocoenological research of MCN Hs DTD chanels in Bačka were found stands of emerged vegetation of alliance Phragmition communis W. Koch 1926 emend. Soó 1947. The aim of this study is anayasis of association stands: Scirpo-Phragmitetum W. Koch 1926, Typhetum angustifoliae Pign. 1953, Typhetum latifoliae G. Lang 1973, Sparganietum erecti Roll 1938 and Glycerietum maximae Hueck 1931 which were found in this anthropogenic ecosystem.*

Key words*: MCN Hs DTD, emerged vegetation, alliance Phragmition communis.*

INTRODUCTION

The hydro system Danube-Tisza-Danube (Hs DTD) is a multi-purpose hydro system that regulates the waters in the regions of Bačka and Banat. Its canal network connects 80 urban locations in the two regions, helps their protection against surface waters, allows ground water management and supplies irrigation water for agricultural production, potable water for urban and industrial facilities and fresh water for fishponds. The canal network is also used for commodity transport by water, it takes and removes industrial wastewater and it also serves for recreation, sport and tourism. The total length of the main canals of Hs DTD is 930 km, of which 600 km are navigable. In terms of water table, Hs DTD is divided into 14 sections, eight of which are in the region of Bačka and six in Banat.

In the region of Bačka, the main canal network (MCN) of Hs DTD consists of the following canals: Bezdan-Baja, Vrbas-Bezdan,Kosančić-Mali Stapar, Prigrevica-Bezdan, Odžaci-Sombor, Bečej-Bogojevo, Bački Petrovac-Karavukovo, Novi Sad-Savino Selo and Jegrička canal. Their total length is 421 km.

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The objective of this study was to analyze stands of the associations *Scirpo-Phragmitetum*, *Typhetum angustifoliae*, *Typhetum latifoliae*, *Sparganietum erecti* and *Glycerietum maximae* registered in the MCN of Hs DTD in the region of Bačka.

MATERIAL AND METHODS

The phytocoenological study conducted in the course of the 2003-2006 growing seasons in the MCN of Hs DTD in the region of Bačka was carried out according to the standards of the Swiss-French school (Braun-Blanquet, 1964). The sampling surface size was 10-25 m^2. The syntaxonomic status of the identified communities was analyzed after Soó (1964-1980). Plant species were determined by standard determination keys (Josifović, ed., 1970-1977; Saric, ed., 1986; Tutin et al., 1964-1980; Felföldy, 1990).

RESULTS AND DISCUSSION

Stands of the following associations were found in the course of the several-year phytocoenological study of the MCN of Hs DTD in the region of Bačka: *Scirpo-Phragmitetum*, *Typhetum angustifoliae*, *Typhetum latifoliae*, *Sparganietum erecti* and *Glycerietum maximae* (Lazić, 2006). These associations belong to the emergent vegetation of the alliance *Phragmition communis* W. Koch 1926 emend. Soó 1947, the order *Phragmitetalia* W. Koch 1926 and the class *Phragmitetea* Tx. et Prsg. 1942.

Table 1. Plant communities of the alliance *Phragmition communis* in MCN DTD in Bačka
Tabela 1. Biljne zajednice iz sveze Phragmition communis u OKM Hs DTD u Bačkoj

	1a	1b	2	3	4	5
Characteristic species of the association / *Karakteristične vrste asocijacije*						
Phragmites communis Trin.	V_{3-5}	IV_{1-3}	III_{+-2}	I_{+}	II_{+-1}	II_{+-2}
Schoenoplectus lacuster (L.) Palla	I_{+}	V_{3-5}	I_{+}	I_{2}	I_{+}	
Typha angustifolia L.	III_{+}	I_{1}	V_{3-5}	II_{+-1}	II_{+-1}	I_{+}
Typha latifolia L.		V_{+-1}	II_{+-1}	V_{3-5}	I_{+}	
Sparganium ramosum Huds.	II_{+}				V_{3-4}	
Glyceria maxima (Hartm.) Holm.	III_{+}	III_{+-2}	III_{+-3}	III_{+-3}	I_{+}	V_{3-5}
Characteristic species of the alliances / *Karakteristične vrste sveze*						
Sagittaria sagittifolia L.	I_{+}		I_{+}	I_{+}		
Acorus calamus L.			I_{+}			
Characteristic species of the class / *Karakteristične vrste klase*						
Rumex hydrolapathum Huds.	IV_{+}	I_{1}	IV_{+-1}	II_{+-1}	III_{+}	IV_{+-2}
Lycopus europeus L.	IV_{+}		III_{+-1}	I_{+}	II_{+-1}	
Iris pseudacorus L.	III_{+-1}	I_{2}		I_{1}	II_{+-1}	II_{+-1}
Butomus umbellatus L.	III_{+-2}		II_{+}	I_{+-2}	III_{+-1}	IV_{+-1}
Sium latifolium L.	II_{1-2}		III_{+-1}	II_{+-1}	I_{1}	
Glyceria fluitans (L.) R. Br.	I_{1}	I_{1}				
Typhoides arundinacea L.	I_{+}					
Roripa amphibia (L.) Bess.		I_{+}				
Oenanthe aquatica (L.) Poiret	I_{+}		I_{+}			II_{+}

Stachys palustris L.	I_{+}				II_{+}	II_{+}
Alisma plantago-aquatica L.	I_{+}		I_{+}	I_{+}		
Accessory species / Vrste pratilice						
Calystegia sepium (L.) Br.	III_{+-1}	I_{+}	III_{+}	II_{+}		II_{+}
Bolboschoenus maritimus (L.) Palla		IV_{+-1}	I_{+}			II_{+-2}
Carex pseudocyperus L.	III_{+}		II_{+}	I_{+}		II_{+-1}
Galium palustre L.	III_{+}	I_{+}	II_{+-2}	II_{+-1}		II_{+}
Solanum dulcamara L.	III_{+}		II_{+}	I_{+}		II_{+}
Juncus articulatus L.	I_{2}					I_{+}
Heleocharis palustris (L.) R. Br.		I_{2}	I_{+}			
Epilobium hirsutum L.	I_{1}		I_{1}			
Catabrosa aquatica (L.) P. B.	I_{1}		I_{+}	I_{2}		II_{+}
Juncus compressus Jacq.		I_{1}	I_{+}			
Eupatorium cannabinum L.	II_{+}		I_{+}	II_{+}		
Lythrum salicaria L.	II_{+}					
Polygonum lapathifolium L.	II_{+}		I_{+}	I_{+}		I_{1}
Scutellaria galericulata L.	II_{+}		I_{+}		I_{+}	
Bidens tripartitus L.	I_{+}		III_{+-1}	I_{+}		I_{+}
Angelica sylvestris (Bess.) Hoffm.	I_{+}					
Typha laxmanni Lepechin	I_{+}					
Polygonum hydropiper L.	I_{+}					
Carex vulpina L.		I_{+}	I_{+}	I_{+-1}		II_{+-1}
Mentha aquatica L.	I_{+}		II_{+-1}	I_{+}		II_{+-1}
Solidago serotina Ait.	I_{+}					
Epilobium adnatum Gris.	I_{+}					I_{+}
Urtica dioica L.	I_{+}					
Lysimachia vulgaris L.	I_{+}					
Poa palustris L.	I_{+}			I_{+}		
Ranunculus sceleratus L.	I_{+}			I_{+}		I_{+}
Polygonum amphibium L.		I_{+}			II_{+-2}	
Inula britannica L.			I_{+}			
Cyperus fuscus L.			I_{+}			
Juncus effusus L.			I_{+}			
Verbena officinalis L.			I_{+}			
Lysimachia nummularia L.			I_{+}			II_{+}
Potamogeton fluitans Roth.					II_{+-3}	
Trapa natans L.					II_{1-2}	
Ceratophyllum demersum L.					II_{+-1}	
Hydrocharis morsus-ranae L.					II_{+-1}	
Salvinia natans (L.) Allioni					II_{+}	
Lemna trisulca L.					I_{2}	
Vallisneria spiralis L.					I_{+}	
Potamogeton perfoliatus L.					I_{+}	
Najas marina L.					I_{+}	

Nuphar lutea (L.) Sm.	I_+	
Myriophyllum spicatum L.	I_+	
Xanthium italicum L. Moretti		I_1
Torilis arvensis (Huds.) Link.		I_+
Rumex conglomeratus Murr.		I_+
Juncus gerardi Lois.		I_+

Legend: 1a - *Scirpo-Phragmitetum* subass. *phragmitetosum*; 1b - *Scirpo-Phragmitetum* subass. *schoenoplectetosum lacustris*; 2 - *Typhetum angustifoliae*; 3 - *Typhetum latifoliae*; 4 – *Sparganietum erecti*; 5 - *Glycerietum maximae*

1) Ass. *Scirpo-Phragmitetum* W. Koch 1926 (Tab. 1, col. 1)

The ass. *Scirpo-Phragmitetum* growing in the MCN of Hs DTD in the region of Bačka was found to form two subassociations: subass. *phragmitetosum* Schmale 1939 (Tab. 1, col. 1a) and subass. *schoenoplectetosum lacustris* Soó 1957 (Tab. 1, col. 1b).

Stands of *Scirpo-Phragmitetum* subass. *phragmitetosum* dominated the shallow waters along the banks of all investigated canals in Bačka. In the section from Zmajevo to Žabalj (Jegrička canal) and near the village of Mali Stapar (Kosančić-Mali Stapar canal), these stands spread to the middle of the canals. The flora of the stand was composed of 38 taxa. In addition to the dominant species *Phragmites communis*, the following species were found in large numbers and percentage cover: *Butomus umbellatus*, *Glyceria maxima*, *Typha angustifolia*, *Rumex hydrolapathum*, *Lycopus europaeus*, *Calystegia sepium*, *Iris pseudacorus*, *Carex pseudocyperus*, *Sium latifolium*.

Stands of *Scirpo-Phragmitetum* subass. *schoenoplectetosum lacustris* were formed at sites with deeper water than it was the case with the previously discussed subassociation, because the codominant species *Schoenoplectus lacuster* requires high moisture throughout the vegetation period. These stands were found only in Jegrička canal (near the village of Sirig and in Žabalj fishpond) where they were interspersed with stands of subass. *phragmitetosum*. The stands were floristically poor (16 taxa), with *Phragmites communis* and *Schoenoplectus lacuster* occurring in increased numbers. *Glyceria maxima*, *Typha latifolia* and *Bolboschoenus maritimus* were other important members of this biocoenosis.

2) Ass. *Typhetum angustifoliae* Pign. 1953 (Tab, 1, col. 2)

Stands of this phytocoenosis, usually small in size, grew in shallow water, up to 0.5 m deep, near canal banks. They were typically interspersed with stands of ass. *Scirpo-Phragmitetum*, less frequently with stands of ass. *Typhetum latifoliae* and ass. *Glycerietum maximae*. Towards the middle of the canals, these stands neighbored stands of the floating associations *Hydrocharidetum morsus-ranae, Nymphaeetum albo-luteae,* and *Lemno-Spirodeletum* and stands of the submerged associations *Elodeetum canadensis* and *Ceratophyllo demersi-Vallisnerietum spiralis* (Džigurski et al., 2009).

The floristic composition of the association included 33 taxa, with the percentage cover generally ranging between 90-100%. The dominant species *Typha angustifolia* was found in large numbers and high percentage cover in all analyzed stands. The characteristic species of the class *Phragmitetea*, *Rumex hydrolapathum*, *Glyceria maxima*, *Phragmites communis*, *Lycopus europeus* and *Sium latifolium*, were present in increased numbers. The species *Typha angustifolia* and *Rumex hydrolapathum* formed the characteristic group of plants.

3) Ass. *Typhetum latifoliae* G. Lang 1973 (Tab. 1, col 3)

Hygrophilous stands of ass. *Typhetum latifoliae* were registered in shallow waters (0.2 to 0.5 m deep) of the studied ecosystem as well as on canal banks. These stands had a limited distribution, forming islands or belts that neighbored stands of ass. *Scirpo-Phragmitetum*. In the parts where they bordered the vegetation-free water surface, these associations were in contact with floating stands of ass. *Hydrocharidetum morsus- ranae*, ass. *Nymphaeetum albo-luteae*, ass. *Trapetum natantis*, ass. *Salvinia-Spirodeletum polyrrhizae* and ass. *Lemno-Spirodeletum* as well as with submerged stands of ass. *Elodeetum canadensis*.

The presence of 24 taxa indicated that the analyzed association was floristically poor. The total percentage cover was 90-100%. The dominant species *Typha latifolia* gave the physiognomic mark to these stands. *Sagittaria sagittifolia,* the characteristic species of the alliance, was registered in only one location and even there its numbers and percentage cover were low. Among the characteristic species of the class, the following species were distinguished for the presence, numbers and percentage cover: *Glyceria maxima, Rumex hydrolapathum, Sium latifolium* and *Typha angustifolia*. The characteristic group included only *Typha latifolia*, the dominant species of the community. The analyzed stands were heterogeneous with regard to their floristic structure.

4) Ass. *Sparganietum erecti* Roll 1938 (Tab. 1, col. 4)

Stand of the community *Sparganietum erecti* grew in the form of islands in water up to 1 m deep. A characteristic feature of the sites occupied by this phytocoenosis was significant fluctuations of water level (Stančić, 2007). In the ecological sequence towards the deeper water, the stands of this association bordered with floating stands of ass. *Hydrocharidetum morsus-ranae* and *ass. Nymphaeetum albo-luteae*. Towards the bank, this association bordered with emersed stands of ass. *Scirpo-Phragmitetum* and ass. *Typhetum angustifoliae*. In the northern part of Jegrička canal, stands of ass. *Sparganietum erecti* covered in some places the entire width of the canal (Lazić et al., 2005).

The floristic composition of the community included 25 plant species, whose total percentage cover was 80-100%. The coverage of the community was due to large numbers and extensive spread of the dominant species of the community, *Sparganium ramosum*. Among the characteristic species of the class, the following species were distinguished for their presence, numbers and percentage cover: *Butomus umbellatus*, *Rumex hydrolapathum*, *Typha angustifolia*, *Iris pseudacorus*, *Phragmites communis*, *Stachys palustris* and *Lycopus europaeus*. As the stands of this phytocoenosis grew in the zone that was continuously covered with water, the number of accompanying plants was small and all of them were hydrophytes.

5) Ass. *Glycerietum maximae* Hueck 1931 (Tab. 1, col. 5)

Stands of this phytocoenosis were tolerant to large changes in water level and they grew at sites rich in organic matter. The dominant species, *Glyceria maxima*, formed very dense stands in which few other plants were able to survive (*Rumex hydrolapathum*, *Stachys palustris*, *Iris pseudacorus*, etc.). Stands of this phytocoenosis formed small or large islands, which bordered with stands of ass. *Scirpo-Phragmitetum* and ass. *Typhetum latifoliae*. Towards the middle of the canals, these stands were in contact with floating stands of ass. *Nymphaeetum albo-luteae* and ass. *Lemno-Spirodeletum* and submerged stands of ass. *Ceratophyllo demersi-Vallisnerietum spiralis* (Džigurski et al., 2009).

The floristic composition of the community *Glycerietum maximae* included 26 plant species. Most of these stands were floristically poor. Their percentage cover that ranged from 80 to 100% was due to the numbers and cover of *Glyceria maxima*. The characteristic

species of the class were: *Rumex hydrolapathum*, *Butomus umbellatus*, *Phragmites communis*, *Iris pseudacorus*, *Oenanthe aquatica*, *Stachys palustris* and *Typha angustifolia*.

CONCLUSION

In the MCN of Hs DTD in the region of Bačka, the stands of the emergent vegetation of the alliance *Phragmition communis* were found to consist of the associations *Scirpo-Phragmitetum*, *Typhetum angustifoliae*, *Typhetum latifoliae*, *Sparganietum erecti* and *Glycerietum maximae*. These associations form belts or islands in shallow waters along the banks of the canals. In the locations of Zmajevo and Žabalj (Jegrička canal) and Mali Stapar (Kosančić-Mali Stapar canal), stands of the above vegetation tended to spread to the middle of the canals.

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EMERZNA VEGETACIJA OKM HS DTD U BAČKOJ

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BRANKA LJEVNAIĆ-MAŠIĆ

Izvod

Višegodišnjim fitocenološkim istraživanjima kanala OKM Hs DTD na području Bačke konstatovane su sastojine emerzne vegetacije sveze *Phragmition communis* W. Koch 1926 emend. Soó 1947. Cilj rada je analiza sastojina asocijacija: *Scirpo-Phragmitetum* W. Koch 1926, *Typhetum angustifoliae* Pign. 1953, *Typhetum latifoliae* G. Lang 1973, *Sparganietum erecti* Roll 1938 i *Glycerietum maximae* Hueck 1931 konstatovanih u ovom ekosistemu.

Ključne reči: OKM Hs DTD, emerzna vegetacija, *Phragmition communis.*

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THE USE OF AZOTOBACTER IN MAIZE PRODUCTION: THE EFFECT ON MICROBIOLOGICAL ACTIVITY OF SOIL, EARLY PLANT GROWTH AND GRAIN YIELD*

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SUMMARY. The experiment focused on examining the effect of a mixture of azotobacter strains on the early growth of maize, grain yield and microbiological activity in rhizospheric soil of three maize hybrids: Tisa, NS5010 and NS444 ultra. Before seeding, the mixture of azotobacter strains was applied to one half of the experimental plot.The use of azotobacter led to an increase in the number of microorganisms. The mixture of azotobacter strains stimulated the early growth of maize to a great extent. With Tisa hybrid, the plants were up to 5 cm higher than the control plants and with NS444 ultra, the plants were up to 19,63 cm taller than the control plants. The grain yield in the variants with azotobacter increased with all three hybrids. The highest increase in yield was in NS5010 hybrid: 641 kg ha^{-1}, in Tisa 403 kg ha^{-1} and in NS444 ultra, the grain yield increased by 396 kg ha^{-1}.

***Key words**: azotobacter, growth of maize, microorganisms, grain yield.*

INTRODUCTION

Thanks to microorganisms in soil, synthesis and transformation of organic matter take place all the time. In this way a part of necesarry nutrients for plants is supplied. In the nutrition of all crops, including maize, nitrogen has an important role. Many investigations have shown that certain amounts of mineral nitrogen can be replaced when nitrogen-fixing bacteria are used (Okon and Itzigson,1995; Govedarica et al., 2001). In the production of field crops and vegetables, azotobacter is the one which is most frequently applied (Jarak et al., 2008; Mrkovački et al., 2006).

The aim of the experiment was to examine the effect of azotobacter on the microbiological activity of rhizospheric soil, early growth of maize and grain yield.

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MATERIAL AND METHODS

Three maize hybrids were used in the experiment: Tisa (FAO 700), NS 5010 (FAO 500) and NS 444 ultra (FAO 400, selection of the Institute of Field and Vegetable Crops, Novi Sad). In autumn, before primary soil treatment, 300kg/ha of the mineral fertilizer NPK (15:15:15) was applied. The type of soil was chernozem soil, in the locality of Futog.

Before seeding, a mixture of azotobacter strains was introduced into one half of the experimental plot. One litre of the inoculum with the cell density of 10^9 in 1ml was dilluted in 300 l of water and sprayed into the soil. After this, the upper layer of soil was additionally prepared for planting with a cultivator so that microorganisms would incorporate into the soil as well as possible. In the growth phase of 6-8 leaves, the length of the plant was measured and microbiological analysis of the rhizospheric soil conducted. The total number of microorganisms was determined by Poshon method, the number of fungi was measured on potato-dextrose medium and the number of azotobacter was determined by Fjodorov method (Jarak and Đurić, 2006). After harvesting, the grain yield (with 14% grain humidity) was measured in t/ha. The data were processed by analysis of variance and the significance was expressed by LSD test.

RESULTS AND DISCUSSION

The hybrids which were examined in this experiment have a high genetic fertility potential and are frequently used in production. In order for the hybrids to realize this potential, it is necessary to provide them with adequate nutrition during the growth period. One part of nutrients is created during microbiological processes in soil. The activity of microorganisms depends on soil characteristics, plants, agrotechnical measures etc.

These investigations were conducted in chernozem soil which has optimum conditions for microbiological processes (Belić et al, 2005; Đurić et al., 2008). Microbiological processes can additionally be stimulated by introducing biofertilizers. These microorganisms reproduce in soil and with their enzymatic activity raise and keep the appropriate level of organic matter in soil (Hajnal et al., 2005; Jarak et al., 2009). In this experiment, in the growth phase of 6-8 leaves, the number of microorganisms was larger when the mixture of *Azotobacter chroococcum* strains was applied than in the control (Table 1).

Table 1. The effect of Azotobacter chroococcum (AZ) on the total number of microorganisms (TN), number of fungi (F) and number of azotobacter (AZ) in rhizospheric soil of maize
Tabela 1. Uticaj Azotobacter chroococcum (AZ) na ukupan broj mikroorganizama (TN), broj gljiva (F) I broj azotobaktera (AZ) u rizosfernom zemljištu pod kukuruzom

Hybrids *Hibridi* (A)	T N (10^6 g^{-1} apsolutly dry soil) *(10^6 g^{-1}apsolutno suvog zemljišta)*		F (10^4 g^{-1} apsolutly dry soil) *(10^4 g^{-1}apsolutno suvog zemljišta)*		AZ *(10^2 g^{-1} apsolutly dry soil) (10^2 g^{-1}apsolutno suvog zemljišta)*	
	Control *Kontrola* (B)	AZ (B)	Control *Kontrola* (B)	AZ (B)	Control *Kontrola* (B)	AZ (B)
NS 5010	5.3	252.5	2.5	2.9	29.5	44.7
NS 444 ultra	11.9	85.5	1.8	3.0	40.5	79.7
Tisa	9.3	23.4	1.4	2.2	42.2	60.1
Average Prosek	8.86	120.52	1.96	2.70	37.4	61.5
A B AxB	LSD 0.05 0.01 26.12 45.50 31.99 37.15 45.24 64.35		LSD 0.05 0.01 1.53 2.17 1.25 1.78 2.16 3.07		LSD 0.05 0.01 7.17 10.20 5.85 8.34 10.14 14.43	

The number of the examined microorganisms was also dependant upon the maize hybrid (Table 1). Namely, plants secrete through the root different organic and mineral substances which are used by rhizospheric microorganisms for nutrition. Even though the plant is the same, the number of microorganisms in rhizospheric soil also depends on hybrids and varieties within the same sort (Walker et al., 2003). A large number of rhizospheric microorganisms, including azotobacter, produce growth substances such as auxins, gibberellins etc.

The use of these microorganisms stimulates the early growth and development of a plant (Jarak et al., 2007; Udovički and Jarak, 2005). In this experiment, the mixture of *Azotobacter chroococcum* strains stimulated the early growth of maize to a great extent. With Tisa hybrid, the plants were up to 5 cm higher than the control plants. With NS444 ultra, the plants were up to 19.63 cm and with NS 5010 were up 16. 33 cm higher than the control plants (P>0.01) (Table 2).

Table 2. The effect of Azotobacter chroococcum on the hight of plant, phase 6-8 leaves
Tabela 2. Uticaj Azotobacter chroococcum na visinu biljke u fazi 6-8 listova

Hybrids *Hibridi* (A)	Hight of plant (cm) / *Visina biljke (cm)*		
	Control *Kontrola* (B)	*A.chroococcum* (B)	Difference between treatment and control *Razlika između tretmana i kontrole*
NS 5010	51.67	68.00	16.33
NS 444 ultra	57.67	77.33	19.63
Tisa	51.83	57.00	5.17
Average / Prosek	53,72	67,44	13,69
LSD A B AxB 0.05 4.53 3.70 6.41 0.01 6.44 5.26 9.11			

In comparison with the control, there was a statistically significant increase of the grain yield in all three hybrids (P>0.05) when azotobacter was used. With NS5010 hybrid, the increase was statistically highly significant (P>0.01). The grain yield was higher by about half a tons per hectare in comparison with the control (Table 3).

Table 3. The effect of *Azotobacter chroococcum* on the yield of seed of maize
Tabela 3. Uticaj Azotobacter chroococcum na prinos zrna kukuruza

Hybrids / *Hibridi* (A)	Yield (t ha^{-1}) / *Prinos (t ha^{-1})*		
	Control - Kontrola (B)	*A. chroococcum* (B)	Difference between treatment and control *Razlika između tretmana i kontrole*
Tisa	9.496	9.889	0.403
NS 5010	9.008	9.649	0.641
NS 444 ultra	9.898	10.294	0.396
Average / *Prosek*	9.467	9.959	0.492
LSD A B 0.05 0.484 0.395 0.01 0.669 0.546			

According to results of Govedarica et al. (2004), with introduction of azotobacter, biological activity in soil increases, and yield of maize depends on hybrids and strains azotobacter.

The increase in yield can be due to the influence of *Azotobacter chroococcum* which fixes up to 90 kg N ha^{-1} a year which increases the nitrogen pool and biological activity of soil (Hajnal et al., 2004; Jeličić et al., 2008).

CONCLUSION

The value of the investigated parameters was dependant on inoculation, hybrids and their interaction.

The use of *Azotobacter chroococcum* led to an increase in the number of microorganisms.

Azotobacter chroococcum stimulated the early plant growth. The increase in height in the inoculated variants ranged from 5 cm in Tisa hybrid to 20 cm in NS444 ultra hybrid. The use of *Azotobacter chroococcum* led to a higher grain yield in all three maize hybrids. The highest increase in yield was in NS5010 hybrid: 641 kg ha^{-1}, in Tisa 403 kg ha^{-1} and in NS444 ultra, the grain yield increased by 396 kg ha^{-1}.

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PRIMENA AZOTOBAKTERA U PROIZVODNJI KUKURUZA: UTICAJ NA MIKROBIOLOŠKU AKTIVNOST U ZEMLJIŠTU, POČETNI RAST BILJKE I PRINOS ZRNA

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Izvod

Ispitivan je uticaj smeše sojeva azotobaktera na početni porast biljke, prinos zrna i mikrobiološku aktivnost u rizosfernom zemljištu kod tri hirida kukuruza: Tisa, NS 5010 i NS 444 ultra. Pre setve na polovini ogledne površine uneta je smeša sojeva *Azotobacter chroococcum* Jedan litar inokuluma gustine ćelija 10^9 u ml, razblažen je u 300 l vode i prskalicom je unet u zemljište. Nakon toga je površinski sloj zemljišta dodatno pripremljen frezom kako bi se uneti mikroorganimi što bolje izmešali sa zemljištem. U fazi porasta 6-8 listova izmerena je dužina biljke i urađena mikrobiološka analiza rizosfernog zemljišta i to ukupan broj mikroorganizama, broj gljiva i broj azotobaktera. Nakon skidanja useva izračunat je prinos zrna u t/ha sa 14 % vlage. Visina biljaka u fazi 5-7 listova na inokulisanim varijantama bila je povećana od 5 d0 20 cm. Prinos zrna kod sva tri hibrida kukuruza bio je povećan na inokulisanim varijantama. Povećanje prinosa je bilo statistički značajno za P>0.05 i kod hibrida NS 5010 bilo je 641 kg ha^{-1}, kod hibrida Tisa 403 kg ha^{-1} i kod hibrida NS 444 396 kg ha^{-1}.

Ključne reči: kukuruz, azotobakter, mikroorganizmi, visina biljke, prinos.

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REPRODUCTIVE EFFICIENCY IN HIGH-MILKING DAIRY COWS AFTER CALVING*

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SUMMARY: The aim of this work was to determine incidence of calving and postcalving reproduction disorders and their effect on the major parameters of cows reproductive efficiency at dairy farm in the R. Serbia. Total number of 1835 high-milking dairy cows (av. milk production >8500L/305d of lactation) records has been reviewed during one year period, at the large dairy farm in area of Vojvodina (R. Serbia). Normal calving and puerperal period without health problems was observed at 583 cows (27,6%), while different abnormalities during and after calving were recorded in 1252 cows (72,4%). The highest percentages of registered peripartal disorders were dystocia and endometritis (62% and 64%, respectively), affecting almost the same percentage of cows. Peripartal disorders had statistically significant influence (P<0.05) on the increase of number of services/conception (3,5 vs. 2,7, reproductive disorders vs. healthy cows, respectively), prolongation of the interval from first postpartal insemination till conception (110 vs. 57 days, reproductive disorders vs. healthy cows, respectively), and increase of days open period (214 vs. 146 days, reproductive disorders vs. healthy cows, respectively). This results have confirmed increased incidence of reproduction disorders during calving and postcalving period that significantly reduces reproductive efficiency in high milking dairy cows after parturition.

***Key words**: reproduction disorders, reproduction efficiency, dairy cows.*

INTRODUCTION

Modern dairy farming with continuous pressure for the increase of milk production is inevitably linked to the dairy cows reproductive efficiency. One of the major

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parameters of reproductive efficiency in dairy cows is calving interval. Maximal milk production and continuous calving in the high-milking dairy farms is achieved if the average calving interval is between 12-13 months (Stevenson and Britt, 1977). However, in the field conditions calving interval is often longer than 14 months. Since pregnancy duration is biologically constant, calving interval has been determined by the period between calving and first successful artificial insemination (AI), namely service period (SP). Servuce period is under the influence of many paragenetic factors like parity, season, housing and nutrition, contact with fertile bulls, as well as dairy cows health status, with special consideration of reproductive tract condition (Petrović, 1976; Stančić, 1989; Stančić and Košarčić, 2007). From the reproduction management view SP period is directly determined by the duration of interval between calving and first ovulatory estrus, and period between very first and first successful insemination. In healthy dairy cows first postpartal ovulation usually occurs between 15-30 days after calving, but unfortunately about 70% of these ovulations are „silent", ie. without manifested signs of estrus. However, from practical standpoint it could be important to detect such ovulations, since it could ease the observation of the next estrous cycle with estrus detection and increase chances for successful insemination. If our goal in dairy farming is to have optimal 12 months calving interval it is necessary to achieve successful conception within maximally 90 days post partum (Bousquet et al., 2000; Crowe, 2008).

Manny researchers during the last decades indicated permanent decline of modern high-milking dairy cows reproductive efficiency (Roche, 2000; Lucy, 2001; Dobson et al., 2007). Decrease of reproductive efficiency is manifested as prolonged postpartal anoestrus (Thacher et al., 2006), increase number of cows in silent estrus with irregular estrous cycle duration, resulting from short luteal phase in the first few postpartal cycles (Darwash et al., 1997), decline in the first insemination conception rate (CR) (Lucy, 2001), as well as an increase number of cows with abnormal early embryonic development and various forms of uterine diseases, increasing embryonic and fetal mortality rate (Diskin, 1987; Fourchon et al., 2000; Bouchard i Du Tremblay, 2003; Lucy, 2007). The final result is decrease in the reproductive efficiency with an increase of number of inseminations needed for successful conception (Sheldon i Dobson, 2003). It was reported that successful CR after the first insemination decreased between 1990-2000 in most European countries from 55% to 45% (Bousquet et al., 2004). This situation had a direct influence on the increase of number of inseminations and Lucy (2001) reported that 20 years before approximately 1,75 inseminations were needed for successful conception while in the last few years it has increased to more than 3. This is important parameter of reproduction efficiency since it is inversely proportional to the reproduction efficiency and directly proportional to DO period (Esslemont et al., 2000).

In the modern high-milking dairy farms milk production is usually between 8000-10000 kg milk/cow/lactation, and it is the result of intensive selection of cows for high milk production, as well as constant improvements in the nutrition and dairy farms management. Decline in the reproductive efficiency is inversely linked to the increase of milk production, and directly connected to the negative effect of different stressors originating in inadequate housing, environmental conditions, lack of contact with fertile bulls etc. (Rodriguez-Martinez et al., 2008). However, there is a general agreement that peripartal diseases are more important factor that causes decline in reproductive efficiency than high-milk production (Lucy, 2001). Aim of this work was to investigate incidence of calving and postcalving reproduction disorders and their effect on

reproductive efficiency at one high-milking dairy cows farm in the region of Vojvodina (Republic of Serbia).

MATERIAL AND METHODS

Total number of 1835 Holstein-Friesian dairy cows health records was examined at the large high-milking dairy cows farm in the region of Vojvodina (Republic of Serbia). All experimental animals health records were analyzed from one calving to the next calving and animals were divided in two groups according to the following criteria: a) animals without reproduction disorders (n=583), and b) animals with reproduction disorders (1252). The two groups of reproduction disorders were investigated: 1) health problems during calving (dead calves, dystocia, assisted calving, other) and 2) health problems after calving (placental retention, endometritis, parametritis, perimetritis, piometra, ovarian cysts, nonfunctional ovaries, other).

About 20 days after calving all investigated animals were monitored for signs of estrus during milking and feeding time. All investigated cows were artificially inseminated (AI) once or twice during the manifested estrus detected after day 40 postcalving. Pregnancy diagnosis was confirmed using rectal palpation at 45-60 days after insemination. Following reproduction parameters were determined: (1) interval between calving and the first recorded estrus, (2) interval between calving and first insemination, (3) interval between calving and successful insemination (service period, SP), (4) number of AI needed for successful conception, and (5) interval between the first and second AI.

Statistical significance of differences between means was determined using Student t-test at the level of $P<0.05$.

RESULTS AND DISSCUSSION

From the total number of 1835 high-milking dairy cows calving and postpartal health disorders were recorded in 1252 dairy cows (72,4%), while 583 (27,6%) dairy cows were healthy. Relative numbers of high-milking dairy cows with reproduction disorders are presented in the Figure 1. The most frequent reproduction disorders after and during calving were endometritis (64%) and dystocia (62%).

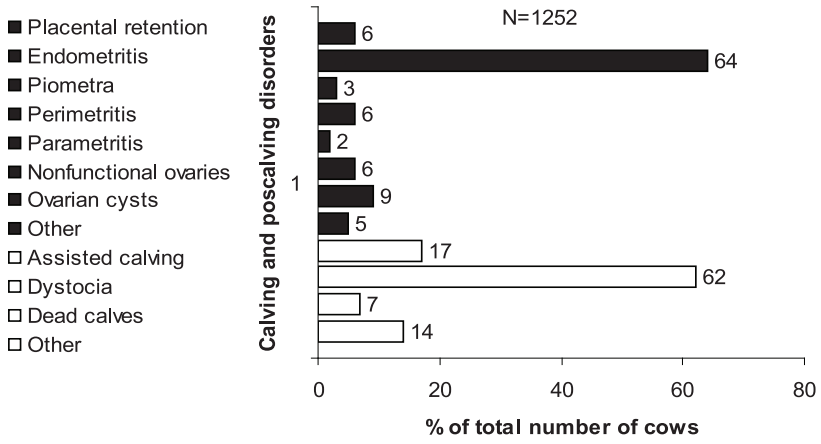

Figure 1. Relative number of high-milking dairy cows with reproduction disorders during and after calving

Relative number of dairy cows (%) with external signs of estrus detected during 45 days after calving was not significantly different between group of cows with and without reproductive disorders (Figure 2). However, it is clear that almost 50% of cows with postcalving reproduction disorders had extremely long interval between calving and the first estrus (49% of cows with postcalving disorders had interval ≥92 days). It is interesting that 38% of healthy dairy cows and similar percentage of cows with calving disorders (40%) also had very long interval between calving and first observed estrus (Figure 2).

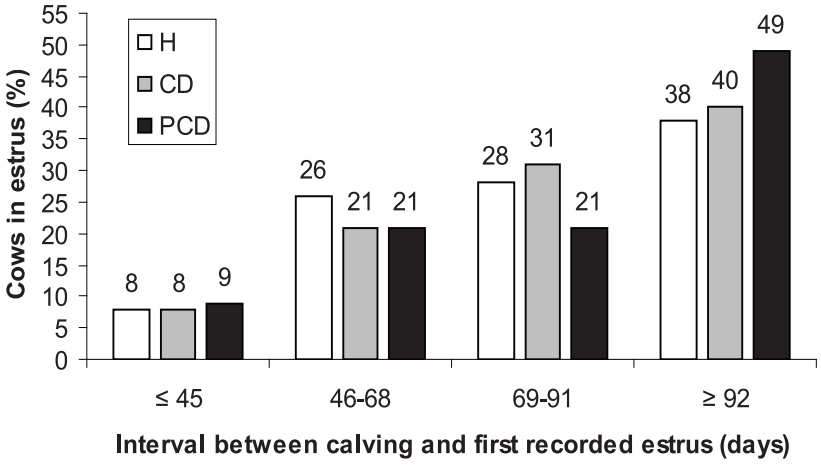

Figure 2. Relative number of dairy cows and the interval from calving to the first recorded estrus; H- healthy cows, CD – calving disorders, PCD – postcalving disorders

High incidence of postcalving disorders significantly prolongs the interval between calving and first insemination. The duration between calving and first insemination is longest in dairy cows with ovarian cysts (120 days), with similar interval in cows

with endometritis (116 days). The data about interval between calving and first insemination relative to the calving and postcalving reproduction disorders are presented in the Figure 3.

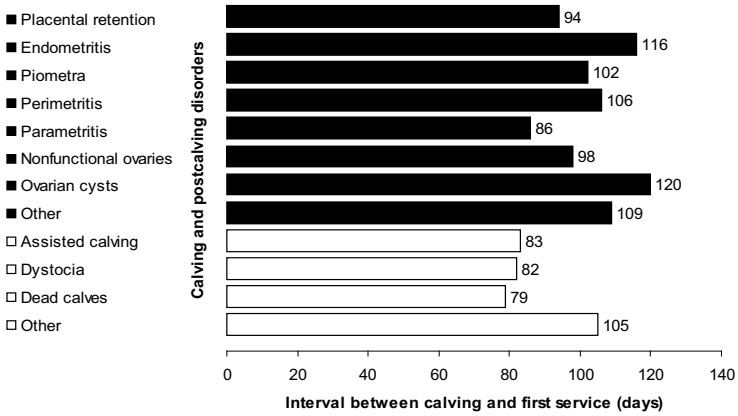

Figure 3. Interval between calving and first AI in high-milking dairy cows (days)

The effect of calving and poscalving reproductive disorders on the number of AI needed for successful insemination (insemination index), relative to the calwing parity is presented in the Table 1. The average insemination index value for healthy and dairy cows with reproductive disorders was significantly different (2.7:3.5, respectively, $P<0.05$). The average insemination index value was not different between first lactation healthy dairy cows and older healthy dairy cows (2.8 : 2.7, respectively, $P>0.05$), and it was significantly higher in first lactation and older dairy cows with reproduction disorders compared to the healthy cows (3.7 and 3.4, respectively).

Table 1. The effect of calving and poscalving reproductive disorders at number of AI per successful conception, relative to the calwing parity
Tabela 1. Uticaj reproduktivnih poremećaja tokom i posle tepenja na broj VO po uspešnoj koncepciju, u zavisnosti od pariteta telenja

			1st calwing cows *Prvotelke*		≥2 calwing cows *Krave sa ≥ 2 telenja*		Total	
			H	CD+PCD	H	CD+PCD	H	CD+PCD
Conception after *Vrednost koncepcije*	1.AI/*VO*	n	27	22	85	34	112	56
		%	15,7	6,7	20,8	3,7	19,2	4,5
	2.AI/*VO*	n	44	36	98	150	142	186
		%	25,4	11,0	23,9	16,2	24,4	14,8
	3.AI/*VO*	n	39	66	69	147	108	213
		%	22,5	20,2	16,8	15,9	18,5	17,0
	≥4.AI/*VO*	n	63	203	158	594	221	797
		%	36,4	62,1	38,5	64,2	37,9	63,7
	Total *Ukupno*	n	173	327	410	925	583	1.252
		%	100,0	100,0	100,0	100,0	100,0	100,0

Average No. of service per conception *Prosečan br. VO po koncepciji*	2,8[a]	3,7[b]	2,7[a]	3,4[b]	2,7[a]	3,5[b]

H – healthy cows / *Zdrave krave*, CD – cows with calving disorders / *Krave sa poremećajima tokom telenja*,
PCD – cows with postcalving disorders / *Krave sa poremećajima posle telenja.*
[a,b]Values in the same row not shearing the same superscript are significantly different (P<0,05).
[a,b]*Vrednosti sa različitim superskriptima, u istom redu, se statistički značajno razlikuju (P<0,05).*

The average interval between the very first and successful AI in first lactation (H, 1st parity) and older healthy (H, ≥2 parity) dairy cows was not significantly different (57:46 days, respectively, P>0.05). This interval was significantly longer (P<0.05) in FL and Old dairy cows with calving and postcalving disorders (93, 106, 110, and 102 days, respectively). Similar relationship exists regarding average servis period that was significantly shorter (P<0.05) in FLH and OldH dairy cows (146 and 137 days, respectively), compared to the same categories of dairy cows with calving and postcalving reproductive disorders (202, 214, 188 and 186 days, respectively). There were no significant differences between two groups of dairy cows with reproductiove disorders (calving and postcalving) regarding the interval between calving and first AI and servis period (Table 2 and Figure 4).

Table 2. Interval between the first ans successful AI and the service period duration
Tabela 2. Interval između prvog i uspešnog VO i trajanje servis perioda

	1st calwing cows *Prvotelke*			≥2 calwing cows *Krave sa ≥ 2 telenja*		
	H	CD	PCD	H	CD	PCD
Aver. interval between the first AI and successful AI (days) *Pros. interval od prvog VO do uspešnog VO (dani)*	57[a]	93 [b]	106[b]	46[a]	110[b]	102[b]
Aver. service period duration (days)* *Pros. trajanje servis perioda (dani)**	146[b]	202[a]	214[a]	137[b]	188[a]	186[a]

H – healthy cows / *Zdrave krave*, CD – cows with calving disorders / *Krave sa poremećajima tokom telenja*,
PCD – cows with postcalving disorders / *Krave sa poremećajima posle telenja.*
[a,b]Values in the same row not shearing the same superscript are significantly different (P<0,05).
[a,b]*Vrednosti sa različitim superskriptima, u istom redu, se statistički značajno razlikuju (P<0,05).*
*Interval from the calwing to successful AI / Interval od telenja do uspešne koncepcije

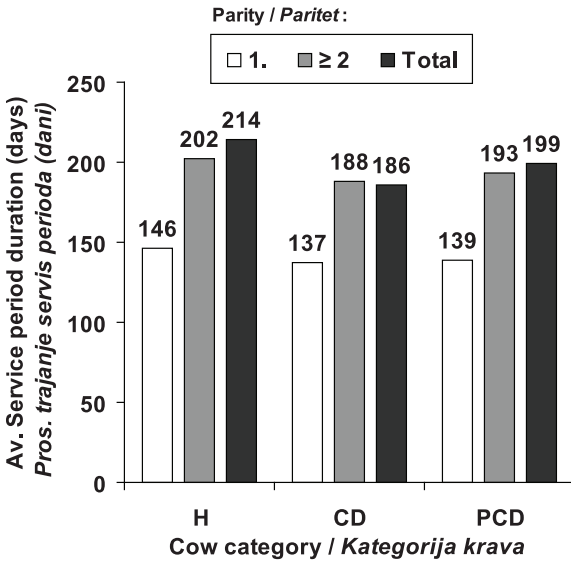

Figure 4. Service period duration in different dairy cows categories
(H – healthy cows, CD – calving disorders, PCD – postcalving disorders)

One of the major parameter of reproduction efficiency is calving interval. Manny authors have indicated that 12-13 months calving interval enables maximal milk and calves production (Stančić, 1989; Walker, 1997; Wattiaux, 2006). Calving interval is determined by the two factors: (1) gestation duration and (2) service period (SP) duration (interval between calving and successful conception). Since gestation duration is biologically constant, the calving interval is influenced by the factors determining servic period duration. Prolongation of the SP is the result of long interval between calving and first estrus, as well as the interval between the first AI and successful AI. Both intervals could be extended because of the numerous non infective and infective etiologic factors (Gordon, 1997; Stančić and Košarčić, 2007).

Our research results indicate that dystocia and assisted calving are the most frequent calving disorders (79% of all calving disorders). The potential causes for this situation could be poor body condition of cows or increased newborn calves body weight. After calving the most frequent reproductive organs disorders were inflammatory processes affecting uterus (endometritis, piometra, perimetritis), with about 75% affected dairy cows. However, inflammatory diseases are direct consequence of complicating calving or postcalving disorders. Between 30-50% of dairy cows could be affected with some kind of calving and postcalving disorder resulting in the decrease of their reproductive efficiency or culling (Fourchon et al., 2000; Grögn and Rajala-Schultz, 2000; Beever, 2004; Dobson et al., 2007).

Our data indicate that average interval between calving and the first registered estrus was 95, 93 and 97 days in H, CD and PCD dairy cows, respectively. This unfortunate finding is the result of small number of dairy cows from all three groups with estrus detected within 68 days post partum (36%, 29% and 30% in H, CD and PCD groups of cows). This was the reason that about 2/3 of cows could not be successfully inseminated within 90 days post partum. In a good managed dairy herds the first post partal

estrus should be detected within 60 days after calving in 90% of dairy cows (Walker, 1997). Estrus detection procedure in our dairy farm is based on checking of estrus signs manifestation in dairy cows, twice daily (during morning and evening milking time), that is probably inadequate, preventing better reproduction efficiency. This estrus detection practice is not able to detection animals in „silent" heat and ovulation, where estrus is not followed with manifest outer signs of sexual receptivity. However, first post partal estrus is without manifest clinical signs in about 70% of dairy cows, declining to 50% during second post partal estrous cycle (Peters et al., 1987; Stančić, 1989; Stančić, 1995; Crowe, 2008). In healthy dairy cows cyclic ovarian activity and ovulation should be established 2-4 weeks after calving (First, 1979), and between 80-90% of dairy cows normally should become cyclic within 30 days after calving (Lamming et al., 1981; Stančić, 1995; Crowe, 2008). Combining careful detection of manifest signs of estrus with rectal palpation it is possible to register first postpartal estrus in more than 90% of dairy cows with initiated cyclic ovarian activity (Petrović, 1976).

Peripartal disorders significantly extended interval between calving and first AI in our research results (between 79 and 120 days, depending on the disorders type). Standard concerning this interval in good dairy herds is between 45-60 days. If the interval between calving and first AI is longer than 60 days there are serious reproduction problems in those herds, and detailed management analysis is needed in order to identify the problems and suggests appropriate corrective measures (Walker, 1997; Stančić, 1989; Petrujkić et al., 1993; Kasimanickam et al., 2002). The decline of first AI conception rate has been reported many times during last 50 years. Data from numerous countries (England, Holland, Canada, USA, Spain, Ireland) indicate that first AI conception rate was about 65% in mid 20th century and decreased between 10-15% during the last few decades. Authors from USA suggested different reasons for this decline, starting from the extended use of deep frozen bulls semen, enlargement of dairy herds, significant increase of milk production, increased number of dairy cows with short luteal phase of the estrous cycle, increased embryonic mortality, decrease immune response and increased frequency of reproduction disorders (Sreenan and Diskin, 1983; Diskin, 1987; Gordon, 1997; Beam and Butler, 1999; Royal et al., 2000a; Lucy, 2001; Bousquet et al., 2004).

Another direct evidence of the decreased reproduction efficiency in post partal dairy cows is increased number of AI needed for successful conception (Esslemont & Kossaibati, 2000). Lucy (2001) reported that average number of AI for successful conception increased from 1,75 to more than 3. The research in Canada has also indicated decrease in dairy cows fertility rate from 44% in 1990. to 39% in the year 2003, that increased number of AI per conception for 0,48/lactation (Bouchard and Du Tremblay, 2003). Similar situation has been reported for Ireland by Mee et al., (2004), where number of services per conception has increased between the years 1990-2000 from 1,54 to 1,75. Maximal success of services (conception) is achieved between days 60-90 postcalving (Salisbury et al., 1978). Our research has also indicated significant increase of number of services per conception, especially in dairy cows with calving and postcalving reproduction disorders (number of services/conception was 3,5).

Service period (SP) is one of the major parameters of reproduction efficiency that directly influences calving interval (Wattiaux, 2006; Stančić, 2008). In the well managed dairy herds average SP is optimally between 85-110 days, with no more than 10% of cows having SP period longer than 120 days. In the case that SP period is above 140

days, or number of cows with SP period longer than 120 days is more than 15% there are serious reproduction issues on that dairy farm (Walker, 1997). In our research we have determined the average SP period of 146 days in the first parity cows and 137 days in older and healthy cows. The days open period was significantly longer ($P<0.05$) in the first parity and older dairy cows with peripartal reproduction disorders (202-214 and 186-188 days, first parity and older CD and PCD cows, respectively). Our results indicate that reproduction disorders after calving significantly extended SP period and calving interval. The most important reproduction disorders were uterine inflammatory diseases, as well as ovarian dysfunctions (ovarian cysts). Similar results concerning decrease of number of dairy cows successfully inseminated in the first poscalving service and extended SP period are reported by other authors (Quin et al.,2002; Lewis, 2003; Peter, 2004; Gvozdić et al., 2004; O'Connor et al., 2006; Wattiaux, 2006).

CONCLUSION

Our present results clearly indicate serious problem with the decreased reproductive efficiency at the examined dairy farm, were we have detected more than 70% of cows with calving and postcalving reproductive system disorders. The result of this situation was significant increase of calving to first recorded estrus interval, number of services/conception and extended SP period. Further research regarding complex etiology and possible corrective management and veterinary measures are needed, in order to decrease SP period, number of services/conception and calving interval.

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REPRODUKTIVNA EFIKASNOST VISOKO-MLEČNIH KRAVA POSLE TELENJA

DRAGAN GVOZDIĆ, IVAN STANČIĆ, MILAN SAVOVIĆ, BLAGOJE STANČIĆ, ALEKSANDAR BOŽIĆ, SVETLANA MILANOVIĆ, IVAN JOVANOVIĆ, TOMISLAV BARNA

Izvod

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su ustanovljeni kod 1.252 krave (72,4%). Najčešće ustanovljeni peripartalni poremećaji kod visoko mlečnih krava su teško teljenje (62%) i endometritis (64%). Peripartalni poremećaji imaju statistički značajan uticaj (P<0,05) na povećanje indeksa osemenjavanja (3,5 prema 2,7), produženje intervala od prvog do fertilnog osemenjavanja (57 prema 110 dana), kao i na produženje trajanja servis perioda (146 prema 214 dana), u poređenju sa kravama bez poremećaja. Dobijeni rezultati pokazuju da na farmama visoko mlečnih krava povišena učestalost peripartalnih reproduktivnih poremećaja koji značajno utiču na smanjenje reproduktivne efikasnosti krava nakon partusa.

Ključne reči: reprodukcija, efikasnost, visoka mlečnost, poremećaji post partum, krava.

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EFFICIENCY EVALUATION OF FLUROXYPYR IN MAIZE CROP

BRANKO KONSTANTINOVIĆ, MILENA KORAĆ[1]

SUMMARY: In the period 2009-20010, efficiency tests in maize crop were carried out with the aim of monitoring of fluroxypyr efficiency in control of broadleaved weed species. Trial was set up at two sites and test type was randomized bloc design. Applications of herbicides were performed in the phase of 3-6 leaves of maize. Efficiency evaluation of the herbicides was performed twice, visually. Results showed that fluroxypyr can be successfully applied for control of great number of broadleaved weeds such as: Amaranthus retroflexus L., Chenopodium album L., C. hybridum L., Datura stramonium L., Solanum nigrum L.

Key words: *efficiency, fluroxypyr, maize, weed control.*

INTRODUCTION

After rice and wheat, maize (*Zea mays* L.) is one of the most important field crop in the world (Sharara et al., 2005). In Serbia this culture also occupies significant place in total agricultural production due to its high yields (over 10t/ha) and volume of production of over 1-1.3 million ha annually (Milosavljević et al., 2010).

In addition to diseases and pests, weed occurrence on arable land has great impact on maize quality and yield (Sharara et al., 2005). Weed control is on of the most important conditions for achievement of stable yields (Casagrande et al., 2010), and measures that are carried out in early phases of crop development when it is the most susceptible to weed competition are especially important (Konstantinović, 2008). Planning and targeted performance of herbicide treatments is of great importance (Stanojević, 2000). Efficiency tests and studies of herbicide spectrum of action are the first precondition for successful protection of crops from weeds.

Last years, post-emergence use of foliar herbicides has become more frequent, both in narrow-row crops of wheat, barley and oats (Shah et al., 2006), and in maize (Sharara, 2005). Fluroxypyr also belongs to this group of herbicides, i.e. auxin-type herbicides, that have important application in agriculture due to wide spectrum of action to against resistant broad-leaved weed species (Tao and Yang, 2010; Zhang et al., 2011). It is translocated systematic herbicide, and selectivity toward maize can be achieved only if it is applied in prescribed crop development phase (Konstantinović, 2008). The pre-

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scribed development phase of maize in which treatments by fluroxypyr were performed is 3-6 leaves (Anonymous, 2008). The aim of the evaluation in this paper was to study fluroxypyr impact to annual and perennial broad-leaved weed species in maize crop.

MATERIAL AND METHODS

In 2009, at sites Bački Maglić and Kovin, a trial with fluroxypyr (herbicide Patrol) was set up in rates of 0.8l/ha for efficiency evaluations and 1.6l/ha for determination of phytotoxicity to maize crop. In the following 2010, at sites Tovariševo and Zmajevo, product Starane 300 in rate of 0.6l/ha was applied for efficiency evaluations and 1.2l/ha for study of phytotoxicity of fluroxypyr to maize. Standard herbicide in all trials was herbicide dicamba (product Banvel 480) at a rate of 0.6l/ha. Trials were set up according to the scheme of randomized block design. At all sites herbicides were applied folliary in maize development of 3-6 leaves, and in the phase of intensive growth of weeds. Herbicide treatments were performed by back-sack sprayer. Efficiency evaluations were performed twice, two weeks and a month after treatments. Evaluations were done by visual assessments of number of weed species distribution on the area of $1m^2$, in 4 replications for each studied plot. For the purpose of herbicide efficiency evaluation, the following scale was used: poor herbicide efficiency (KE < 75%); good herbicide efficiency (KE = 75-90%); high herbicide efficiency. (KE > 90%). Phytotoxicity of herbicides was simultaneously also assessed. All evaluations were performed in accordance to OEPP/EPPO methods (EPPO Standards, 2004). The final results were obtained by ratio of the mean value of samples taken from 4 plots of each treatment with mean value of samples taken from four control plots.

RESULTS

In 2009 at site Bački Maglić in maize crop, presence of nine weed species was determined: *Amaranthus retroflexus* L.*, Chenopodium hybridum* L.*, Cirsium arvense* (L.) Scop.*, Datura stramonium* L.*, Polygonum aviculare* L.*, Polygonum persicaria* (L.) Small.*, Sinapis arvensis* L.*, Solanum nigrum* L. and *Xantium strumarium* L. In both evaluations, herbicide Patrol used at a rate of 0.8l/ha showed good efficiency (KE > 90%) in relation to weed species *Amaranthus retroflexus, Chenopodium hybridum, Datura stramonium, Polygonum aviculare, Polygonum persicaria, Sinapis arvensis, Solanum nigrum*. In relation to weed species *Cirsium arvense*, at rate of 0.8l/ha the herbicide showed good efficiency (KE = 75-90%), while in both evaluations it showed poor performance to weed species *Xantium strumarium*.

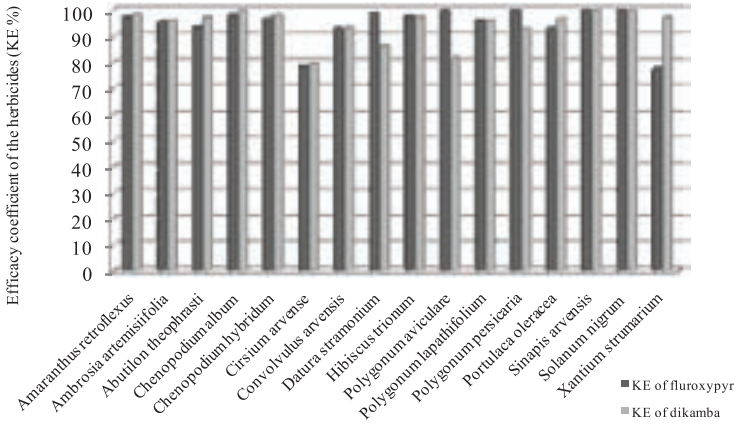
The same year, on the trial plot in Kovin, the following weeds were determined: *Amaranthus retroflexus, Chenopodium album* L.*, Chenopodium hybridum, Datura stramonium, Hibiscus trionum* L.*, Portulaca oleracea* L.*, Polygonum lapathifolium* L.*, Solanum nigrum* and *Xantium strumarium*. In both evaluations, herbicide Patrol used at a rate of 0.8l/ha, showed good efficiency (KE > 90%) to weed species: *Amaranthus retroflexus, Chenopodium album, Chenopodium hybridum, Hibiscus trionum, Portulaca oleracea, Polygonum lapathifolium* and *Solanum nigrum.* In both evaluations, the herbicide showed poor efficiency (KE < 75%) to the weed species *Xantium strumarium*. The product Banvel 480 used as standard had approximately the same results as the

tested herbicide (Graph. 1). In both trials, fluroxypyr used at a rate of 1.6l/ha, showed no signs of phytotoxicity on maize.

During 2010, at experimental plot in Tovariševo, the following weed species were determined: *Amaranthus retroflexus, Ambrosia artemisiifolia, Chenopodium album, Convolvulus arvensis, Datura stramonium, Hibiscus trionum, Polygonum lapathifolium, Solanum nigrum* and *Xantium strumarium.* The product Starane 300 used at a rate of 0.6l/ha, showed good efficiency in control of the following weed species: *Amaranthus retroflexus, Ambrosia artemisiifolia, Chenopodium album, Convolvulus arvensis, Datura stramonium, Hibiscus trionum, Polygonum lapathifolium* and *Solanum nigrum*. In both evaluations the herbicide showed satisfactory efficiency (KE = 75-90%) in control of weed species *Xantium strumarium.*

Before herbicide application, at experimental plots in Zmajevo nine weed species were determined: *Abutilon theophrasti* Med.*, Amaranthus retroflexus, Ambrosia artemisiifolia, Chenopodium album, Datura stramonium, Hibiscus trionum, Polygonum aviculare, Solanum nigrum* and *Xantium strumarium.* In both evaluations the product Starane 300, used at a rate of 0.6l/ha showed good efficiency (KE > 90%) in relation to all determined weed species except to *Abutilon theophrasti* and *Xantium strumarium* to which it showed satisfactory efficiency.

In both trials, standard herbicide achieved similar results, as well as the studied one (Graph. 1). During experiments, no signs of phytotoxicity were observed on the maize crop.



Graph. 1. Efficiency of the studied herbicide fluroxypyr and standard herbicide dicamba after second evaluation of treatments (average for both years and all 4 studied sites)

Graf. 1. Efikasnost ispitivanog herbicida fluroksipira i standardnog preparata dikambe nakon II ocene tretmana (prosek za obe godine i sva 4 ispitivana lokaliteta)

DISCUSSION

During 2009, evaluations of fluroxypyr efficiency showed the following: at both studied sites, i.e. Bački Maglić and Kovin the highest susceptibility to fluroxypyr showed weed species *Amaranthus retroflexus, Chenopodium hybridum, Datura stramonium* and *Solanum nigrum.* At site Bački Maglić the additional three weed spe-

cies, i.e. *Polygonum aviculare, Polygonum persicaria* and *Sinapis arvensis* showed extreme susceptibility to the applied herbicide with the maximum efficiency coefficient of 100%, while at site Kovin this level of susceptibility showed *Hibiscus trionum* and *Polygonum lapathifolium*. Weed species *Portulaca oleracea* and *Chenopodium album* also showed high level of susceptibility to fluroxypyr with efficiency coefficient of over 90%. Exceptional susceptibility of weed species *Sinapis arvensis* and *Chenopodium album* to fluroxypyr (KE = 100%) in wheat crop was observed in studies of Radivojević et al., 2002, and in barley Roibu et al., 2000. At site Bački Maglić, perennial weed species *Cirsium arvense* showed somewhat higher resistance to the studied herbicide, and efficiency coefficient to this weed species was less than 80%. Weed species *Xantium strumarium* proved to have the lowest susceptibility. Efficiency coefficient for this weed species was 66-71%.

During 2010, evaluations of fluroxypyr efficiency provided the following results: at sites Tovariševo and Zmajevo the highest susceptibility to the applied herbicide showed weed species *Chenopodium album, Datura stramonium, Polygonum aviculare, Solanum nigrum* (KE = 100%) and *Amaranthus retroflexus, Ambrosia artemisiifolia, Abutilon theophrasti, Convolvulus arvensis, Hibiscus trionum* and *Polygonum lapathifolium* (KE = 90-100%). At these sites, *Xantium strumarium* also showed the lowest susceptibility to fluroxypyr. Efficiency coefficient for this weed species was 85-86% (Table 1).

Table 1. The average fluroxypyr efficiency (herbicides Patrol and Starane 300) and standard herbicide Banvel 480 to different weed species
Tabela 1. Prosečna efikasnost herbicida fluroksipir (preparata Patrol i Starane 300) i standaradnog preparata Banvel 480 na različite korovske vrste

Weed species	Product Patrol	Standard Banvel 480	Product Starane 300
	0.8l/ha	0.6l/ha	0.6l/ha
	Herbicide efficiency (%) after second evaluation		
Annual dicotyledonous			
Abutilon theophrasti Med.	-	97	93
Amaranthus retroflexus L.	97	99	97
Ambrosia artemisiifolia L.	-	96	96
Chenopodium album L.	94	100	100
Chenopodium hybridum L.	97	98	-
Datura stramonium L.	97	86	100
Hibiscus trionum L.	100	97	96
Polygonum aviculare L.	100	81	100
Polygonum lapathifolium L.	100	96	92
P. persicaria (L.) Small.	100	93	-
Portulaca oleracea L.	93	97	-
Sinapis arvensis L.	100	100	-
Solanum nigrum L.	100	100	100
Xantium strumarium L.	69	97	86
Perennial dicotyledonous			
Cirsium arvense (L.) Scop.	78	79	-
Convolvulus arvensis L.	-	93	93

CONCLUSION

The above given results showed that in recommended rates fluroxypyr can be successfully applied for control of great number of broadleaved weed species in maize, without phytotoxicity to the crop. Good efficiency (KE > 90%) the herbicide showed in relation to weed species: *Amaranthus retroflexus, Ambrosia artemisiifolia, Abutilon theophrasti, Convolvulus arvensis, Chenopodium album, Chenopodium hybridum, Datura stramonium, Hibiscus trionum, Portulaca oleracea, Polygonum lapathifolium, Polygonum aviculare, Polygonum persicaria, Sinapis arvensis* and *Solanum nigrum*. It showed satisfactory efficiency (KE = 75 - 90%) in relation to *Cirsium arvense* and weak in relation to *Xantium strumarium*. Considering the fact that *Xantium strumarium* is one of the most dangerous competitors in maize (Karimmojeni et al., 2010), it is necessary to use fluroxypyr in combination with other active ingredients, more efficient to this weed species.

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ISPITIVANJE EFIKASNOSTI FLUROKSIPIRA U USEVU KUKURUZA

BRANKO KONSTANTINOVIĆ, MILENA KORAĆ

Izvod

Tokom 2009-2010 godine, u usevu kukuruza, sprovedena su istraživanja u cilju praćenja efikasnosti fluroksipira u suzbijanju širokolisnih korovskih vrsta. Ogled je postavljen na dva lokaliteta po šemi slučajnog blok sistema. Herbicidi su primenjeni kada je kukuruz bio u fazi 3-6 listova. Ocena efikasnosti preparata izvršena je dvokratno, vizuelnom procenom. Rezultati su pokazali da se fluroksipir uspešno može primeniti za suzbijanje velikog broja širokolisnih korovskih vrsta kao što su: *Amaranthus retroflexus* L., *Chenopodium album* L., *C. hybridum* L., *Datura stramonium* L., *Solanum nigrum* L.

Ključne reči: efikasnost, fluroksipir, kukuruz, suzbijanje korova.

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RELATIONSHIP BETWEEN ELECTRICAL RESISTANCE OF VAGINAL MUCUS IN THE MOMENT OF INSEMINATION AND COW'S PREGNANCY RATE

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SUMMARY: This paper presents our results about the relationship between vaginal mucus electrical resistance values at the time of insemination and achieved cow's pregnancy rate. We examined total of 130 cows, divided into three groups, based on the values of vaginal mucus electrical resistance (less than 179, from 180 to 320 and more than 321 units). Our three groups of cows achieved pregnancy rates of 87,5%, 73,6% and 75%, respectively. Pregnancy rate for all examined cows was 74,6%. The obtained results in this study showed that there was no statistically significant difference in pregnancy rate among analyzed groups.

Key words: *vaginal mucus, electrical resistence, pregnancy rate, cow.*

INTRODUCTION

Oestrus, as the most visible phase of the oestrous cycle, is characterised by nervousness, roaring, mounting activity, standing to be mounted by another cow, reduced milk production and food refusing. In addition to these, relatively insecure signs, appearance of the vaginal mucus is considered to be the most characteristic sign of oestrus. (Miljković and Veselinović, 2000; Matarugić et al., 2007). This phase of cycle is characterized by follicular maturation and ovulation and by proliferation of endometrial mucosa and its preparation for the acceptance of early embryos. Along with changes in ovaries and uterine mucosa, cyclical changes are occurring also in cervix, with opening of cervical canal to facilitate sperm transport and secretion of cervical mucus as a medium in which the spermatozoa move (Miljković and Veselinović, 2000; Stančić and Veselinović, 2002). All these events are controlled by the neuroendocrine system of animal. During oestrus, the cervical canal is open, relaxed and filled with oestrous mucus. Oestrous mucus is a product of cervical canal glands and fills cervix during oestrus, than pass through vagina and vulva and expands in the form of long threads

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Original scientific paper / *Originalni naučni rad*

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(Matarugić et al., 2007). Vaginal mucus, except epithelial cells and substances necessary for the survival of sperm, also contains electrolytes (salts of sodium, magnesium, calcium and other cations), which determine its electrochemical reaction. Electrolytes in smears of oestrous mucus can be observed as crystals (Miljković, 1990). During the various stages of oestrous cycle and oestruos itself, certain changes are occuring in composition and relationship of some electrolytes in oestrous mucus. The presence of electrolytes leads to ionization of oestrous mucus, which also affects its electrical conductivity. Based on these characteristic of oestrous mucus, method for determining the electrical resistance of oestrous mucus is been developed, as a method for detecting the optimum time for insemination or mating (Carter and Dufty, 1980; Elving et al., 1983; Fossen, 1991; Rorrie et al., 2002). With changes in composition of oestrous mucus, there are parallel changes in electrical resistance, which represents the base of this diagnostic method. During oestrus, the electric resistance of oestrous mucus decline (Tasal et al., 2005). In the next period, value of resistance increase, which indicates that the moment of ovulation is approaching. Firk et al. (2002) report that some pathological conditions of the reproductive tract, such as vaginitis and cervicitis, can give a values of electrical resistance of vaginal mucus similar to those in oestrus.

This study aims to determine the connection of pregnancy rate and values of electric resistance of the vaginal mucus at the time of insemination.

MATERIAL AND METHODS

The examination included a total of 130 cows of domestic mix-breed in Simmental breed type, aged 3 to 16 years, in breeding condition, with an average parity of 5,13 (1-13) calvings. The average length of service period of inseminated cows was 80,62 days (30-135). The cows were inseminated on average 9,32 (3-20) hours after the first observed signs of oestrus. At the time of insemination oestrous mucus was observed in 46% cows.

To determine the values of electric resistance of the vaginal mucus oestrous detector Draminski (Olsztyn, Poland) was used. The value of the electric resistance of the vaginal mucus was determined as the average value of three measurements taken immediately prior to insemination. According to the manufacturer of oestrous detector, oestrus occurs when the value of resistance is about 180 units, and ovulation occurs about 24 hours later, when the value of resistance begins to grow closer to 320 units. In accordance with the manufacturer's recommendations, all examined cows were divided into three groups. The first group consisted of cows with values of the electric resistance of vaginal mucus less than 179 units, in second group were cows with values from 180 to 320 units, and third group were cows with values higher than 321 units. All cows were inseminated by bimanual method, and the gravidity was confirmed by rectal examination 9-12 weeks later. Repeat breeding cows were not the subject of further research.

RESULTS

The values of the electric resistance of vaginal mucus in examined cows at the time of insemination are shown in Table 1.

Table 1. The values of the electric resistance of the vaginal mucus at the time of insemination
Tabela 1. Vrijednosti otpora vaginalne sluzi u momentu osjemenjavanja

Group / Grupa	n	X±Sx	±S	IV	CV
First / Prva	8	162,9±5.04	14,27	140-176,7	8,76
Second / Druga	110	240,5±3.53	37,08	180-320	15,42
Third / Treća	12	404,7±28.61	99,13	326,7-650	24,49

Pregnancy rate and its relation to the values the electric resistance of vaginal mucus at the time of insemination is shown in Table 2.

Table 2. Pregnancy rates in relation to the values of resistance of vaginal mucus at the time of insemination
Tabela 2. Procenat graviditeta u odnosu na vrijednost otpora vaginalne sluzi u momentu osjemenjavanja

Group / Grupa	*Inseminated cows* Osjemenjene krave		*Pregnancy rate* Procenat graviditeta	
	n	%	n	%
First / Prva	8	6,2	7	87,5±11,69[a]
Second / Druga	110	84,6	81	73,6±4,20[a]
Third / Treća	12	9,2	9	75±12,5[a]
Total / Ukupno	130	100,0	97	74,6

[a,b,c] Values with different superscripts within column are significantly different (P<0,05).
Vrednosti sa različitim superskriptima su signifikantno različite (P<0,05).

In the first group of cows average value of the electric resistance of the vaginal mucus at the time of insemination was 162,9±5,04 units. Pregnancy rate in this group cows was 87,5%. An average value of the electric resistance of the oestrous mucus in the second group of cows was 240,5±3,53 units, with pregnancy rate of 73,6%. The third group of cows had an average value of the electric resistance of the oestrous mucus 404,7±28,61units, with pregnancy rate of 75%. Pregnancy rate for all observed cows was 74,6%. The obtained results in this study showed that there was no statistically significant difference in pregnancy rate among analyzed groups.

DISCUSSION

Intensive selection for milk production negatively affected the reproductive efficiency of high yielding dairy cows. Oestrus detection is a common problem on farms or in households that kept dairy cows. The reasons for that are insufficiently pronounced signs of estrus, and lack of producers knowledge. Therefore, a series of studies were conducted to find instrumental or other method which would succesfully determine the optimum time for insemination or mating. Potential solution for overcoming these problems is a oestrus detection device, used in our study.

Fossen (1991) found a positive correlation between the value of vaginal mucus resistance and progesterone concentration in milk, as a diagnostic method to detect the optimal moment for insemination. McCaughey and Patterson (1981) found a connection between the value of vaginal electrical resistance, the level of progesterone in milk and

observed signs of oestrus. The values of the vaginal mucus resistance of cows and heifers were significantly correlated with the concentration of progesterone in gravid and non-gravid cows. McCaughey (1981) noted positive experiences with measuring the electrical resistance of vaginal mucus, while Cavestany and Foote (1985) described this method as insufficiently reliable in large farms conditions. Zuluaga et al. (2008) concluded that this method is not reliable in detecting the optimum time for insemination after induction and synchronization of ovulation in cows.

Elving et al. (1983) state that the method of measuring electrical resistance of vaginal mucus is not effective in oestrus detection, due to variations in consecutive oestrus and between the animals in the same phase of oestrous cycle. Kitwood et al. (1993) state that, when using a method of measuring resistance of vaginal mucus in cows, it is needed to determine the lowest value of resistance of vaginal mucus. According to results of Carter and Dufty (1980) the method of measuring resistance of vaginal mucus is not suitable for the detection of the optimal moment for insemination, because the recorded lowest values of resistance varied between animals in different times of oestrus. Leidl and Table (1976) observed a clear correlation between the value of resistance of vaginal mucus and phase of oestrous cycle. According to these authors, the lowest values of resistance were found during oestrus. The same authors found that ovariooctomised animals showed no change in values of resistance, as a result of the absence of hormonal regulation of mucus secretion. According to these authors, disadvantage of this method is that some pathological conditions of genital tract can give values similar to those observed during oestrus. On this basis, they state that the diagnosis of estrus and the optimum time for insemination can not be based solely on the determination of resistance of vaginal mucus, but it should serve as an auxiliary method. The same authors found that pregnancy rate was 82% in cows with vaginal mucus resistance values less than 300 units. The value of electrical resistance of vaginal mucus was lowest during estrus and increases during dioestrus, which confirmed the results of Tasal et al. (2005). According to Fossen (1991), the best conception is achieved, when cows were inseminated within 12 hours after the established minimum value of resistance of vaginal mucus. Although they found different values of resistance of vaginal mucus, Foote et al. (1979) have achieved the same pregancy rates group of cows which were inseminated following a value of resistance, and the other group using a common visual method for detecting of oestrus. Aboul-Ela et al. (1983) have shown that resistance of vaginal mucus fluctuated during dioestrus, with marked decline (25%) during oestrus. The lowest value of resistance was observed in the second half of oestrus, which coincides with the peak of LH.

Soto (2008) using Draminski oestrus detector, found that the lowest value of resistance of vaginal mucus at oestrus was average 181 units, while in subsequent stages of oestrus cycle values was increased, ie during postestrus the value of resistance 214, proestrus 239 and in dioestrus 254 units. Kostov et al. (1984) showed that the optimum time for insemination of cows is in the period when the vaginal mucus resistance is less than 250 units. Our results are consistent with the findings of these authors, as confirmed by the fact that the highest pregnancy rate was established in cows with the lowest value of resistance of vaginal mucus.

CONCLUSION

Timely and accurate oestrus detection is the basis for managing the reproductive performance of cows. One of the methods for estrus detection is the measurement of resistance of vaginal mucus. By monitoring of values of vaginal mucus resistance, we can determine the stage of oestrous cycle. In our study we found that the largest pregancy rate was established in the cows with the values of resistance less than 179 units. However, differences in the pregnancy rate of analysed groups were not statistically significant. In order to investigate the efficacy of this method further research is needed.

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POVEZANOST ELEKTRIČNOG OTPORA VAGINALNE SLUZI U MOMENTU OSEMENJAVANJA I PROCENTA GRAVIDITETA KRAVA

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Izvod

U radu su predstavljeni rezultati istraživanja povezanosti vrednosti električnog otpora vaginalne sluzi krava u momentu osemenjavanja i ostvarenog procenta graviditeta. Ukupno je ispitano 130 krava koje su na osnovu vrednosti električnog otpora vaginalne sluzi podeljene u tri grupe (manje od 179, od 180 do 320 i više od 321 jedinice). U prvoj grupi krava ostvaren je procenat graviditeta od 87,5%, u drugoj 73,6% i trećoj 75%. Procenat graviditeta za sve ispitane krave bio je 74,6%. Nije ustanovljena statistički značajna razlika u procentu graviditeta između ispitanih grupa krava.

Ključne reči: vaginalna sluz, električni otpor, procenat graviditeta, krava.

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THE EFFECT OF LIQUID AND SOLUBLE FERTILIZERS ON LETTUCE YIELD*

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SUMMARY: The aim of this study was to examine the effect of five liquid/soluble fertilizers on yield and yield contributing characters in plastic house grown lettuce. Bioactiv, Hascon M-10 AD and Herbagreen were splashed onto the plants; Foliacon 22 was applied within watering and Drin was used in both ways. The yield was significantly increased by all treatments, except for Bioactiv. Considering both the yield and other traits analyzed, the best results have been achieved by application of Drin within watering and it *could be recommended for plastic house grown winter lettuce.*

Key words: *lettuce, liquid and soluble fertilizers, yield.*

INTRODUCTION

The optimized application of mineral fertilizers provides increased and stabile yields in agricultural plants (Latković et al., 2009; Bogdanović et al., 2010). However, unnecessarily high rates of mineral fertilizers affect environment, since the excess that has not been used by crops remains in the soil or washes in groundwater and surface water (Moreno et al., 2003; Li et al., 2005; Fernandez-Escobar et al., 2011). In addition, inadequate fertilizing may affect the balance of soil micro and macro elements causing significant crop nutrient disorders (Boroujerdnia and Ansari, 2007; Oljača, 2008).

One of the possibilities for solving the problems related to excessive use of mineral fertilizers in vegetable crops production may be the use of liquid or soluble fertilizers, applied foliarly or within watering. Foliar fertilizing facilitates rapid absorption and

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efficient use of nutrients; therefore, it may be applied as both preventive and curative measure to compensate for nutrient deficiency (Fernandez et al., 2008; Hossain and Ryu, 2009; Seshadri, 2010; Del Amor and Cuadra-Crespo, 2011). Foliar nutrient supply may play significant role in intensive production of vegetables.

This study was undertaken in order to investigate the effect of five liquid and soluble fertilizers on yield and yield contributing characters in plastic house grown lettuce (*Lactuca sativa* L.), cultivar Neva. Applying the results of this study may enhance intensive lettuce production.

MATERIAL AND METHODS

The plastic house experiment has been set during the winter of 2008/09 at the Institute for Vegetable Crops, Smederevska Palanka, Serbia. The trial included five liquid and soluble fertilizers (Table 1) combined in six treatments (Table 2) and control (without fertilizing), which were applied on lettuce cultivar Neva. The cultivar has been developed at the Institute.

Table 1. Nutrient content in the applied fertilizers
Tabela 1. Sadržaj hranljivih materija u đubrivima koja su korišćena u ogledu

Fertilizer / *Đubrivo*	Nutrient content / *Sadržaj hranljivih materija*
Drin	Aminoacids / *Aminokiseline* 39%, C 19%, N 6.3%
Foliacon 22	CaO 15%, N 13.2%, MgO 7.5%
Hascon M-10 AD	K_2O 28%, P_2O_5 21%; B, Mn and Mo in traces / *u tragovima*
Bioactiv	Mixture of microorganisms / *Smeša mikroorganizama*
Herbagreen	CaO 44.1%, SiO_2 9.1%, MgO 2.2%, Fe_2O_3 1.2%, SO_4 0.11%, Mn 132 ppm, Zn 60 ppm, Cu 22.5 ppm, Ni 3.3 ppm, Cr 3.25 ppm

Table 2. The applied fertilizer treatments
Tabela 2. Primenjeni tretmani đubrivima

Treatment / *Tretman*	Application / *Primena*	Concentration / *Koncentracija*
Drin (1)	Foliar / *Folijarno*	0.2 %
Drin (2)	Watering / *Zalivanjem*	17 ml/m^2
Drin + Foliacon 22 (3)	Watering / *Zalivanjem*	0.1 % + 0.2 %
Hascon M-10 AD (4)	Foliar / *Folijarno*	0.15 %
Bioactiv (5)	Foliar / *Folijarno*	5 g/l
Herbagreen (6)	Foliar / *Folijarno*	5 g/l

The lettuce was planted as a subsequent crop, without previous fertilization. The main crop was tomato, which was fertilized following the standard procedures. Lettuce seedlings were transplanted from nursery on mulch foil on November 18th and harvested on March 11th. The plastic house was not heated during the experiment. Air temperature was recorded on the daily basis and compared to multiyear average (Figure 1). The main plot area was 5 m^2 and it consisted of 100 plants. The crop was watered by the micro irrigation system.

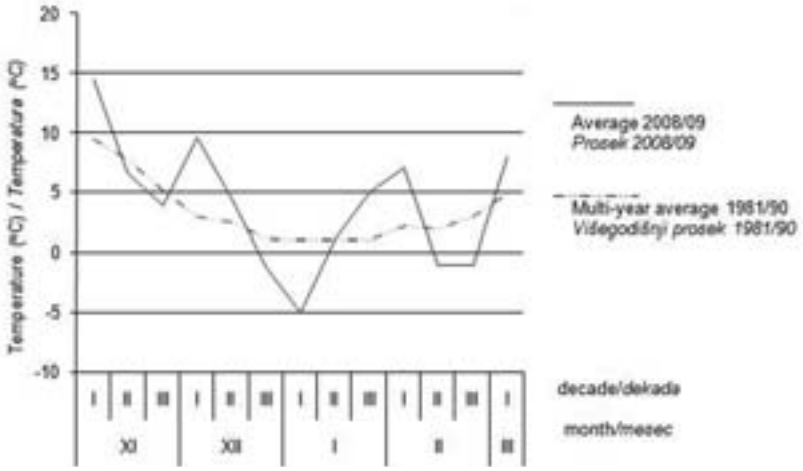

Figure 1. Plastic house air temperature recorded during the experiment
Grafikon 1. Temperatura vazduha u plasteniku merena tokom ogleda

The treatments were imposed following the dynamics recommended by the producers (Table 3). Every main plot was treated with 2l of fertilizer solution. All treatments were replicated 5 times.

Table 3. Dynamics of treatment application
Tabela 3. Dinamika primene tretmana

Treatment / *Tretman*	Date / *Datum*	Treatment / *Tretman*	Date / *Datum*
(1), (2)	December 04th, 2008	(1), (6)	January 14th, 2009
(1)	December 15th, 2008	(6)	January 30th, 2009
(1), (2), (3), (4), (5), (6)	December 24th, 2008	(6)	February 10th, 2009

The following traits have been analyzed: yield per plant (g), number of leaves per plant, leaf rosette weight (g), head weight (g) and head index, which was calculated as the ratio of head weight and leaf rosette weight. Samples included 10 plants per replication. The data was processed by ANOVA and LSD test was used for comparison of the means of treatments.

RESULTS AND DISCUSSION

The average effects of the applied liquid and soluble fertilizers on the analyzed lettuce traits are listed in Table 4. Significant differences have been found among the applied treatments for all investigated lettuce traits.

Table 4. The effect of the applied fertilizer treatments on lettuce yield per plant (g), number of leaves per plant, leaf rosette weight (g), head weight (g) and head index
Tabela 4. Uticaj primenjenih tretmana đubriva na prinos salate po biljci (g), broj listova po biljci, masu listova rozete (g), masu glavice (g) i indeks glavice

Treatment *Tretman*	Yield *Prinos*	No of leaves *Broj listova*	Rosette weight *Masa rozete*	Head weight *Masa glavice*	Head index *Indeks glavice*
(1)	134.4**	15.4**	67.5**	66.9**	0.99
(2)	142.9**	15.4**	69.8**	73.1**	1.05
(3)	127.6**	12.2	66.8**	60.8	0.91**
(4)	132.8**	12.0	69.9**	62.9**	0.90**
(5)	119.6	12.2	62.2**	57.4	0.92**
(6)	136.0**	16.4**	72.1**	63.9**	0.89**
Control/*Kontrola*	114.1	12.2	56.3	57.8	1.03
$LSD_{0.05}$	7.0	1.5	4.6	4.0	0.05
$LSD_{0.01}$	8.4	1.8	5.5	4.8	0.07

**significantly different with the respect to control (0.01 level of probability)

Yielding capacity of the lettuce cultivar Neva was not fully realized in this experiment (Marković et al., 1997); either on unfertilized, or on fertilized plots. Although the plants were grown in a plastic house, low temperatures during the vegetation slowed their development. The temperatures were extremely low in January (Graph 1). Nevertheless, the application of liquid and soluble fertilizers significantly increased lettuce yield per plant with respect to unfertilized control. Similar results have been reported by Saglam et al. (2002), Vetrano et al. (2009) and Luz et al. (2010). The only exception was fertilizer Bioactiv, which practically had no effect on yield. Therefore, the application of this fertilizer is not recommended for plastic house winter lettuce production.

Other lettuce traits had been analyzed primarily with the aim to investigate the possible effects of different fertilizers on the head and rosette weight ratio (head index). Since the lettuce with highly developed rosette is not preferred by the consumers, head index should be as high as possible.

Drin + Foliacon 22, Hascon M-10 AD and Herbagreen treatments significantly increased lettuce yield. However, Drin + Foliacon 22 increased leaf rosette weight and had no effect on head weight. Hascon M-10 AD and Herbagreen increased both head and rosette weight, however rosette weight was increased to the greater extent comparing to head weight. Consequently, head indexes calculated for the plots treated with Drin + Foliacon 22, Hascon M-10 AD and Herbagreen were significantly lower with the respect to the control. Out of three treatments mentioned, only Herbagreen increased the number of leaves, however, those were rosette leaves. Since lower head index may make lettuce less attractive to the consumers, fertilizing plastic house grown winter lettuce with the above substances may not be recommended.

On the other hand, treatment Drin increased significantly the yield per plant, the number of leaves per plant, leaf rosette weight and head weight, having no effect on head index. The increases in the studied traits have been observed for both foliar application and application by watering; however, the positive effects of fertilizer Drin on lettuce were more pronounced when applied by watering. Therefore, among the six studied liquid and soluble fertilizer treatments, application of Drin within watering could be recommended for plastic house grown winter lettuce. Yield per plant and head weight

were increased by Drin for approximately 25% with respect to the control, even in the conditions of unfavorably low winter temperatures.

CONCLUSION

Application of liquid and soluble fertilizers completes basic fertilization through the soil and it is especially useful in stress conditions (low temperature, physiological drought, lack of light, etc.). The best results have been achieved by application of fertilizer Drin within watering; therefore, it can be recommended for plastic house winter lettuce production. Application of microbiological fertilizer Bioactive is not recommended for the mentioned production system.

Further investigations on the levels and dynamics of fertilizer applications, as well as on the chemical composition of the lettuce fertilized with liquid and soluble preparations should be undertaken.

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UTICAJ TEČNIH I LAKO RASTVORLJIVIH SREDSTAVA ZA ISHRANU BILJAKA NA PRINOS ZELENE SALATE

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Izvod

Cilj ovog istraživanja bilo je ispitivanje uticaja pet tečnih i lako rastvorljivih sredstava za ishranu biljaka na prinos i svojstva u vezi sa prinosom kod zelene salate gajene u zaštićenom prostoru u toku zime. Bioactiv, Hascon M-10 AD i Herbagreen primenjeni su folijarno; Foliacon 22 je zalivanjem a Drin je korišćen na oba načina. Zabeleženi su značajno viši prinosi po biljci na svim ispitivanim tretmanima, izuzev na tretmanu Bioactiv. Uzevši u obzir prinos i druga ispitivana svojstva najbolji rezultati su postignuti primenom preparata Drin zalivanjem, te bi se ovaj tretman mogao preporučiti kod plasteničke proizvodnje zelene salate u toku zime.

Ključne reči: zelena salata, tečna i lako rastvorljiva đubriva, prinos.

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PROPERTIES OF FRENCH WALNUT CULTIVARS GROWN UNDER ENVIRONMENTAL CONDITIONS OF THE ČAČAK REGION

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SUMMARY: In this paper, we have studied major pomo-technological properties and productivity of standard French walnut cultivars ('Marbot', 'Parisisenne' and 'Franqete'), newly developed ones ('Fernor', 'Fernet') and standard cv. 'Elit', grown in the region of Čačak. Newly bred cultivars had more favourable vegetation onset, although their fruits were smaller, with lower fruit weight. The average yield per tree ranged from 8.7 kg in 'Elit' to 13.7 kg in 'Fernete' (the highest yield was in 'Fernor', 15.0 kg), i.e. from 870 to 1370 kg (1500 kg/ha). The lowest kernel ratio per tree was in 'Elit' (4.5 kg, 450 kg) while it was the highest in 'Fernete' (6.6 kg, 660 kg/ha) and 'Fernor' (7.4 kg, 740 kg/ha).

***Key words**: walnut, cultivars, phenology, fruit, productivity.*

INTRODUCTION

Walnut assortment both on the global scale and in Serbia is not rich, and it is mainly concentrated in major walnut growing regions. The largest walnut producers, Hungary, Romania and Bulgaria, are primarily focused on the assortment of their regions and they grow local, domestic cultivars (Mitrović et al., 2007). Interestingly enough, there are differences in the assortment in terms of vegetation onset. Owing to frequent occurrence of late spring frosts, there has been pronounced interest in mid-late and late cultivars among walnut producers (Korać et al., 1998).

Introduced French walnut cultivars with late vegetation onset (after May 5) grown in the Čačak region are the subject of a serious study. Their properties are uncommonly interesting for our region, i.e. besides late vegetation onset, they have sturdy trees, healthy leaves (no occurrence of *Gnomonia leptostyla* has been observed), their resistance to low winter temperatures is good, they are excellent croppers bearing quality fruits (Germain (1996) and Germain et al. (1997)). It is for the above reasons that major pomo-technological properties and productivity of newly bred French cultivars

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('Fernor', 'Fernet') have been investigated along with those of 'Marbot', 'Parisienne' and 'Franquette', and Slovenian cv 'Elit'.

MATERIALS AND METHODS

The studied walnut cultivars ('Marbot', 'Parisienne', 'Franquette', 'Fernor', 'Fernet', and 'Elit' as standard) were planted in 2001 (planting distance 10 x 10 m) in a trial field at a location in the region of Čačak. The introduced walnut cultivars were obtained from INRA Institute (Bordeaux, France). Grafted two-year old plants were of exquisite health status, trees in the planting were well maintained and all cultural practices were applied.

Monitoring of phenology included different stages, i.e. onset, full and end of leafing, inflorescence onset and shedding, emergence of female flowers and their pollination capacity, and fruit ripening. Productivity of the studied cultivars was monitored by successive collecting of ripe fruits (split shuck) and measuring. At the season's end, yield per tree was checked and calculated per unit of land. The paper presents the average three-year results (2005 – 2007) and includes phenological and pomological properties, and fruit and kernel yield per unit of land for each season. The results were subjected to statistical analysis, analysis of variance and LSD test.

RESULTS

The earliest leafing was observed in 'Parisienne' (May 2), and the latest in 'Fernor' and 'Elit' (May 6). Full flowering was first reported in 'Parisienne' (May 7), and the latest in 'Elit' (May 10), whereas the end of leafing was first in 'Marbot' and 'Parisienne' (May 10), and the latest in 'Elit' (May 13). In conclusion, in terms of leafing, control cv. 'Elit' was somewhat later than the studied French walnut cultivars (Table 1).

Inflorescence was the earliest in 'Marbot' (May 3), and the latest in 'Fernete' (May 9), whereas inflorescence onset was the first in 'Marbot' (May 9), and the latest Franqete (May 14). Female flowers with pollination capacity emerged first in 'Marbot' and 'Parisiene' (May 7), and latest in 'Fernete' and 'Elit' (May 9). As for fruit ripening (chuck split), 'Marbot' was the earliest (October 2) and 'Franquette' was the latest (October 10). In terms of inflorescence and flowering, some of the studied cultivars performed a few days earlier than standard cultivar Elit.

Table 1. Phenological properties of the studied walnut cultivars
Tabela 1. Fenološke osobine ispitivanih sorti oraha

Cultivar *Sorta*	Leafing *Listanje*			Inflores cence *Rese*		Female flower parts *ženski cvetovi Ženski cvetovi*		Ripe ning *Sazr evanje*
	Onset *Poče tak*	Full *puno*	End *kraj*	Pollin ation *praše njee*	Pollination *Opraši vanje*	Occurrence *pojava*	Pollin ation Capacity *Sposobni za opraš ivanje*	
Marbot	4.05	8.05	10.05	3.05	9.05	7.05	10.05	2.10

Parisienne	2.05	7.05	10.05	6.05	11.05	7.05	9.05	4.10
Franquette	4.05	8.05	11.05	9.05	14.05	8.05	10.05	6.10
Fernor	6.05	9.05	12.05	8.05	13.05	8.05	10.05	9.10
Fernete	5.05	8.05	12.05	7.05	12.05	9.05	11.05	10.10
Elit	6.05	10.05	13.05	8.05	12.05	9.05	11.05	8.10

Fruit length was the lowest in 'Parisienne' (36.8 mm), and the highest in 'Fernor' (43.0 mm). As for fruit width and thickness, it was the greatest in 'Marbot' (34.1 mm and 39.7 mm, respectively) and the lowest in 'Elit' (30.6 mm and 31.0 mm, respectively). The investigation revealed that the French cultivars were larger than standard 'Elit'. Analysis of variance and LSD test pointed to highly significant differences among the studied cultivars. In contrast, as regards thickness, and width in particular, high significances were observed only among certain cultivars.

Fruit weight was lowest in 'Elit' (10.6 g), and highest in 'Marbot' (12.4 g), whereas 'Fernete' and 'Franquette' had the highest kernel mass (5.3 g and 5.8 g). Kernel ratio was lowest in 'Fernete' (44.0%) and highest in 'Elit' (51.7%). Analysis of variance and LCD test revealed that only fruit weight of 'Marbot' and 'Fernor' had highly significant difference in comparison with the other cultivars. In respect to kernel mass, high significances were identified among all the studied cultivars.

As regards kernel content, high significances were observed in 'Elit' and 'Marbot'. Shell was the thinnest in fruits of 'Fernete' (1.0 mm), and the thickest in 'Fernor' and 'Franquette' (1.3 mm). Shell thickness was negligible, therefore statistical significances were not determined. Shell was in the shades of yellow, while kernel was light brown in all the studied cultivars (Table 2).

The cropping of walnuts grown in our region has not been particularly investigated. In our study, cropping was the lowest in 'Marbot' and 'Elit' (in their fifth leaf), i.e. (3 kg/tree), and it was the highest in 'Fernor' (6.0 kg/tree). In the following year, production was the lowest in 'Elit' (9.0 kg/tree), and the highest in 'Fernor' and 'Fernete' (14.0 kg/tree). In their third year, production per tree in 'Elit' and 'Fernor' was 14 kg/tree and 25.0 kg/tree, respectively. Analysis of variance and LSD test showed highly significant differences of 'Fernor', 'Fernete' and 'Franquette' as compared to the other cultivars in all the years of study (Table 3).

Table 2. Pomo-technological properties of the studied walnut cultivars
Tabela 2. Pomološko-tehnološke osobine ispitivanih sorti oraha

Properties *Osobine*		Cultivar/*Sortas*						LSD	
		Marbot	Fernor	Fernete	Franquette	Parisienne	Elit	0.05	0.01
Fruit *Plod* (mm)	Length *visina*	39.8	43.0	39.5	39.4	36.8	38.9	0.82	1.11
	Width *širina*	34.1	33.4	34.0	33.8	33.6	30.6	0.78	1.06
	Thickness *debljina*	39.7	33.7	33.1	31.1	32.4	31.0	0.99	1.35
Fruit weight (g) *Masa ploda*		12.4	11.8	11.9	12.0	11.0	10.6	1.34	1.82
Kernel mass (g) *Masa jezgre*		5.7	5.8	5.3	5.8	5.7	5.5	0.87	1.18

Ratio (%) *Randman*	46.0	49.3	44.0	48.0	51.5	51.7	7.28	9.86
Shell thickness (mm) *Debljina ljuske*	1.2	1.3	1.0	1.3	1.1	1.2	0.20	0.28
Shell colour *Boja ljuske*	SY	BY	SY	SY	BY	L		
Kernel colour *Boja jezgre*	LB	LB	LB	LB	LB	LY		

SY – straw yellow/*slamasto žuta;* LB- light brown/*svetlo braon;* BY – bright yellow/*svetlo žuta;* LY – Light yellow/*svetlo žuta;* L – Light/*svetla.*

Over the entire period of study, the total yield per tree was the lowest in 'Elit' (26.0) and the highest in 'Fernor' (45.0 kg). From the aspect of unit of land, the total production ranged between 2,600 and 4,500 kg/ha in the same cultivars. Similarly, statistical data analysis inferred highly significant differences between 'Fernor', 'Fernete' and Franquette' on the one hand, and the other cultivars on the other hand. Over the three-year period, the average yield per tree ranged between 8.7 kg ('Elit') and 13.7 kg ('Fernete'), while 'Fernor' (15.0 kg, i.e. from 870 to 1,370 kg, 1,500 kg/ha) had the highest average production per tree, which is in accordance with the previous results and statistical analysis. Measuring of kernel ratio showed the average yield per tree and unit of land. The lowest kernel content per tree was observed in 'Elit' (4.5 kg), and the highest in 'Fernete' (6.6 kg, 660 kg/ha) and 'Fernor' (7.4 kg, 740 kg/ha). In this case, kernel production in 'Fernor', 'Fernete' and 'Franquette' was significantly different in comparison with the other cultivars.

Table 3. Productivity of the studied walnut cultivars

Tabela 3. Rodnost ispitivanih sorti oraha

Season *Godina*		Productivity /*Rodnos*t kg/tree						LSD	
		Marbot	Fernor	Fernete	Franquette	Parisienne	Elit	0.05	0.01
V		3	6	5	5	4	3	0.90	1.22
VI		11	14	14	12	10	9	1.19	1.62
VII		16	25	22	21	15	14	1.36	1.84
Total yield *Ukupno plodova*	kg/tree *kg/stab.*	30	45	41	38	29	26	1.71	2.32
	kg/ha *kg/ha*	3,000	4,500	4,100	3,800	2,900	2,600	440.4	596.8
Average yield *Prosečno plodova*	kg/tree *kg/stab*	10	15	13.7	12.7	9.7	8.7	0.91	1.23
	kg/ha *kg/ha*	100	1,500	1,370	1270	970	870	82.13	111.3
Kernel ratio (%) *Randman jezgra*		46	49.3	44	48	51.5	51.7	7.28	9.86

Average kernel yield *Prosečno jezgre*	kg/tree *kg/stab*	4.6	7.4	6.6	6.1	5	4.5	0.76	1.03
	kg/ha *kg/ha*	460	740	660	610	500	450	76.21	103.27

DISCUSSION

The first results about newly developed French walnut cultivars Fernor and Fernet were published by Germain et al. (1996 and 1997). The cultivars were moderately vigorous, of late flowering and low susceptibility to bacteria. These cultivars bear fruits on lateral flowers, bear fruits early but more abundantly in comparison with 'Franquette'. Fruit weight in 'Fernor' and 'Fernete' ranges from 10 to 12 g and 11 to 13 g respectively, whereas kernel content in these cultivars is 49% and 54%, respectively. Similar results about these cultivars have been reported by Džuvinov et al., (2004), kernel ratio ranging from 42 to 47% and 48 to 52%, respectively. Under our conditions of growing, fruit weight and kernel ratio of fruits of these cultivars were lower, which is the result of the old age of studied tree, applied cultural practices and duration of the study. All the reports are in accordance with the results obtained by Mitrović et al. (2005) in the study of these cultivars.

Cultivar specificities of the other studied cultivars were reported when grown under our conditions, in terms of phenological and fruit properties. Observed differences in respect to fruit weight and size are due to the applied cultural practices (Korać et al., 1998).

Besides phenological properties (late vegetation onset), cvs 'Fernor' and 'Fernet' have very high cropping performance. Among the studied cultivars, these cultivars had the most abundant cropping and kernel yield. Thus, according to Korać et al. (1998), the average walnut production in walnut growing regions of Serbia amounted to 3, 5 and 8 kg/tree (in the fifth, sixth and seventh year of production, respectively).

CONCLUSION

Comparative analysis of major pomo-technological properties of newly developed French cultivars 'Fernor' and 'Fernete' and standard cvs 'Marbot', 'Parisienne', 'Franquette' and 'Elit' revealed that the former have more favourable phenological characteristics and higher production capacity. In comparison with the latter, 'Fernor' and 'Fernete' also had more favourable vegetation onset and smaller fruits with lower fruit weight. These cultivars also displayed the highest production and kernel yield, which is their most pronounced specificity. Over the three-year period of study, the average yields per tree ranged from 8.7 kg ('Elit') to 13.7 kg ('Fernete'), and the highest production per tree was 15.0 kg ('Fernor'), i.e. (870 to 1370 kg, 1500 kg/ha). The lowest kernel content per tree was observed in 'Elit' (4.5 kg), and the highest in 'Fernete' (6.6 kg, 660 kg/ha) and 'Fernor' (7.4 kg, 740 kg/ha).

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OSOBINE FRANCUSKIH SORTI ORAHA U AGROEKOLOŠKIM USLOVIMA ČAČKA

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Izvod

Ispitivane su važnije pomološko-tehnološke i produktivne osobine standarnih francuskih soti oraha (Marbot, Parisienne i Franqete), novijih (Fernor, Fernet) uporedo sa standarnom sortom Elit, na području Čačka (centralna Srbija). Novije sorte (Fernor, Fernet) su u odnosu na ostale sorte imale povoljnije, vreme početka vegetacije i sitnije plodove, sa manjom masom. Među ispitivanim, ove sorte su ostvarile najveću rodnost i najveći prinos jezgre, što je njihova posebna vrednost.. Za trogodišnji period ispitivanja, prosečni prinosi po stablu bili su od 8,7 kg (Elit) do 13,7 kg (Fernete) i najviše 15,0 kg (Fernor), ili od 870 do 1370 kg, odnosno 1500 kg/ha. Najmanji sadržaj jezgre po stablu bio je kod sorte Elit (4,5 kg), a najveći kod sorte Fernete 6,6 kg, (660 kg/ha) i sorte Fernor (7,4 kg) ili 740 kg/ha.

Ključne reči: orah, sorte, fenologija, plod, rodnost.

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APPLICATION OF PRINCIPAL COMPONENT ANALYSIS TO REALIZE VARIABILITY OF CHEMICAL PROPERTIES OF CHERNOZEM*

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SUMMARY: This paper presents a practical application of the principal components analysis (PCA), as one of the methods of multivariate analysis used for reducing the dimensionality of the problem (reducing a set of a large number of variables down to a smaller number of mutually uncorrelated linear combinations). Seventeen soil properties from 95 samples were analyzed in order to single out the soil properties which have significant impact on soil variability. The paper also presents a comparison of certain results obtained by using this method and by the application of factor analysis. This research showed that heavy metals contribute most to the first principal component, organic matter contributes most to the second, while inorganic compounds (oxides) contribute most to the third component. Accordingly, it is these variables that contribute most also to accountability of the total variability of the observed phenomenon. The determined accountability of the total variability by the first principal component was around 35%, by the second around 20%, while with the third it was 14%, which means that the first three dimensions together account for around 70% of the total variability of the observed set of variables.

***Key words**: multivariate analysis, principal component analysis, variability, eigenvalues, soil, soil properties.*

INTRODUCTION

One of the main objectives in soil analysis is an integrated overview of its properties as indicators of soil fertility. The methods used in multivariate statistics are considered to be powerful tools for obtaining these integrated approaches helping researchers to obtain as much information as possible from available data. Sena et al. (2002) argue that soil scientists should be better acquainted with multivariate methods, which, in

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comparison to univariate and bivariate methods, greatly increase the capacity of extraction and interpretation of soil-related data.

Multivariate analysis is an analysis of a large number of variables, which enables their examination and quantification, as well as identification of their dependence, *i.e.* links between a larger number of variables.

Principal components analysis (PCA) is one of many methods of multivariate analysis. This method enables transformation of a large number of variables into a smaller number of latent variables (principal components, PCs) which are not inter-correlated. These transformed variables represent linear functions of input variables.

Kovačić (1994) stresses the importance of application of the PCA in socio-economic research where it is necessary to reduce an exceptionally large number of indicators of this type to a small number of representative indicators. Many studies (Chang et al., 2001; Visconti et al., 2009; Kummer et al., 2010) showed purposefulness of applying the PCA in examining interrelation of soil properties and their impact on soil variability.

According to Koutsoyiannis (1977), the PCA is just a special case of a more general factor analysis (FA) model. This author argues that formation of a series of variables from the existing variables is the objective of the PCA. The first linear combination called the principal component PC takes the maximum possible share of the total variability in the series consisting of all input variables, the second PC takes the maximal possible remaining variability, and the third takes then the maximal possible remaining variability, etc.

The PCA has a wide application in sociological, economic, biological research as well as the research connected to crop and animal production. The advantages of applying the PCA in such studies are especially important when a large number of variables are to be analyzed. The benefits of PCA analysis and biplot are even greater when applied to larger sets of soil-related data (Sena et al., 2002).

One of the most important applications of the PCA is in regression analysis, where, apart from reducing dimensionality in multiple regression analysis, it also reduces multicollinearity. This stems from the fact that multiple regression analysis is performed using a small number of transformed mutually uncorrelated variables.

The aim of this research is to apply the PCA on a practical example related to soil research and to single out soil proprieties which have statistically significant impact on soil variability.

MATERIAL AND METHODS

Research site and sampling methods

Sampling was conducted on the territory of Srem while the localities on which the samples were taken were determined with a net of squares of 4 x 4 km, which was drawn on the existing pedologic map of Vojvodina R 1: 50,000. In this way, every sample is representative for 1 600 ha of soil. In this research the properties of different soil types were examined, and for the purposes of this paper the data for the soil type chernozem were used. Chernozem belongs to the class of humus-accumulative soils (Miljković, 1996) with the average humus accumulation in Vojvodina about 165 t C ha^{-1} up to 100 cm of depth (Manojlović et al., 2010). Chernozem is naturally fertile soil but

it requires significant amount of fertilizers in order to obtain high yield (Bogdanović et al., 2010).

The research included a total of 95 samples. Considering high diversity of the parent material of soil and the climate in Srem, the samples collected in this way are significantly different regarding their physical and chemical properties, thus representing a broad spectrum of different properties for the analysis even within the same soil type. Soil samples were collected in disturbed state in accordance with the propositions of the System of Soil Fertility Control, *i.e.* with an soil probe, up to the depth of 30 cm, in accordance with the system of circular control plots. Prior to chemical analysis, the samples were sieved through a 2 mm sieve and homogenized. Each sample is also accompanied by the data on its precise position with the coordinates and altitude, since GPS technology was used for location of the sampling sites.

Soil analyses

In each analyzed sample, 17 soil properties, *i.e.* variables were analyzed: pH in KCl, pH in water, $CaCO_3$, humus, total nitrogen, P_2O_5, K_2O, copper, cobalt, manganese, arsenic, chromium, nickel, cadmium, lead, zinc and iron. The pH values in KCl and in water were determined potentiometrically, while the values of $CaCO_3$ content were determined by volumetric method on Scheibler's calcimeter. The humus content was determined by wet oxidation of organic carbon with $K_2Cr_2O_7$ and multiplying the carbon with a coefficient (1.724), while the content of the total nitrogen was obtained by calculation from the humus content. The amount of easily soluble P_2O_5 and K_2O in soil was determined by Al-method. The total content of microelements and heavy metals (Cu, Zn, Mn, Pb, Co, Cr, Ni, Cd, As and Hg) extracted with concentrated HNO_3, was determined using inductively coupled plasma on ICP-OES VistaPro Varian, while the Fe content was determined using AAS.

Statistical analysis

Detailed descriptive statistics of the results of examined samples was conducted. The principal components analysis (PCA) was performed in the following way: a) correlation matrix of soil variables was developed; b) varimax rotation was applied on the resulting matrix; c) component defining variables were selected (variables that saturate most the PC). Descriptive analysis of the data and the PCA were performed using the software package STATISTICA 10.0.

RESULTS AND DISCUSSION

Descriptive statistics of analyzed soil properties (variables) are given in Table 1. Projection of the variables in the factorial plane (Graph. 1) indicates that the variables cobalt, lead and cadmium contribute most to the first PC (which accounts for 34.79% of variability), and thus to the total variability of the basic set. The second PC (which accounts for 19.93% of variability) is contributed most by the variables pH in KCl, pH in H_2O, humus and the total nitrogen. In total, the first and second PCs account for 54.72% of the total variability of the observed set. The third PC accounts for 14% of the total variability of the observed set. Accordingly, three PCs together account for around 70% of the total variability of the initial set of variables. With the first three components (Giuffré et al. 2006) explained 90% of the overall variation in Argentinian Argiudolls. The greatest contribution to the third PC is given by the variables P_2O_5 and K_2O. Onweremadu et al. (2007) argue that by applying PCA on 12 variables in the soils of Nigeria,

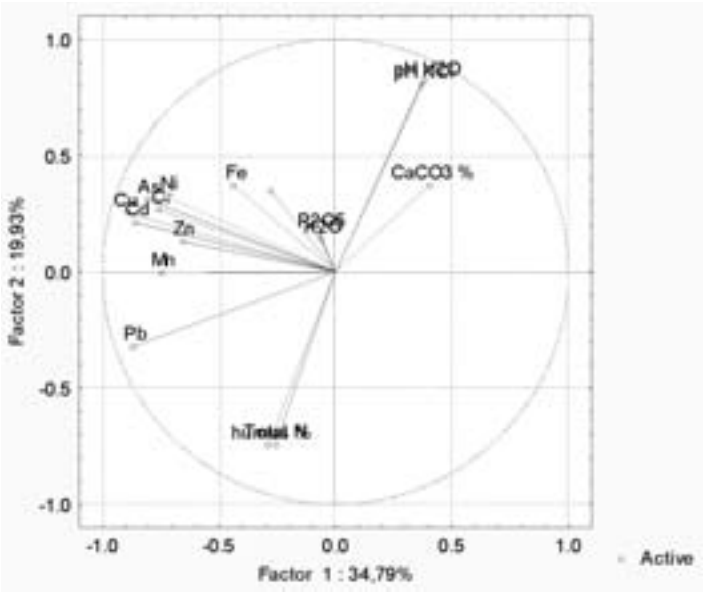
28.5% of the total variance was accounted for with pH value (first PC), and 27.3% with organic carbon (second PC), which means that pH values and organic carbon are highly important for sustainability of these soils.

Table 1. Descriptive statistics of soil properties
Tabela 1. Deskriptivna statistika osobina zemljišta

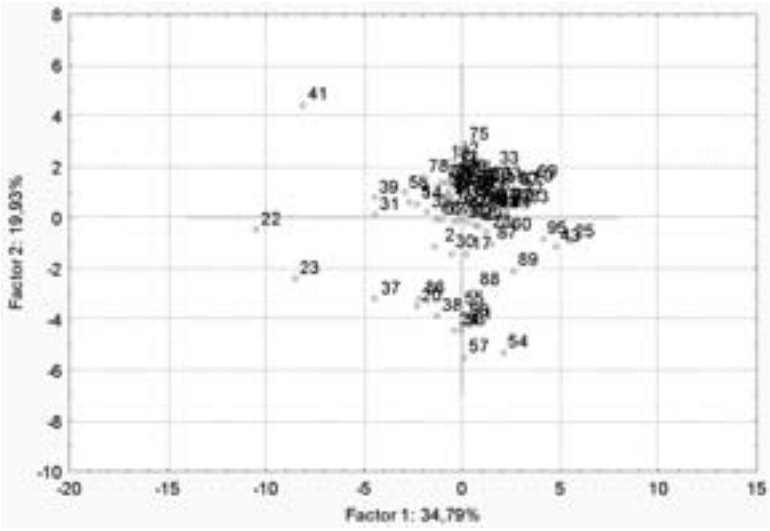
Soil properties / *Osobine zemljišta*	Mean *Srednja vrednost*	Standard deviation *Standardna devijacija*
pH (KCl)	6.60	1.117
pH (H_2O)	7.79	1.003
$CaCO_3$ (%)	5.88	8.060
Humus (%)	2.90	1.060
Total / *Ukupni* N(%)	0.21	0.058
P_2O_5 (mg/100g)	18.62	33.644
K_2O (mg/100g)	21.25	15.905
Cu (mg/kg)	24.34	13.792
Co (mg/kg)	12.50	3.609
Mn (mg/kg)	627.10	367.605
As (mg/kg)	7.74	2.949
Cr (mg/kg)	31.92	14.309
Ni (mg/kg)	46.55	32.330
Cd (mg/kg)	0.34	0.094
Pb (mg/kg)	22.13	5.714
Zn (mg/kg)	48.40	18.086
Fe (mg/kg)	25258.42	3349.903

Factor coordinates of variables based on the coefficients of correlation indicate that the highest values of these coefficients are for the variables cobalt (-0.90), lead (-0.87) and cadmium (-0.86) (compared with the factor no. 1) and variables pH in KCl (0.82), pH in H_2O (0.81) and humus and the total nitrogen (-0.74 each) (compared with the factor 2).

Factor coordinates of individual observations (Graph. 2) indicate that the total variability is influenced most by the samples numbered 22 (-10.5), 23 (-8.5), 41 (-8.1), 25 (5.5) and 43 (4.8) (for factor no. 1) and the samples numbered 57 (5.51), 54 (5.32), 41 (4.44), 24 and 53 (4.43) and 21 (4.23) (for factor no. 2).



Graph. 1. Projection of variables in factorial plane
Grafikon 1. Prikaz promenljivih u faktorskoj ravni

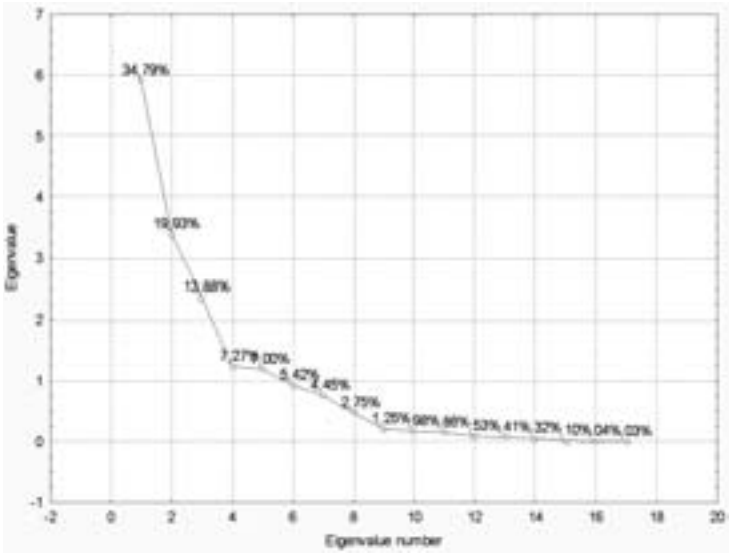


Graph. 2. Projection of cases in factorial plane
Grafikon 2. Prikaz pojedinačnih posmatranja u faktorskoj ravni

Eigenvalues (Graph. 3) indicate that the first factor accounts for slightly over one third of the total variability of the observed set (34.78 %). The second factor accounts for 19.93 % variability, the third for 13.88 %, the forth 7.27 %, the fifth 7.00 %, the sixth 5.42 %, the seventh 4.45 %, the eighth 2.74 %, the ninth 2.03 %, the tenth 0.98 %, the eleventh 0.86 %, the twelfth 0.53 %, the thirteenth 0.41 %, the fourteenth 0.32 %, the

fifteenth 0.10 %, the sixteenth 0.04 % and the seventeenth 0.03 %. The first three factors account for slightly over two third of the total variability (68.60 %) (Table 2).

Input values (unrotated) for the factors in application of FA show that variability is accounted for by the same variables as with the PCA.



Graph. 3. Scree- plot of eigenvalues
Grafikon. 3. Scree dijagram svojstvenih vrednosti

Rotated input values (varimax rotation) for the factors in FA indicate that the first factor is largely accounted for by the variables cobalt (0.93), cadmium (0.88) and arsenic (0.86). The second factor is most accounted for by the variables pH in H_2O (-0.91), pH in KCl (-0.89) and humus (0.80). The factor in FA represents linear combination of a larger number of variables.

Table 2. Eigenvalues on the basis of correlation correlation matrix
Tabela 2. Svojstvene vrednosti na bazi korelacione matrice

Eigenvalue *Svojstvena vrednost*	% Total variance *% Ukupna varijacija*	Cumulative eigenvalue *Kumulativna sv. vrednost*	Cumulative % *Kumulativ %*
5.913948	34.78793	5.91395	34.7879
3.388275	19.93103	9.30222	54.7190
2.359530	13.87959	11.66175	68.5985
1.235977	7.27045	12.89773	75.8690
1.190190	7.00112	14.08792	82.8701
0.921563	5.42096	15.00948	88.2911
0.756603	4.45060	15.76609	92.7417
0.467134	2.74784	16.23322	95.4895
0.211650	1.24500	16.44487	96.7345
0.166863	0.98155	16.61173	97.7161
0.146395	0.86114	16.75813	98.5772
0.089806	0.52827	16.84793	99.1055

0.068879	0.40517	16.91681	99.5107
0.054654	0.32150	16.97147	99.8322
0.017010	0.10006	16.98848	99.9322
0.007134	0.04197	16.99561	99.9742
0.004389	0.02581	17.00000	100.0000

CONCLUSION

On the basis of the performed statistical analysis using PCA it was determined that the variables cobalt, lead and cadmium (making the smallest angle with the axis of the first component), contribute most to the first PC (which accounts for 34.79% of variability), and thus also the total variability of the basic set. The second PC (which accounts for 19.93% of variability) is contributed most by the variables pH in KCl, pH in H_2O, humus and the total nitrogen. The third PC is most contributed by the variables P_2O_5 and K_2O.

In total, the first, second and third PCs account for around 70% of the total variability of the observed set of variables.

This research showed that heavy metals contribute most to the first PC, organic matter contributes most to the second PC, while inorganic compounds (oxides) contribute most to the third PC. Accordingly, these components contribute most also to accountability of the total variability of the observed phenomenon.

Application of the PCA in this research points to the conclusion that the observed and quantified variables can be used in scientific and experimental work for examining the impact of a larger number of variables in a specific case, *i.e.* in examples related to soil research.

The practical significance of this paper is that the results obtained by application of the PCA can provide better and more detailed understanding of the impact of certain factors on the observed phenomenon.

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ANALIZA HEMIJSKIH SVOJSTAVA ČERNOZEMA PRIMENOM METODE GLAVNIH KOMPONENATA

SLOBODAN NIĆIN, LJILJANA NEŠIĆ, VLADIMIR ĆIRIĆ

Izvod

U ovom radu prikazana je praktična primena metoda glavnih komponenata (PCA) kao jednog od metoda multivarijacione analize koji se koristi kod smanjenja dimenzionalnosti problema (svođenja skupa velikog broja promenljivih na manji broj međusobno nekoreliranih linearnih kombinacija). Sedamnaest svojstava iz 95 uzoraka zemljišta je analizirano da bi se izdvojila svojstva zemljišta koja imaju značajan uticaj na njegovu varijabilnost. Takođe, u radu je izvršeno i upoređenje nekih od rezultata dobijenih primenom ovog metoda i dobijenih primenom faktorske analize.

U ovom proučavanju pokazalo se da teški metali najviše doprinose prvoj glavnoj komponenti, organska materija najviše doprinosi drugoj, dok neorganska jedinjenja (oksidi) najviše doprinose trećoj glavnoj komponenti. Samim tim, ove promenljive najviše doprinose i objašnjenosti ukupnog varijabiliteta posmatrane pojave. Konstatovana objašnjenost ukupne varijabilnosti pomoću prve glavne komponente iznosila je oko 35 %, kod druge oko 20 %, dok je kod treće iznosila oko 14 %, što znači da je ukupno pomoću prve tri dimenzije objašnjeno oko 70 % ukupne varijabilnosti posmatranog skupa promenljivih.

Ključne reči: multivarijaciona analiza, metod glavnih komponenata, varijabilitet, svojstvene vrednosti, zemljište, osobine zemljišta.

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DEVELOPMENT OF THE FIRST SPRING CANOLA LINES WITH RESISTANCE TO SCLEROTINIA STEM ROT

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SUMMARY: Spring canola is susceptible to Sclerotinia stem rot (SSR) and fungicides are used to control disease in the field. S1 per se recurrent selection and greenhouse and field screening were utilized in order to improve resistance of spring canola to SSR. Due to an apparent lack of trait improvement in Cycles 1 to 4, Population T was opened to convergent introgression of new resistance sources and subjected to selection utilizing Field Limited-Term Incubation (FLTI) method. These changes resulted in trait improvement beginning in Cycle 5, gradually leading to a near complete shift towards resistant field reaction by Cycle 10. On a scale of 1 (highly susceptible) to 9 (completely resistant) for SSDIS (Sclerotinia sclerotiorum Disease Incidence Severity), the Population T mean increased significantly from 2.4 in Cycle 5 to 6.7 in Cycle 10. The improved lines have potential for use in developing hybrids with field resistance to Sclerotinia.

Key words: *Sclerotinia, canola, screening, FLTI, Population, resistance.*

INTRODUCTION

Sclerotinia sclerotiorum infects over 400 species of plants, including numerous economically important crops such as *Brassica* species, sunflowers, dry beans, soybeans, field peas, lentils, lettuce, and potatoes (Boland and Hall, 1994). The fungus causes Sclerotinia stem rot of canola (SSR). Canola is a *Brassica napus* oilseed type having a low level of glucosinolates and erucic acid in the seed (Daun, 1984).

Fungal sclerotia germinate carpogenically producing apothecia that release windborne ascospores. Ascospores cannot infect leaves and stems of canola directly but use dropped flower petals as a nutrient source for germination and infection of leaves (Heran et al., 1999). The disease is favoured by moist soil conditions and temperatures of 15-25 ^{o}C, prior to and during canola flowering. On average, yield losses equal 0.5 times the percentage of infection (Del Rio et al., 2007). Canola quality spring and winter products in North America and Europe are considered susceptible to SSR and no

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significant improvements have been achieved through breeding. Therefore, fungicide applications are used to control disease (Morall et al., 1985).

Differences in field resistance to SSR in crops like white beans and soybeans can be attributed to canopy differences and/or partial resistance. Hunter et al. (1981) and Cline and Jacobsen (1983) described the use of a Limited-Term Inoculation (LTI) indoor method on white beans and soybeans. LTI was developed after observations that Sclerotinia kills all plants under favorable conditions irrespective of their level of resistance. Limited exposure to the fungus under favorable conditions was used to determine if partial resistance was involved in reduction of field symptoms.

Some semi-winter Japanese cultivars of rapeseed have partial stem resistance to SSR. Partial stem resistance was detected with stem testing in comparison with winter canola variaties (Brun et al., 1987). Some Chinese semi-winter varieties of rapeseed are partially resistant to SSR as well. The partial field resistance in Chinese varieties originated mostly from the rapeseed variety Zhong you 821. Despite improvements in partial resistance in Zhong you 821, its reaction to disease is variable under environmental conditions favorable for development of SSR (Li et al., 1999). Fungicide application is recomended to protect rapeseed crops against SSR in China.

Patel et al. (1991) demonstrated that recurrent selection can succesfully improve yield and % oil in populations of canola. The introduction of strict quality and blackleg resistance standards in the Canadian registration system after 1991 prompted the use of S1 *per se* as a strategy for trait improvement (Patel et al., 1999). S1 *per se* recurrent selection is suitable for trait improvements and was used successfully to improve a number of traits including blackleg resistance and seed oil and protein content.

Between 1986 and 1988, Pioneer Hi-Bred acquired a collection of semi-winter rapeseed Germplasm from Plant Genetic Resources (PGR) Canada, The Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan and USDA North Central Regional Plant Introduction Station at Iowa State University in the USA. Initial screening work and some of the sources of resistance were based on the research of Brun et al. (1987). The goal of the efforts was to develop spring canola lines with partial stem resistance to SSR.

Lack of available resistance to SSR in elite spring canola varieties and the apparent success of S1 *per se* in trait improvement triggered initiation of this trait development project in 1993.

MATERIAL AND METHOD OF THE STUDY

Population T development

Lines originating from two partially stem resistant Japanese sources as well as elite lines with low field susceptibility were used to assemble Population T. The synthesis of cycle zero (C_0) was as described by Patel *et al.* (1991). Each cycle was completed in one year following three steps: (a) S0 seed production via intercrossing in the greenhouse of S1 field selections during fall; (b) S1 seed production in the greenhouse during winter and (c) S_1 plant evaluation in the field during summer. SSR pressure was applied at all three steps in each Cycle through indoor stem screening (a, b) and field selection (c).

The first three cycles of Population T were tested under moderate SSR pressure in the field using a relatively small number of lines (90-150). The fourth cycle was evaluated under high pressure for lodging which increased disease severity in the field. The

results of the first three cycles did not show a significant improvement in resistance in the Population T compared with the control varieties. The number of lines in the fourth cycle was increased to 1100 in order to confirm the trends of the first three cycles, and prepare for an opening of Population T to new introgressions. Material in Cycle 4 was selected for better resistance to SSR and stem lodging as well as other agronomic and quality characteristics.

Cycles 0 to 4 were closed Cycles. Selections from Cycle 4 were used for the production of Cycle 5 with the opening of Population T and BC1 introgression of a third Japanese source of partial stem resistance. New sources were incorporated into Cycles 5 to 8 via BC1 convergent introgression.

Lines for intercrossing were selected based on *Sclerotinia sclerotiorum* Disease Incidence Severity value (SSDIS), agronomic performance of plants and quality characteristics. Selections were further reduced indoors before intercrossing on the basis of individual stem reaction to SSR. Plants originating from intercrossed seed were also subjected to SSR testing and selection indoors, thus producing S_1 seed for field evaluation.

In each cycle, the S_1 plant evaluation was carried out in replicated nursery rows planted at two locations in Ontario. One location was used for SSR screening while the other was utilized for visual agronomic score (1=poor, 9=excellent). At harvest, 15 g of S2 seed was harvested from each selected entry and analyzed for oil % and protein % using Near Infrared Spectroscopy.

During each year, two check varieties were included in field Sclerotinia trial as running checks (augmented RCBD design) while evaluating the S_1s. 46A65 susceptible (S) check was used from Cycle 1 to Cycle 10. 46A76 moderately susceptible (MS) check was used from Cycle 5 to Cycle 10. 46A65 (S), 46A76 (MS) and 44A89 highly susceptible (HS) were used as check entries covering the range of existing variation (Table 1). As such, they were randomized within experiment and their mean (X_C) was calculated (Table 2).

Analysis of variance (ANOVA) was performed for the SSDIS trait after adjusting for alternating running check deviations (running check every 6th row in the field). Since SSDIS frequency values (Figure 1) were adjusted with running checks, no adjustment was done with X_C. Thus, deviation of X_C from expected mean shows actual variation in exerting extreme disease pressure in the field in a given season.

For SSDIS in each cycle, the Population T mean (μ) and phenotypic standard deviation (σ) were calculated as well as other standard deviation-based parameters. Heritability % was derived from ANOVA output based on genetic and phenotypic variance values. The heritability estimates are not available for Cycle 10 due to a loss of one replicate in the field.

Indoor disease stem screening

Indoor screening for stem reaction was conducted using the LTI principles of Hunter et al. (1981) and Cline and Jacobsen (1983). The basic premise was development of 20mm long lesions on stems of susceptible plants with a removal of inoculum and cessation of high humidity after that. This target was adequate for a differentiation of partial stem resistance (based on unreported data). Over the span of 11 years (1995-2005) the methodology has evolved from the use of mycelial PDA agar plugs attached to stems via parafilm into the usage of low nutrient PDA mycelial agar plugs attached to the stem with an entomological needle.

Field disease screening

Field screening based on natural inoculum commenced with Cycle 1 in 1996 and finished with Cycle 4 in 1999. This type of screening was conducted under moderate SSR pressure in an irrigated field with a heavy load of sclerotia in the soil. Cycle 5 testing in 2000, as well as the opening of Population T for convergent introgressions, was a starting point for standardized field LTI (FLTI) testing until Cycle 10 evaluation in 2005.

FLTI method

A mycelial seed carrier was produced as per Kim et al. (1999). Instead of oat seeds, Niger seed (*Guizotia abissinica*) was utilized as a carrier. The application of *Sclerotinia*-colonized Niger seeds was done by fertilizer spreader at the rate of 20kg/ha during late flowering, to mimic petal distribution in the leaf canopy. A sprinkler system with nozzles that produce misting droplets of water was used to maintain moisture in the field. The system was controlled with leaf moisture sensors (Campbell Scientific (Canada) Corp, Edmonton, Alberta, Canada). Ground water was used for irrigation of fields. Ground water was found to be inhibitory to *Sclerotinia* in years without natural rainfall. Deionized water (DI) treatment (Siemens Canada Limited, Brampton, Ontario, Canada) enabled *Sclerotinia* development under such conditions. Finally, the field was covered with fine netting to modify the environment under unfavourable conditions for the development of SSR.

Rating Field Reactions

The FLTI method is based on monitoring the reactions of running checks 46A65/46A76 planted at regular frequency within testing material with five rows of tested material and the sixth row being an alternating check. When these lines reached disease incidence thresholds achieved under natural conditions of extreme pressure (Table 1), rating was performed. Each plant was individually assessed for disease severity (*Sclerotinia sclerotiorum* disease severity-SSDS) according to the scale 1 to 9 (1=dead plant, 8=small lesion, 9=no symptoms). Such data allow calculation of % of diseased plants (*Sclerotinia sclerotiorum* disease incidence % - SSDI %). Parameters SSDI% and SSDS were used to derive an index parameter SSDIS (*Sclerotinia sclerotiorum* disease incidence severity) relative to location adjusted performance of 46A65 and 46A76 (expected SSDI%=70% and 60% respectively).

Table 1. Measuring field performance under extreme disease pressure
Tabela 1. Ocene intenziteta oboljenja pod ekstremnim pritiskom parazita

Rating SSDIS *Ocena SSDIS*	Category *Kategorija*	Disease incidence SSDI% *% obolelih biljaka SSDI%*	Checks *Kontrolne sorte*
1.0	Highly susceptible *Visoko osetljiva*	≥80	44A89=1
1.1 – 2.0	Susceptible *Osetljiva*	79 – 70	46A65=2
2.1 – 3.0	Moderately susceptible *Umereno osetljiva*	69 – 60	46A76=3
3.1 – 4.0		59 – 50	
4.1 – 5.0	Moderately resistant *Umereno otporna*	49 – 40	
5.1 – 6.0		39 – 30	Single fungicide application *Jedno prskanje fungicidom*
6.1 – 7.0	Resistant *Otporna*	29 – 20	
7.1 – 8.0		19 – 10	Two fungicide applications *Dva prskanja fungicidom*
8.1 – 9.0	Highly resistant *Visoko otporna*	9 – 0	

RESULTS

Table 2 shows an increase in the number of field-tested S1 progenies from the lowest number of 176 in Cycle 5 to the highest number of 600 in Cycle 7. The number of lines in Cycle 10 was lowered to 390 as it became obvious that trait improvemen,w was progressing. The number of intercrossed selections was determined by the number of available S1 lines meeting upward moving thresholds of SSDIS without compromising other traits. The lowest number of intercrossed lines was recorded in the synthesis of Cycle 8 (16) and the highest in the synthesis of Cycle 6 (38). Cycles opened for convergent introgressions (5-8) had either one (5 and 6) or two sources introgressed (Cycle 7) while Cycle 8 had six sources introgressed within its synthesis. The analysis of variance for each cycle showed mean squares due to genotypes to be significant for SSDIS. The population mean (μ), standard deviation (σ), mean of the selected lines (X_S), and mean of the checks (X_C) are presented in Table 2. Standard deviation range was 1.0 to 1.1 with the exception of Cycle 7 (1.5). SSDIS improvement trends, based on (μ-X_C) and (μ-X_C)/σ comparisons between the cycles, showed that the recurrent selection was gradually improving SSDIS values over Cycles 5 to 10. Broad sense heritability estimates ranged from as low as 21.5 for Cycle 7 to as high as 50.8 for Cycle 8.

Cycles 5 to 8 were opened for significant introgressions. Population T was closed for Cycles 9 and 10. SSDIS Population T-based results are shown in Table 2. The SSDIS frequency distribution is presented within Figure 1. In contrast to previous cycles, where there was no significant increase in resistance, Cycle 5 resulted in the first S1 lines with SSDIS values of 5 and higher. The trend of increasing resistance continued until the 10th cycle where almost the entire Population T exceeded the best lines of Cycle 5. On the scale 1 (highly susceptible) to 9 (completely resistant) for the SSDIS parameter, the Population T mean increased significantly from 2.4 in Cycle 5 to 6.7 in Cycle 10. The SSDIS population mean increased gradually across Cycles with the exception of Cycle 7 where it was 4.3 vs. 4.5 for Cycle 6. The SSDIS mean of the selected lines (X_S) was increased steadily from 3.6 for Cycle 5 to 7.9 for Cycle 10.

Table 2. SSDIS parameter for Population T – Cycles 5 (2000) to Cycle 10 (2005)
Tabela 2. Srednje vrednosti parametra SSDIS Populacije T tokom ciklusa od 5 (2000.god) do 10 (2005.god)

Pop T Cycles *Ciklusi populacije T*	5	6	7	8	9	10
Number of S1 lines *Broj S1 linija*	176	238	600	585	585	390
Mean of S_1 (μ) *Srednja vrednost SSDIS S_1 linija(μ)*	2.4	4.5	4.3	5.6	5.9	6.7
Range of SSDIS values *Raspon SSDIS vrednosti*	1.0-6.0	1.5-7.0	1.0-8.2	1.9-8.1	1.7-8.1	1.1-8.4
Std. Dev. (σ) *Standardna devijacija (σ)*	1.1	1.1	1.5	1.1	1.0	1.1
Mean of Checks (X_C) *Sred. vrednost SSDIS kontrolnih linija(X_C)*	2.0	3.2	2.8	2.9	2.6	1.7
Number of intercrossed selections *Broj ukrštenih selekcija*	38	24	16	26	32	23
Mean of intercrossed selections S_1 (X_S) *Sred. vrednost ukrštenih selekcija S_1 (X_S)*	3.6	5.6	6.9	7.1	7.4	7.9

Number of new sources introgressed *Broj unešenih novih izvora otpornosti*	1	1	2	6	N/A	N/A
Heritability % / *Heritabilnost*	46.7	38.2	25.1	50.8	46.4	N/A
$(X_S-\mu)/\sigma$	1.07	1.07	1.73	1.37	1.51	1.12
$(X_S-X_C)/\sigma$	1.4	2.3	2.7	3.9	5.0	5.6
$(\mu-X_C)/\sigma$	0.4	1.2	1.0	2.6	3.5	4.5

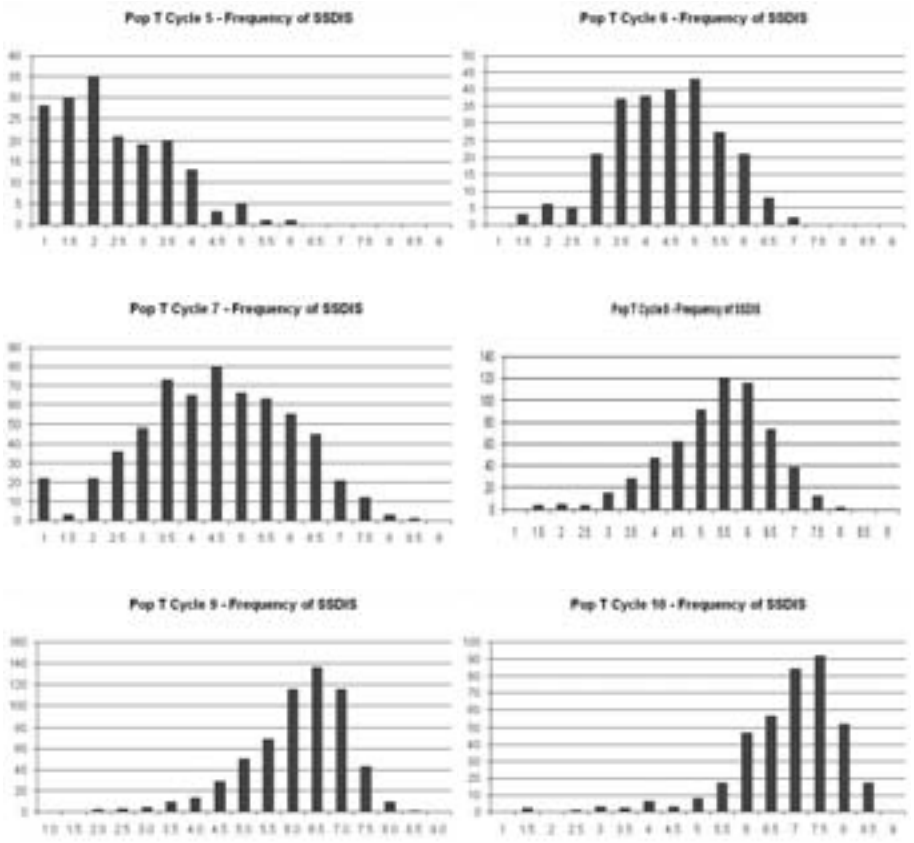

Figure 1. Distribution of SSDIS parameter through Population T Cycles 5 to 10 (2000-2005) Y axes - Frequency of S1lines; X axes – SSDIS (1-9)

Grafikon 1. Frekvencije vrednosti SSDIS Populacije T i njihovih selekcija u Ciklusima 5,6,7,8,9 i 10 Kordinatna osa Y – Frekvencija S1 linija;Kordinatna osa X – SSDIS (1-9)

DISCUSSION

Patel et al. (1999) used *S1 per se* recurrent selection to improve canola traits successfully. Spring canola is susceptible to SSR. Population T work begun in 1993 with the specific goal of increasing field resistance to SSR. The initial Population T was based on a combination of less susceptible elite lines and lines carrying partial stem resistance from two Japanese sources. Despite the selection and testing in Cycles 1 to 4,

there was no obvious improvement in the level of field resistance compared to the control lines. A limited level of genetic resistance in Population T and an inadequate selection pressure/methodology were identified as the main reasons for the lack of progress. Access to further sources of resistance, their convergent introgression and use of the FLTI methodology elevated the SSDIS performance in Cycles 5 to 10.

The FLTI method with its running check thresholds enabled the establishment of the extreme disease pressure while avoiding excessive SSR incidence and severity in the field. Hunter et al. (1983) pointed out that Sclerotinia kills all plants under continuous exposure and favourable conditions. On the other hand, lower pressure in the field has a potential to enable escapes, diluting selection quality and ability to make sustainable phenotypic/genetic gains. Table 1 describes the extreme pressure of disease in the field. One application of fungicide protects the crop for two weeks as per label. Extreme disease pressure occurs in years where flowering is extended due to wet/cooler conditions and two fungicide applications are needed. On top of that disease pressure, the FLTI method standardizes data collection so comparable data is generated across years. Other aspects of the FLTI method, like sensor-based irrigation, water treatment and netting enclosure were critical in years with unfavourable weather conditions. The methodology of infection is important for success in developing canola lines with resistance to SSR. By mimicking the natural infection pathway of Sclerotinia, FLTI enables selection of material with field resistance, ultimately the most important parameter in farmers' fields.

Population T was opened for convergent introgressions after Cycle 4 and exposed to a combination of extreme pressure and the FLTI method. This led to significant improvements in the resistance of Population T and selection of improved S1 lines in Cycle 5 (SSDIS highest value 5.5) to cycle 10, where 348 lines out of total of 390 had SSDIS score greater than 5.5. Artificial selection for high levels of horizontal resistance should provide almost complete control of disease, provided there is adequate genetic variability (Robinson, 1996). Considering that FLTI detects performance under highest possible field pressure, near-complete resistance in the best progenies of cycle 10 is evident. Population T yielded canola lines with field resistance significantly reducing disease when compared with the mean of checks in Cycle 10 (8.4 vs. 2.6). The purpose of indoor screening was important in preparation of sources with partial stem resistance as well as for elevating frequency of the progenies with better stem resistance before intercrossing of selections (S1to S0) and before field testing (S0 to S1).

Population T (Figure 1.) shows a relatively small shift in resistance in Cycle 7, and Cycle 8 to a lower extent. This may have been partly due to significant introgressions of new sources of resistance as well as their dissimilarity to existing sources within Population T. It is also possible that opening of Population T to convergent introgressions may have introduced some less desirable alleles, as it may be seen from Cycles 7 and 8. Table 2 as well as Figure 1 show that Cycle 7 is an 'outlier' relative to other Cycles. The SSDIS standard deviation in Cycle 7 was higher than for other Cycles (1.5 vs. 1.0-1.1), demonstrating a dispersed population as can be seen from Figure 1. Although the population mean value was reduced from 4.5 (Cycle 6) to 4.3 (Cycle 7), selection improvement was uninterrupted as it increased from 5.6 (Cycle 6) to 6.9 (Cycle 7). The broad sense heritability estimates were lower (25.1) than other Cycles as well (38.2-50.8).

After the closing of Population T in cycle 8, level of resistance was elevated in Cycles 9 and 10. S1 *per se* enables the accumulation of positive alleles over time within

the closed Population T. Once adequate genetic variability was introgressed, trait improvement results were achieved similar to non-modified S1 *per se* (Patel et al., 1999). Population T's aim was not only to increase the resistance, but also generate lines that could be used to produce hybrids with resistance to SSR which resulted in release of the first canola hybrid with improved field resistance to SSR in 2008. Dickson and Petzddt (1996) found that the resistance in cabbage *Brassica oleracea* was based on a single recessive gene. Selection of the S0 and S1 plant generations generally leads to lines that show resistance in the heterozygous state. Resistance based on such lines should be better suited for the production of hybrids.

CONCLUSION

The first spring canola lines with resistance to Sclerotinia stem rot were generated using modified S1 *per se* recurrent selection and Field Limited-Term Inoculation method. These lines will be used in the development of canola products with a 'built in' level of genetic resistance, such that fungicide applications may be reduced or eliminated entirely.

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STVARANJE PRVIH LINIJA JARE ULJANE REPICE OTPORNIH NA BELU TRULEŽ STABLJIKE

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Izvod

Jara uljana repica je osetljiva prema beloj truleži stabljike pa se fungicidi koriste za zaštitu od obolenja u polju. S1 per se rekurentna selekcija, u kombinaciji sa testiranjem otpornosti u staklari i polju, je korištena za poboljšanje otpornosti prema obolenju. Pošto ciklusi 1 do 4 nisu doneli značajno poboljšanje otpornosti, populacija je otvorena za konvergentno unošenje novih izvora otpornosti i podložena selekciji korištenjem poljske metode ograničene inkubacije bolesti (FLTI). Ove promene su rezultirale poboljšanjem otpornosti u ciklusu 5. Postepena poboljšanja otpornosti su pomerila gotovo celu populaciju u ciklusu 10 u otpornu kategoriju. SSDIS parameter (Sclerotinia sclerotiorum Disease Incidence Severity) je korišten za praćenje nivoa otpornosti gde 1 predstavlja jako osetljivu reakciju a 9 kompletnu otprornost. Srednja SSDIS vrednost populacije u ciklusu 5 (2.4) je značajno poboljšana u odnosu na srednju vrednost populacije u ciklusu 10 (6.7). Pošto se FLTI metod zasniva na ekstremonom pritisku obolenja u polju, koji traži primenu dva prskanja fungicidom, otporne linije imaju potencijal za razvoj proizvoda uljane repice sa otpornošću na belu trulež stabljike.

Ključne reči: *Sclerotinia*, uljana repica, testiranje, FLTI, populacija, otpornost.

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EFFECT OF DIFFERENT LENGTH DUCTUS PAPILLARIS ON PATHOMORPHOLOGICAL CHANGES IN UDDER PARENCHYMA

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SUMMARY: Pathomorphological changes in the mammary glands are usually caused by the interaction of macroorganism, microorganism and the environment. A very important processs during intramammary infection is the penetration of microorganisms in a tank papilla. In the mammary gland, there are several mechanisms to prevent penetration of bacteria into the mammary gland. The first line of defence against penetration of pathogens into the mammary gland is ductus papillaris. The interior of the ductus papillaris filled with similar wax, which contains long-chain fatty acids and basic proteins. It was found that these substances in vitro bactericidal effect and bacteriostatic toward specific mastitis pathogens. Previously it was thought that the musculus sphincter papillae most important obstacle to the penetration of microorganisms into the mammary gland. Research tasks are focused on monitoring the length of the ductus papillaris and its impact on the occurrence of pathomorphological changes in the parenchyma of the udder.

***Key wards**: ductus papillaris, udder, pathomorphology, cow.*

INTRODUCTION

Udder, the histological structure, belongs to tubuloalveolaran type of glands, and it has skin origin (Pantić 1980, Boboš 2005, Pobrić 2000). It is functionally closely related to the genital organs, well-developed in female animals, while with the males it is rudimentary (mamma masculina). The cow's udder is divided into four quarters completely separated. Glandular parenchyma was pink-gray in colour, firmer consistency as opposed to fatty tissue that surrounds the gland. Parenchyma is a complex character and consists of glandular tubes with alveolar enlargement (Hurly 1985, Simić 1997, Davidov 2010). *Papilla mammae* cows are cylindrical and slightly inclined towards the ventrocranial. Their function is the secretion of milk into the environment, as well as

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suckling, which is the only way of feeding calves. On the *papilla mammae* no hair, sebaceous or sweat glands or. Their shape and size depend on the shape and size of udder and milk production. Papillae cow has only one channel running-*ductus papillaris* with one hole-*ostium papillae* and the link between the internal system of milk secretion and the environment. *Ductus papillaris* is the main barrier to infection. Closure of *ductus papillaris* and is enabled through the *musculus sphincter* that surrounds a channel (Simić, 1997; Boboš, 2005; Davidov, 2010). Length of *duct papillaris* a proper length of *papillae* varies and increases with increasing number of lactation, so the author is different. Most authors (Claude, 1983; Simić, 1997; Pobrić and al., 1998) reported that the length of the *ductus papillaris* is about 10 mm, but varies from 3 to 18 mm (McDonald ,1973; Hamann, 1987; Geishauser and Querengasser, 2000; Paulrud and Rasmussen, 2004; Paulrud et al., 2004), depending on the breed, and even the stage of lactation (McDonald, 1975). *Ductus papillaris* in last *papilla* is 5-10% longer than the *ductus papilaris* on frontal *papillae* (Paulrud et al., 2004). In dairy cows, mammary gland has a simple defence system consisting of *papillae* and udder. When pathogenic microorganisms penetrate this barrier, they are in the parenchyma of the mammary gland and produce the toxin, leading to damage to the wall causing inflammation and tissue injury. Mc Donald (1979) argued that the length of the *ductus papillaris* is not associated with the emergence of new infections because udder quarters that were infected had a longer *duct papillaris* of four who were infected. Milking microorganisms located in the immediate vicinity papilla take the opportunity to penetrate the *ductus papillaris*, causing trauma and damage to the keratin layer or even mucosal channels (Capuco et al., 1992). *Ductus papillaris* may remain partially open for 1-2 hours after milking and during this period microorganisms can get on. Pathogenic microorganisms are able to enter through an open *ostium papilla*, avoiding antibacterial activity (Khan, 2006). If microorganisms pass the first line of defense and penetrate into the tank, they get to the second line of defense consisting of polymorphonuclear leukocytes, macrophages, which are the main phagocytic cells of the mammary gland were polymorphonuclear leukocytes and macrophages. Macrophages are more in uninfected and infected polymorphonuclear leukocytes in the mammary gland (Davidov, 2010).

MATERIAL AND METHODS

Udder of Holstein-Friesian cows were taken for histological examination. We tested 104 mammary complexes which are measured by length of *ductus papillaris*, and from which tissue samples were taken for histological examination. All samples were fixed in buffered 10% formalin, and then dehydrated through a series of growing concentrations of ethanol and xylol treatment infused as a medium for the introduction of paraffin wax-molding means. Made paraffin molds were cut at a thickness of 5 μm microtome, and stained with hematoxylin eosin, and all were performed microscopic light microscope and photographed with a digital camera Canon.

RESULTS

Papillaris ductus (Figure 1.) represents the relationship between the environment and the interior of the mammary gland. Coated with a substance grey color, similar

to wax, which helps close the *ductus papillaris*. Central muscle layer, especially at its peak, is composed of smooth muscle and collagen fibers, which some *ductus papillaris* and become stronger to ultimately form a *musculus sphincter papillae*, which tightens and closes the *ductus papillaris*.

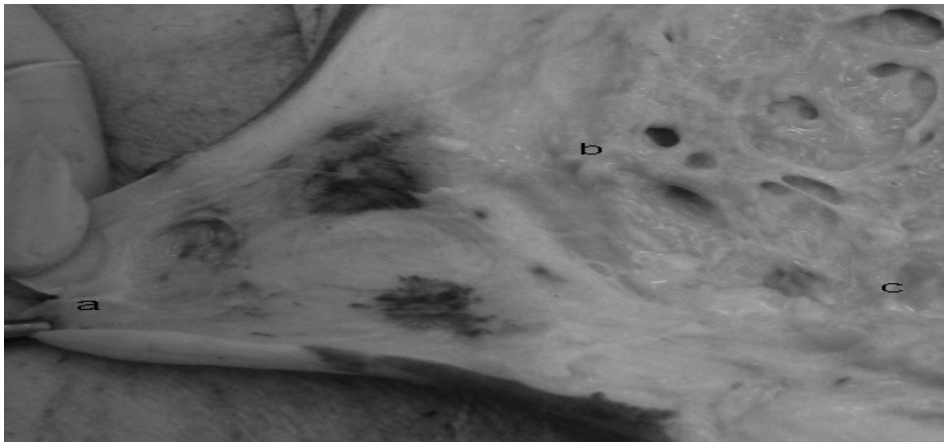

Figure 1. a) ductus papillaris, b) sinus lactiferus, c) parenchyma gl. mammae *Slika 1. a) ductus papillaris, b) sinus lactiferus, c) parenchyma gl. mammae*

By measuring the length of the *ductus papillaris*, we found that the number of *papillae*-84 (80.77%) has a length of *ductus papillaris* up to 10 mm, and only 3 (2.88%) *papillae* length to 5 mm (Table 1). Taking into account the mammary complexes as separate functional units of an udder, we found differences in the length of *ductus papillaris*.

Table 1. Length of ductus papillaris of udder distribution
Tabela 1. Distribucija dužine sisnog kanala vimena

Length of *ductus papillaris*/ *Dužina ductus-a papillaris-a*		≤ 5mm ≤ *5mm*	5,1-10mm 5,1- *10mm*	>10mm > *10mm*	Total *Ukupno*
Papillae mammae/ *Papila*	n	3	84	17	104
	%	2,88	80,77	16,35	100%

Table 2 shows the values calculated mass of leukocytes in the parenchyma of the mammary gland, where in 15 (14.42%) mammary complexes weight leukocyte infiltration was from 0 to 25%, and 45 (43.27%) mass leukocyte infiltrate was of 50.1 to 75%.

Table 2. Distribution of leukocyte infiltration in mammary gland parenchyma
Tabela 2. Distribucija leukocitarne infiltracije parenhima mlečne žlezde

Leukocyte infiltrate *Leukocitarni infiltrat*		0-25%	25,1-50%	50,1-75%	75,1-100%	Total/ *Ukupno*
Mammary glands *Mlečnih žlezda*	n	15	19	45	25	104
	%	14.42	18.27	43.27	24.04	100.0

DISCUSSION

Since the mammary gland is characterized by their fine structure and function, it has adequate and complex defence system of nonspecific and specific reactions involved in the protection of organs from microorganisms. The non-specific defense mechanism, the essential role belongs to morphological traits. (Avdić et al., 2008).. Natural hygiene is changing with the age of individuals, and there are differences within individual lines of dairy cows. Taking into account everything that belongs to the security system in the first place is the anatomy of the udder, rather *papillae mammae*. *Papillae* cow has only one channel running-*ductus papillaris* with one hole-*ostium papillae* and the link between the internal system of milk secretion and the environment. *Ductus papillaris* is the main barrier to infection. Closure of *ductus papillaris* and is enabled through the *musculus sphincter* that surrounds a channel. Length of *ductus papillaris* a proper length of *papillae* varies, so the author is different. Most authors (Claude, 1983; Simić, 1997; Pobrić and et al., 1998) Reported that the length of the *ductus papillaris* and about 10 mm, but varies from 3 to 18 mm (McDonald, 1973; Hamann, 1987; Geishauser and Querengasser, 2000; Paulrud and Rasmussen, 2004; Paulrud et al., 2004), depending on the breed, and even the stage of lactation (McDonald, 1975). Length of *ductus papillaris* is morphological characteristic of each mammary gland. According to our findings, most of the *ductus papillaris* with a length of 10 mm (Table 1.), which agrees with the findings of many authors (Claude, 1983; Simić, 1997; Pobrić et al., 1998; McDonald, 1973; Hamann, 1987; Geishauser and Querengasser, 2000; Paulrud and Rasmussen, 2004; Paulrud et al., 2004). Mc Donald (1979) Argued that the length of the *ductus papillaris* is not associated with the emergence of new pathomorphological changes in the mammary gland, udder quarter because you were not with the pathomorphological changes, had a longer *ductus papillaris* of four who were with the pathomorphological changes in the mammary gland. Unlike McDonald's claims, our research we found that of 104 histologically examined mammary complexes, in 45 (43.27%) (Table 2.) tanks mammary complexes with the length of the *ductus papillaris* and up to 5 mm was found dominant mass leukocyte infiltrate, indicating that the length of the *ductus papillaris* and is important in the development of pathomorphological changes in the mammary gland itself. Pathomorphological changes of the mammary gland of dairy cows are caused when the break through a barrier teat and when pathogenic microorganisms reach the tanks or the parenchyma of the mammary gland. That the degradation of histological structure of tanks and parenchyma. According to the Trinidad-in 1990. Histological examination are valuable in revealing the degree of damage to the tissues of the mammary gland of cows. Histomorphological examinations were recorded in the works of several authors (Chander et al., 1973; Heald, 1979; Nickerson and Head, 1981; Sordillo and Nickerson, 1988). In the altered parts of the mammary gland interlobular connective tissue spreads. Chander and Reid 1973 examined samples of mammary gland parenchyma cows and came to the conclusion that the tissue of infected mammary gland has a large number of polymorphonuclear leukocytes (PMN) and secretory tissue necrosis, which corresponds to our results.

CONCLUSION

Based on the results of our research, we found an uneven length of *ductus papillaris*, so that most of the papillae 84 (80.77%) had a length of 5 to 10 mm, a minimum of changes in the parenchyma (14.42%) of the mammary gland was the finding leukocyte infiltrate 0-25% and 43.27% of the mammary gland parenchyma were the findings leukocyte infiltrate from 50.1 to 75%. What can be concluded from our study is that the length of the *ductus papillaris* udder is important to the appearance of pathomorphological changes in the parenchyma of the mammary gland.

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UTICAJ RAZLIČITIH DUŽINA *DUCTUS PAPILLARIS*-A NA POJAVU PATOMORFOLOŠKIH PROMENA PARENHIMA VIMENA KRAVA

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Izvod

Patomorfološke promene u mlečnoj žlezdi najčešće nastaju interakcijom makroorganizma, mikroorganizma i spoljašnje sredine. Veoma važan proces u mehanizmu nastajanja intramamarnih infekcija je penetracija mikroorganizma u cisternu papile. U mlečnoj žlezdi postoji nekoliko mehanizama kojima se sprečava prodor uzročnika u mlečnu žlezdu. Prva linija odbrane od prodora uzročnika u mlečnu žlezdu je *ductus papillaris*. Unutrašnjost *ductus papillaris* je ispunjena masom sličnom vosku, koja sadrži duge lance masnih kiselina i bazične proteine. Utvrđeno je da ove materije *in vitro* pokazuju baktericidan efekat prema ambijentnoj mikroflori, a bakteriostatski prema specifičnim uzročnicima mastitisa. Ranije se smatralo da je *musculus sphincter papillae* najvažnija prepreka za prodor mikroorganizama u mlečnu žlezdu. Zadatke istraživanja smo usmerili na praćenje dužine *ductus papillaris*-a i njegov uticaj na pojavu patomorfoloških promena u parenhimu vimena.

Ključne reči: ductus papillaris, vime, patomorfologija, krava.

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REFERENCE VALUES AND FREQUENCY DISTRIBUTION OF HEMATOLOGICAL PARAMETERS IN COWS DURING LACTATION AND PREGNANCY*

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SUMMARY: The hematological parameters were investigated in 40 Holstein-Friesian. The cows were in the second and third lactation. The blood samples were taken in four productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2-3 weeks before the start of drying). The findings of hematological values in Holstein-Friesian cows corresponded with reference values for a given type of animal. The periparturient period is characterized by a reduced number of red blood cells, decreased concentrations of hemoglobin, reducing the total number of leukocytes with the growth of neutrophils. The characteristic findings of the entire population of cows, regardless of the period of lactation is to draw the number of neutrophils and lymphocytes to the right and turn left into hemoglobin. This supports the exposure to stress in daily production. All parameters are arranged in the normal (Gaussian) distribution.

Key words: *hematology, dairy cows, the reference values, frequency distribution of parameter.*

INTRODUCTION

Hemogram testing in animals is an indispensable diagnostic procedure. For the evaluation of hematologic findings, it is important to know the reference values for blood elements. Reference values depend on many elements, such as the period of lactation, breed, sex, age, etc. (Mirzadeh et al., 2010). A large comparative analysis (George et al., 2010) showed that the most recent literature defines the limits of normal values in the widest variations were found in healthy cows. The study was used to compare

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Original scientific paper / *Originalni naučni rad*

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hematological results obtained from the literature of the fifties of last century and contemporary sources. It was found that the concentration of hemoglobin and the number of neutrophils, eosinophils and lymphocytes and neutrophils ratio is statistically significantly different between these two sets of data.

The aim of this study was to investigate the effect of lactation on haematological values in dairy cows, and to form a frequency distribution for each value, especially compared to the average reference values from recent world literature, as well as Schalms veterinary hematology (Weiss and Wardrop Ed, 2010) and Large Animal Internal Medicine (Morris, 2009-Smith B. Ed).

MATERIAL AND METHOD

Biochemical parameters were examined in 40 Holstein-Friesian cows. The cows were in the second and third lactation and were grown under the same conditions of diet (standard recipe meals based on corn silage, TMR) and care (free rearing system on deep litter). The blood samples were taken in four main productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2-3 weeks before the start of drying). The blood was collected and quickly processed in the semi-automated hematology analyzer Hemavet 950th directly to a number: RBC (x 10^{12}/ L), WBC (x10^9/L), neutrophils, basophils, eosinophils, lymphocytes, monocytes and platelets (x10^9 /L). The concentration of hemoglobin was determined by mentioned analyzer (g / l). The computation is determined by the erythrocyte indices: MCV (fl), MCH (pg) and MCHC (%).The following formula was used:

MCV = (hematocrit / red blood cells of millions in mL) x 10

MCH = (Hb (g / dl) of millions of red blood cells in mL) x 10

MCHC = (Hb (g / dl) / hematocrit) x 100.

RESULTS

The most pronounced haematological changes were seen in cows in the period after calving. Immediately after calving , the hematological picture is characterized by: the fall in erythrocyte count, hemoglobin level and decrease of the calculated parameters, decreasing the number of leukocytes, but the number of neutrophils increases (Table 1).

Table 1. Average values of haematological parameters of blood under the stage of lactation
Tabela 1. Prosečne vrednosti hematoloških parametara krvi shodno stadijumu laktacije

Parameters *Parametri*	Precalving *Zasušene*	Fresh cows *Početak laktacije*	Mid lactation *Sredina laktacije*	End lactat. *Kraj laktacije*	Ref.value *Ref.vredn. literatura*
Erithrocytes x 10^{12}/L	6.2	4.82*	7.04	8.11	*5-10*
Hemoglobin (g/l)	100.44	85.06*	90.5	103.3	*80-150*
MCV (fl)	50.5	48.21*	52.3	54.8	*40-60*
MCH (pg)	14.4	12.89*	13.22	14.11	*11-17*
MCHC (%)	35.1	34.4	35.2	35.4	*30-36*

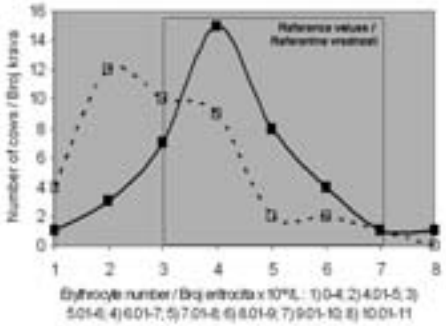
Leukocytes x 10^9/L	12.55	12.03	13.5	13.02	*5-13*
Neutrophils x 10^9/L	4.45	5.19**	4.23	4.3	*1.7-6*
Basophils x 10^9/L	/	/	/	/	*Rare/Retki*
Eosinophils x 10^9/L	0.67	0.58**	0.77	0.89	*0.1-1.1*
Monocytes x 10^9/L	0.71	0.65	0.67	0.74	*0.1-0.7*
Lymphocytes x 10^9/L	5.12	4.83*	5.38	6.99	*1.8-8.1*
Platelet x 10^9/L	410	370	390	405	*100-800*

*p<0.05, ** p<0.01.

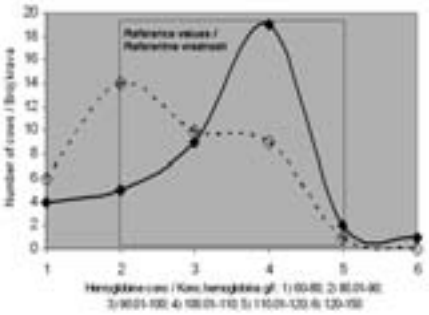
Table 2. The average number of cows whose hematological parameter values outside the reference (+ turn to the right, - turn left)
Tabela 2. Prosečan broj krava čije su vrednosti hematološkog metaboličkog parametra van referentnih (+ skretanje u desno, - skretanje u levo)

Parameters *Parametri*	Precalving *Zasušene*		Fresh cows Početak laktacije		Mid lactation Sredina laktacije		End lactat. Kraj laktacije	
Non referent values *Vrednost izvan referentnih*	+	-	+	-	+	-	+	-
Erithrocytes x 10^{12}/L	0	0	0	16	0	3	1	1
Hemoglobin (g/l)	0	0	0	6	0	0	1	0
MCV (fl)	0	1	0	1	1	0	1	0
MCH (pg)	0	0	0	1	1	0	2	0
MCHC (%)	0	1	0	1	2	0	1	0
Leukocytes x 10^9/L	0	0	1	0	0	0	2	0
Neutrophils x 10^9/L	3	0	8	1	2	0	1	1
Basophils x 10^9/L	/	/	/	/	/	/	/	/
Eosinophils x 10^9/L	0	0	1	0	2	0	0	0
Monocytes x 10^9/L	0	0	4	0	5	0	2	0
Lymphocytes x 10^9/L	1	1	5	4	4	0	4	0
Platelet x 10^9/L	0	0	0	0	0	0	0	0

The value of these parameters after calving was significantly different compared to other periods of lactation (p<0.05, p<0.01). In addition to the original values of parameters to be examined and the number of cows which were investigated parameters are outside normal ranges. These results are presented in Table 2 and show that most cows have values outside the reference period around calving. In order to accurately determine the haematological values important to examine the frequency distribution of parameters in cows. These results are presented in Graph. 1 to 11 It may be noted that most parameters showing normal distribution with a slight turn to the left in the periparturient period.


Reference values /
Referentne vrednosti
Number of cows / Broj krava
Erythrocyte number / Broj eritrocita x 10^6/L: 1) 0-4; 2) 4.01-5; 3) 5.01-6; 4) 6.01-7; 5) 7.01-8; 6) 8.01-9; 7) 9.01-10; 8) 10.01-11

1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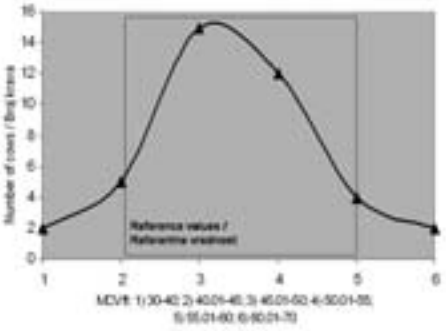
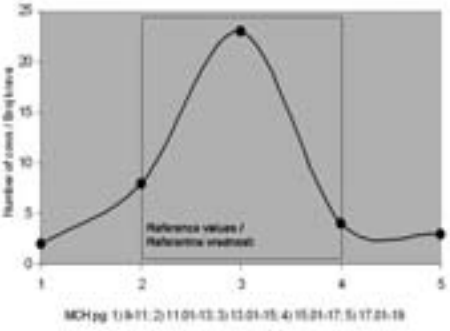
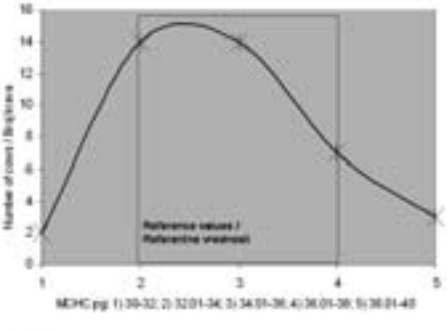

Reference values /
Referentne vrednosti
Number of cows / Broj krava
MCV fl: 1) 30-40; 2) 40.01-45; 3) 45.01-50; 4) 50.01-55; 5) 55.01-60; 6) 60.01-70

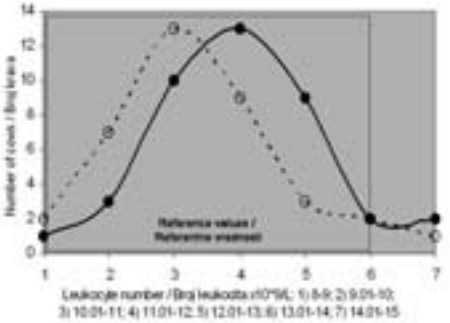
3


Reference values /
Referentne vrednosti
Number of cows / Broj krava
MCH pg: 1) 9-11; 2) 11.01-13; 3) 13.01-15; 4) 15.01-17; 5) 17.01-19

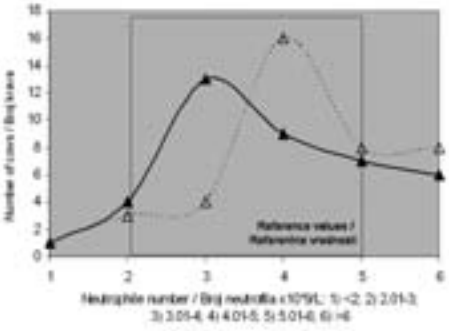
4


Reference values /
Referentne vrednosti
Number of cows / Broj krava
MCHC pg: 1) 30-32; 2) 32.01-34; 3) 34.01-36; 4) 36.01-38; 5) 38.01-40

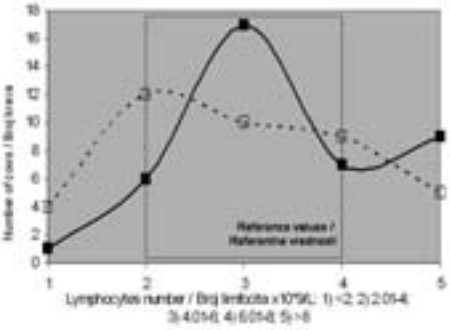
5


Reference values /
Referentne vrednosti
Number of cows / Broj krava
Leukocyte number / Broj leukocita x10^9/L: 1) 5-9; 2) 9.01-10; 3) 10.01-11; 4) 11.01-12; 5) 12.01-13; 6) 13.01-14; 7) 14.01-15

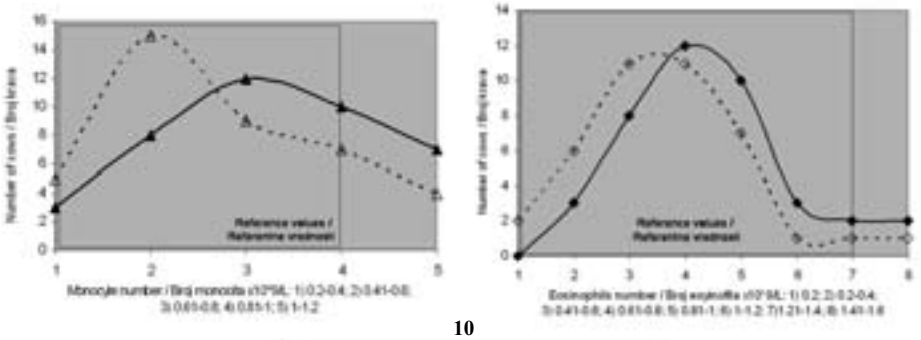
6


Reference values /
Referentne vrednosti
Number of cows / Broj krava
Neutrophile number / Broj neutrofila x10^9/L: 1) <2; 2) 2.01-3; 3) 3.01-4; 4) 4.01-5; 5) 5.01-6; 6) >6

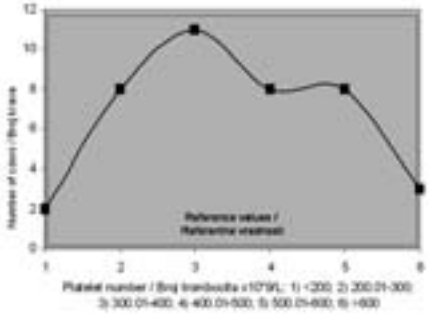
7


Reference values /
Referentne vrednosti
Number of cows / Broj krava
Lymphocytes number / Broj limfocita x10^9/L: 1) <2; 2) 2.01-4; 3) 4.01-6; 4) 6.01-8; 5) >8

8



9 **10**



11

Graph 1-11. Frequency distribution of average values of the parameters examined in 40 cows (solid line), with a distinctive distribution of trends in the periparturient period for the parameters whose values are significantly different from the general average (dashed line), a rectangle shows the reference values obtained by analysis of the literature.

Grafici 1-11. Distribucija frekvencije prosečnih vrednosti parametara kod 40 ispitivanih krava (puna linija), sa naglašenom distribucijom kretanja u peripartalnom periodu za parametre čije se vrednosti signifikantno razlikuju za ovaj period (isprekidana linija), pravougaonik pokazuje referentne vrednosti dobijene anlizom literature.

DISCUSSION

The average values of the studied haematological parameters in accordance with the recommended reference values from world-class literature (Table 1).

The frequency distribution of these parameters (Graph. 1-11) enters the normal (Gaussian) distribution, as confirmed by statistical analysis of previous studies (George et al., 2010). Regardless of the lactation period a number of cows (average 2) will have values higher or lower than the reference (Table 2), but this does not affect significantly the parameters fatnesses and symmetry of distribution (parameters α and β, unrepresented results). Erythrocyte count and hemoglobin concentration significantly turns to the left in the periparturient period (Graph.1 and 2). The number of neutrophils and white blood cell count turns slightly to the right in the whole cow population (Graph. 7 and 8), while the diversion of neutrophils and prominent periparturient period. The value of monocytes decreases and turns to the left in the periparturient period (Graph. 9).

Previous studies have shown that the concentration of hemoglobin and red blood cells depend on the number of lactation in dairy cows (Belic et al., 2010). It was shown that during the period around calving erythrocyte count and hemoglobin concentration in cows decreased. This situation can be associated with milk production, because it was found that heifers have a significantly higher concentration of hemoglobin in relation to dairy cows (Satar and Mirza, 2009). Also, the concentration of hemoglobin and red blood cell count may be associated with stress in dairy cows, when their value declines (Belic et al., 2010). Gavan et al (2010) and Mirzadeh et al (2010) found significantly lower hemoglobin concentration and erythrocyte counts in the periparturient period, which agrees with our results. Reducied concentration of hemoglobin and erythrocyte counts during lactation was illustrated in the much older results (Rowlands et al., 1977).

The influence of lactation on the movement of white blood cells is minimal, except in the periparturient period (Detilleux et al, 1995). Another important indicator is the ratio of neutrophil to lymphocyte (N: L ratio). The neutrophil to lymphocyte ratio greater than 1 is considered that the cows were loaded inflammation or other stress (Latimer et al, 2003), which is present in the periparturient period in cows in our study (table 1). However, an important indicator of inflammation is the burden of certain elements of white grapes, and we have already mentioned that the number of neutrophils and lymphocytes slightly turns to the right. If the periparturient period is dominated by neutrophilia with monocytopenia cows more likely can to develop signs of metritis, while eosinopenia can lead to periparturient mastitis (Belic et al., 2010).

Establishing the reference values and factors that affect these values is important for clinical veterinary practitioners.

CONCLUSION

Hematologic values in Holstein-Friesian cows in our territory correspond with reference values for cattle. Some discrepancies exist in the periparturient period, at the beginning of lactation. All parameters are arranged according to the normal (Gaussian) distribution.

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REERENTNE VREDNOSTI I DISTRIBUCIJA FREKVENCIJE HEMATOLOŠKIH PARAMETARA KOD KRAVA TOKOM LAKTACIJE I GRAVIDITETA

BRANISLAVA BELIĆ, MARKO R.CINCOVIĆ,
LJUBICA KRČMAR, BOJANA VIDOVIĆ

Izvod

Ispitivani su hematološki parametri kod 40 krava Holstajn-Frizijske rase. Krave su bile u drugoj i trecoj laktaciji. Krv je uzorkovana u okviru četiri osnovna produktivna perioda i to: u zasušenju (2-3 nedelje pred porođaj), u postpartalnom periodu (0-2 nedelje postpartum), u sredini laktacije (15-17 nedelja postpartum) i u kasnoj laktaciji (2-3 nedelje pred početak zasušenja). Nalaz hematoloških vrednosti kod Holštaj-frizijskih na našoj teritoriji krava odgovara referentnm vrednostima za datu vrstu. Karakteristika nalaza jeste skretanje broja neutrofila i limfocita u desno i skretanje koncentracije hemoglobina u levo. Ovo govori u prilog izloženosti stresu u svakodnevnoj proizvodnji. Peripartalni period odlikuje se smanjenim brojem eritrocita, sniženom koncentracijom hemoglobina, smanjenjem ukupnog broja leukocita uz porast broja neutrofila. Svi parametri su raspoređeni u okviru normalne (Gausove) distribucije.

Ključne reči: hematološki parametri, mlečne krave, referentne vrednosti, distribucija frekvencije parametara.

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INDUCED SPAWNING OF KOI CARP*

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SUMMARY: Artificial spawning is the most effective and the most reliable method of eggs and fingerlings production, and control of infectious and parasitic diseases. The aim of this study was to investigate the possibility and efficiency of out-of-season induced spawning of KOI carp, the impact of body mass of females on the number of eggs, as well as the correlation between the percentage of migrated oocysts and spawning success. To induce and synchronize ovulation and spermiation by hormonal stimulants, fish receive injection of pituitary gland, calibrated pituitary extract or a synthetic GnRH. In our trial of induced spawning of carp, we used cCPE because of winter time and out-of-season spawning. There was significant correlation (F=0.709) between the body weight of the female to the weight of eggs and a significant correlation (F=0.642) between the body weight of the female and the number of the eggs. We found negative correlation (F = -0.530) between the percentage of migrating oocytes to spawning success. We assume that the reason can be related due to environmental circumstances, as well as the quality of spawners. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had satisfactory success because it was done out of season.

Key words: *induced spawning, KOI carp, cCPE, off-season spawning, females, milt, eggs.*

INTRODUCTION

Induced spawning is the most effective and the most reliable method of eggs and fingerlings production, and control of infectious and parasitic diseases. Controlled

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Original scientific paper / *Originalni naučni rad*

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spawning began around the year of 1725 when L. Jacobi succeeded to control fertilization of eggs of salmon and trout (Nesh et al., 2011). The major breakthrough in fish breeding came with the finding that dopamine acts as inhibitory factor for synthesis of gonadotropin (Zohar and Mylonas, 2001). This breakthrough led to the development of the artificial spawning. Common carp matures in subtropical climate zone in six months (Brzuska and Bialowas, 2002). An adult common carp may spawn four or five times per year in subtropical conditions if temperature is maintained at 20 to 22°C (Horvath, 1986; Chemineau, 2007). Diversity of fish reproductive strategy involves diversity in the timing of fish spawning (Webb and McLay, 1996). Thus, it occurs in late spring to summer in carp in Europe (Brzuska, 2004). In Israel the spawning seasons starts with common carp at the end of February and the beginning of March, when the water temperatures reach 19-21°C (Brzuska and Bialowas, 2002). Since the middle of 1980s, hypophysation has improved through the introduction of standardized dry carp pituitary extract in which the luteinizing hormone (LH) content and activity have been calibrated (calibrated carp pituitary extract = cCPE) (Yaron et al., 2009). Approximately 300 000 to 800 000 newly hatched fry can be expected from a single female (Ćirković et al., 2002; FAO, 2006). The aim of this study was to investigate the possibility and efficiency of out-of-season induced spawning of KOI carp, the impact of body mass of females on the number of eggs, as well as the correlation between the percentage of migrated oocysts and spawning success.

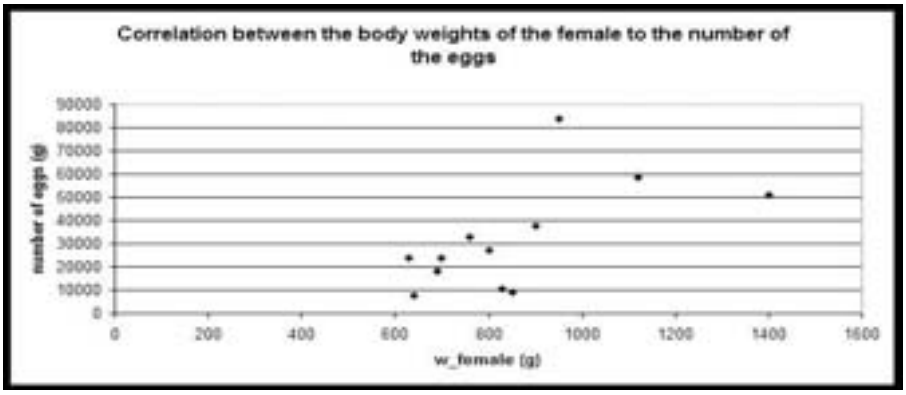
MATERIAL AND METHODS

The spawning was done at Gan Shumel Fisher Hatchery and Breeding Centre in Israel in December. Thirty females KOI-carp (*Cyprinus carpio L.*) were placed in 10 plastic tanks, cca 450 liter. Carp males were kept in separated ponds of carp females brood fish, in order to avoid uncontrolled reproduction. The brood fish were kept in water saturated with oxygen, within the temperature range of 20-24 ºC. The tanks were covered with a net, to prevent the fish from jumping out of the tank. From each tank, one female was biopsied. Females were anesthetized by 99% 2 phenoxyethanol and the biopsy of ovary was done via genital opening by inserting a 3 mm plastic catheter into the gonad. Carp eggs are opaque and the only way to examine the position of GV (germinal vesicle) under the binocular microscope is to make them transparent. The ovarian sample (of about 100 oocytes) was cleared in SERA solution (ethanol 60%, formalin 30% and acetic-acid 10%). Within 3 min the oocytes became translucent and remained so for an additional 5 min. The position of the germinal vesicle and ripeness of female was determined and the number of oocytes at each stage was recorded. Total weight of all females was calculated and it was around 40 kg. Hormones for injection were prepared for all fish together. 10 ml of 0,9%NaCl saline was added to marked vial with "10 kg of CPE" (CPE-carp pituitary extract) and was mixed by hand. Final concentration was 1 kg/ml for each kg of fish that was injected with 1 ml of solution. The dose of hormones was divided in two doses. After the anesthesia of the fish, around 11 00h, intramuscular injection of cCPE hormone was given in the base of the dorsal fin - priming injection. Around 24 00 h fish received a second - inducting injection. The area of injecting was gently massaged followed with the withdrawal of the needle after injection to aid distribution of the extract into musculature and prevention of its backflow. 10 males were injected with 70% of the dose CPE that used for females. The day after, the

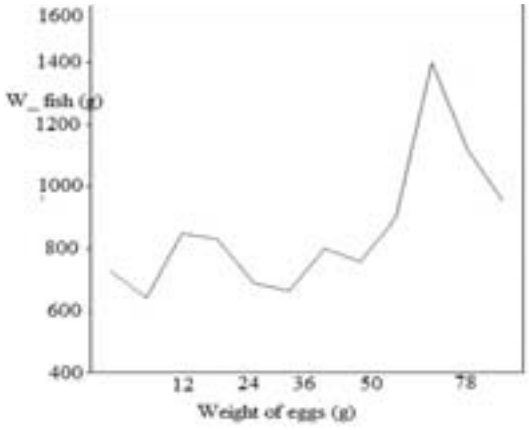
spawning was occurred little bit later than we expected. Eggs were gently squeezed in the dry bowl and letter a small volume of sperm was added and mixed carefully together „the dry method". Physiological solution-saline was used to prolong the fertilization and rinsing solution (dilution of milk) to remove the stickiness of common carp eggs was added afterwards. Incubation was carried out in Zoug jars. For statistical analyses of correlations were used SPSS for Windows and Excel (MS Office).

RESULTS AND DISCUSSION

With total number of fishes n = 30 and number of spawned females n = 13 spawning success was 43.33 %.The Spearman's correlating coefficient between body weight of fish and number of the eggs was measured (Graph 1), correlation between body weight of fish and weight of eggs (Graph 2) and the correlation between the % of migrating oocytes to spawning success (Graph 3). In correlation between the percent of migrating oocytes to spawning success, only eight of the females with biopsy were spawned.

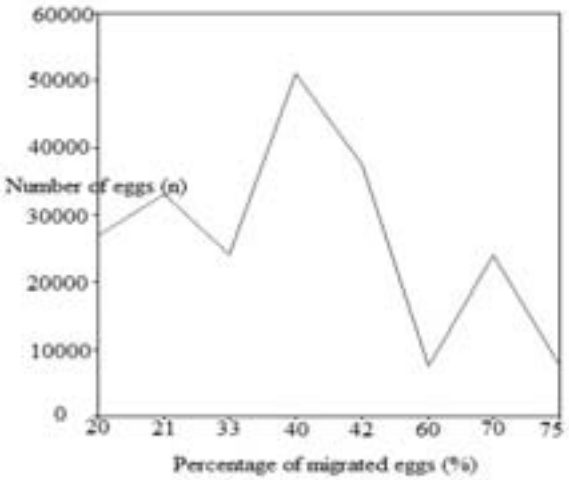


Graph 1. Correlation between the body weights of the females to the number of the eggs
Grafik 1: Korelacija između telesne težine ženki i broja jaja



Graph 2. Correlation between the body weight of the female to the weight of eggs
Grafik 2. Korelacija između telesne težine matica i mase jaja

There was a significant correlation (F=0.709) between the body weight of the female to the weight of eggs and a significant correlation (F=0.642) between the body weight of the female to the number of the eggs. We found negative correlation (F = -0.530) between the percentage of migrating oocytes to spawning success which contradict with many spawning researches.



Graph 3. Correlation between the % of migrating oocytes to spawning success
Grafik 3. Korelacija između % migriranih oocista i uspešnosti mresta

We assume that the reason can be related to environmental circumstances, as well as the quality of spawners. To induce and synchronize ovulation and spermiation by hormonal stimulants, fish receive injection of pituitary gland, calibrated pituitary extract or a synthetic GnRH (Arabaci et al., 2004; Dorafshan, 2003). It is recommended to use cCPE at the beginning and end of the spawning season when the LH content in the pituitary is low and synthetic GnRH in mid-season and in field spawning. (Yaron et al., 2009). In our trial of induced spawning of carp, we used cCPE because of winter time and out off-season of spawning. Concerning hatching performance, hatching rate of 43.33% was slightly lower compared to 95% reported by Horvath and Lukowicz (1982) and 88% reported by Kucharczyk et al. (2008). Latency time is also highly dependable on water temperature. The latency time of our treated carps was little postponed due to small system heating error. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had a satisfactory success because it was done out of season. The carp pituitary extract (CPE) has been used in most hatcheries, however the increased production targets and the cost of this biological material led to consider alternative approaches (Yaron, 1995).

CONCLUSION

Concerning hatching performance, hatching rate of 43.33% was slightly lower compared to results reported by others. This could be attributed to immaturity of some individuals, since the experiment was performed before the reproductive period, in order to secure the spawning response in fully mature fish. The recognition of the best

moment for applying hormonal induction in cyprinid artificial spawning is very important. Before any action on fish is taken, they must be anesthetized and handling must be done very gently. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had a very big success because it was done out of season.

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INDUKOVANI MREST KOI ŠARANA

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Izvod

Veštački mrest predstavlja najefikasniji i najpouzdaniji metod za proizvodnju ikre i ribljeg podmladka i za sprečavanje pojave infektivnih i parazitskih bolesti, koje se prenose sa roditelja na potomstvo. Cilj rada je bilo ispitivanje mogućnosti i efikasnosti vansezonskog indukovanog mresta KOI šarana, uticaja telesne mase ženki na broj jaja, kao i utvrđivanje korelacije između procenta migriranih oocista i uspešnosti mresta. Da bi se indukovala i sinhronizovala ovulacija i spermatogeneza kod riba, koriste se injekcije hormona hipofize, kalibrisani ekstrakt hipofize ili sintetički GnRH. U sprovedenom ogledu, korišćen je cCPE zbog zimske sezone i vansezonskog mresta.Uočena je značajna korelacija (F=0,709) između telesne mase matica i mase ikre i značajna korelacija (F=0,642) između telesne mase matica i broja jaja. Negativna korelacija (F=-0,530) između procenta migriranih oocista i uspešnosti mresta je u suprotnosti sa mnogim ranijim istraživanjima. Petpostavka je da bi razlog ovome mogao biti u faktorima okoline, kao i u kvalitetu samih matica. Iako je procenat izmrešćenih matica bio niži od uobičajenog procenta uspešnosti veštačkog mresta šarana, uspešnost je ipak bila zadovoljavajuća s obzirom da je mrest izvršen van uobičajene sezone.

Ključne reči: indukovani mrest, KOI šaran, cCPE, vansezonski mrest, matice, mleč, jaja

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Thelohanellus hovorkai - IN DIFFERENT CATEGORIES OF CARP*

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SUMMARY: Thelohanellus hovorkai was designated as "hemorrhages thelohanellus" because of severe haemorrhages on the body surface of the affected common carp. The diagnosis of this disease is related to the detection of spores, whose size varies, according to researches, in fish from open water and carp fish raised in aquaculture. The disease is manifested by disturbances in the central nervous system, anemia and absence of progress in fingerlings, where numerous spores are diagnosed in the tissues of fish. The investigations were conducted in the northern Serbia in 15 fish ponds during the period 2007-2009. The methods that have been used were clinical observations, light microscopy and classical pathohistology with H&E stain. The presence of Thelohanellus hovorkai was detected in common carp fingerlings older than 90 days and female carp fish. Effective therapeutic measures are not known but the general preventive measures related to the preparation of facilities for the cultivation of carp fingerlings give favorable results.

Key words: *telohanelosis, common carp, Thelohanellus hovorkai, spores.*

INTRODUCTION

Telohanelosis is a disease of cyprinids, which are clinically observed in the fins and scales *(Thelohanellus nikolskii) and all other tissues and especially in the central nervous system* (*Thelohanellus hovorkai*) (Molnár, 2002a). The biology and histopatohology of *Thelohanellus hovorkai* was described by Ćirković (1986) and Ćirković et al. (1997). Its pathology was described by Yokoyama et al. (1998) who found severe

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Original scientific paper / *Originalni naučni rad*

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haemorrhages on the body surface of the affected common carp and marked the disease as "haemorrhages thelohanellosis". The diagnosis of this disease is related to the detection of spores, whose size varies, according to research descriptions, in fish from open water and carp fish raised in aquaculture (Molnár et al., 2006). Application of molecular techniques can help in diagnosis and can determine whether the parasite corresponds to this species or another species or subspecies (Anderson et al., 2000). In GenBank, there is a short DNA sequence (710 bp) of *T. hovorkai* (AJ133419) collected from the common carp *Cyprinus carpio* in Japan (Anderson et al., 2000). The disease is manifested by disturbances in the central nervous system, anemia and absence of progress in fingerlings, where numerous spores are diagnosed *in the tissues of fish. (Molnár, 2002b; Ćirković et al., 2010). Apart from the investigations made by* Yokoyama et al. (1999) efficient therapeutic measures are not known, but general preventive measures related to the preparation of facilities for the cultivation of carp fingerlings give favorable results (Ćirković et al., 2010).

The subject of the investigation was myxosporidia detection in carps. In this paper, thelohanellosis of one-year old common carp fingerlings and female carp fish caused by *Thelohanellus hovorkai* is described.

MATERIAL AND METHODS

The investigations were conducted in the northern Serbia (Vojvodina province) in 15 fish ponds during the period 2007-2010. All investigated fish ponds provide water from channel network, the rivers Tisa, Tamiš, and Danube, and wells. The methods that have been used were clinical observations, light microscopy and classical pathohistology with H&E stain.

RESULTS AND DISCUSSION

The presence of *Thelohanellus hovorkai* was detected in common carp fingerlings older than 90 days and female carp fish. The infection intensity and number of *T. hovorkai* carriers differed among ponds. It has been noted that the most serious infections and the majority of infected fingerlings were from ponds where they reared with older fish, ponds with natural spawning, and ponds which served for older carp winter storage. This infection of the common carp occurs in the Serbian and Hungarian fish farms every year (Molanár, 2002a; Molanár, 2002b; Ćirković et al., 2010). Microscopic observation of compressed samples of brain tissue reveals small or large groups of spores (Fig. 1 and 2).

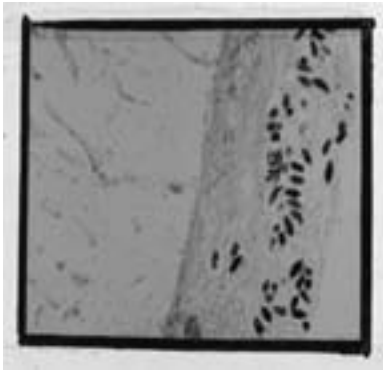

Figure 1. Spores of *T. hovorkai* in brain
Slika 1. Spore T. hovorkai u mozgu

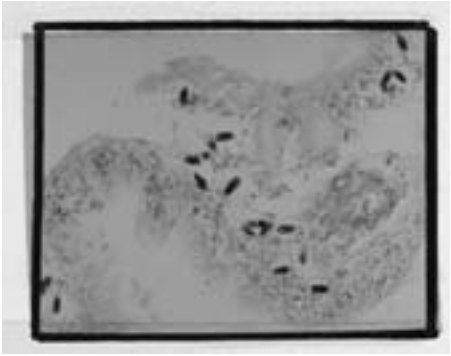

Figure 2. *T. hovorkai* spores
Slika 2. Spore T. hovorkai

According to our investigations, spores show next values: spore length 20-21 μm, spore width 14 μm, polar capsule length 12.5-14μm polar capsule width 11.5-12.5μm, spore length with mucal layer 22.5-25 μm, spore width with mucal layer 14.5-17.5μm. Spores were rounded by mucoid layer (Fig. 3).

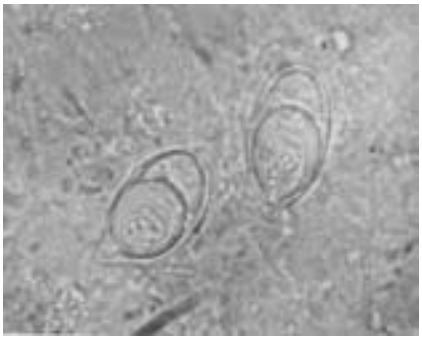

Figure 3. Fresh spores of *T. Hovorkai*
Slika 3. Sveže spore T. Hovorkai

The spores of *T. hovorkai* were present in gills, skin, muscules, liver, spleen, kidneys, intestines, bile and swim bladder, spinal cord and brain where were present the hihgest number of spores (Fig. 4). The results of Lianage et al. (2003a) reveal that the gill is primarily the portal of entry of *T. hovorkai* in fish immersed in waterborne actinospore suspension. Also, their successful transmission of *T. hovorkai* using the intubation method strongly suggests that the gut is another and important site of entry for this species, although actinospore penetration through the gut tissue had not been explored. It is probable that actinospores can utilize multiple sites of entry in the fish host. Yokoyama et al. (2006) were observed that *Thelohanellus hovorki actino spores exhibited a slow response of sporoplasm release to common carp mucus as well as penetration into the gills of common carp.*

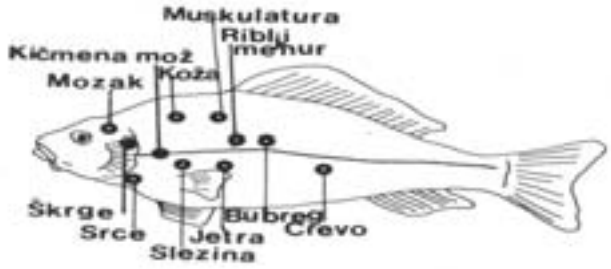

Figure 4. Organs where spores of *T. hovorkai* were detected
Slika 4. Organi u kojima su detektovane spore T. hovorkai

The disease was manifested with disturbances in central nervous system, anemia, and absence of fingerlings progression especially when higher number of spores is diagnosticated. Lianage et al. (2003b) concluded that the developmental cycle of *T. hovorkai* was completed within 3–5months at 20–25ºC, and that the ingestion of large numbers of actinospores orally, possibly by feeding on infected oligochaetes, resulted in a disease condition in carp. The life cycle of *Thelohanellus hovorkai* (Myxozoa), the causative agent of haemorrhagic thelohanellosis of carp *Cyprinus carpio*, involves the alternate oligochaete host *Branchiura sowerbyi*, which plays the role of vector in the parasite's transmission (Ćirković, 1987; Liyanage et al., 2003a). Myxosporean and actinosporean stages of *Thelohanellus hovorkai* develop in common carp and water oligochaete, respectively (Ćirković and Jovanović, 1987; Antychowicz et al., 2005). Furthermore, Liyanage, Yokoyama and Wakabayashi (2003b) revealed that a high prevalence of actinosporean infection (max. 92%) in high population densities of *B. sowerbyi* was another aetiological factor responsible for the disease outbreak. Diseases in fish populations is a dynamic phenomenon that depends on complex interactions among the host, pathogen and the environment (Hedrick, 1998). Alternatively, the biological control of oligochaete abundance using benthoseating fishes is now under trial (Yokoyama et al. 2002). The best preventive measure is still accurate preparation of pond.

CONCLUSION

Natural spawning of fish is not convenient for more intensive common carp production. It is recommended to rear carp fingerlings of the same age group. During the winter, fish ponds bottom soil should be dried up and frozen.

After 3-5 years of exploitation, the surface layer of the soil from ponds should be removed. During pond preparation it is recommended to use rotary ploughs. For fish pond disinfection, burnt lime (1000 kg/ha) or hydrated lime (2000 kg/ha) should be applied.

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Thelohanellus hovorkai – KOD RAZLIČITIH KATEGORIJA ŠARANA

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Izvod

Thelohanellus hovorkai je opisana i kao "hemoragična telohaneloza" zbog čestih nalaza hemoragičnih lezija na površini tela zaraženih šarana. Dijagnostika ovog oboljenja vezana je za detekciju spora čija se veličina razlikuje u opisima istraživača kod riba iz otvorenih voda i šaranskih riba gajenih u akvakulturi. Oboljenje se manifestuje poremećajima u centralnom nervnom sistemu, malokrvnosti i slabijem napredovanju mladunaca a naročito kada se dijagnostikuje veći broj spora u tkivima riba. Istraživanja su sprovedena u Vojvodini na 15 ribnjaka u razdoblju 2007-2010. Metode koje su korištene su klinička posmatranja, svetlosna mikroskopija i klasična patohistologija sa H & E bojenjem. Prisustvo ovog parazita zabeleženo je kod mladunaca šarana starijih od 90 dana i kod matica šarana. Specifične terapijske mere nisu poznate, ali opšte preventivne mere vezane za pripremu objekata za gajenje mladunaca šarana daju povoljne rezultate.

Ključne reči: telohaneloza, šaran, *Thelohanellus hovorkai*, spore.

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BASIC NUTRIENTS AND MINERALS IN THE SAMPLES OF COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA L.*) FROM DIFFERENT SITES*

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SUMMARY: The content of basic nutrients and minerals in the samples of common ragweed (A.artemisiifolia L.) from seven different sites was investigated. The investigation was performed by standard chemical methods. Test results indicate that this weed plant has good nutritional properties both in the content of basic nutrients and the content of macro and microelements. The greatest variability in the content of microelements: iron, cobalt and zinc was found. The average value of protein content (12.02±1.25%) is higher than the protein content in unshelled oats and lower than the content in dehydrated alfalfa hay. The average value of crude fiber (30.30±3.62%) in the samples of common ragweed is greater than its contents in unshelled oats and dehydrated alfalfa (with 15% protein). The average values of investigated microelements were higher than their values in oats and dehydrated alfalfa hay (with 17% protein). The same ratio is with macroelements: magnesium and potassium. In the case of calcium and phosphorus, the average values in samples of common ragweed are similar to the values of these elements in dehydrated alfalfa hay (with 15% protein). The results indicate the need for further studies of this weed.

***Key words**: Ambrosia artemisiifolia L., nutrients, macroelements, microelements.*

INTRODUCTION

Ambrosia artemisiifolia L. is a weedly-ruderal plant from the family Asteraceae. It is known as common ragweed or „limundžik" – in Serbian (Konstantinović et al., 2008). This annual plant is 20-80 cm high (SANU, 1975), according to some sources

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Original scientific paper / *Originalni naučni rad*
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(http://sr. wikipedia.org/sr, 2010) sometimes over 100 cm, with spindle-shaped root. The stem is erect, branched and densely covered with tiny hair. The leaf arrangement is usually opposite at the base (http://en.wikipedia.org/wiki/Ragweed, 2011). Leaves are egg-shaped and feather-like, on each side with 2-3 oblong lanceolate lobes divided. The front side is dark green, and the back side is grayish-green with thick, flattened hair. *A.artemisiifolia L.* is monoecious plant (http://en.wikipedia.org/wiki/Ragweed, 2011) with the flowers gathered in inflorescence heads on the top of the stem and branches. Male flower heads are hemispherical with short handles, hanging, about 4-5 mm in diameter, gathered in densely terminal inflorescence classes. Female flowers heads are situated below the male ones, in the leaf axils and female flowers are single. Male flowers are pale yellow, 10-15 in a head, with a tubular crown. The fruit is enclosed, with involucrum (http://bs.Wikipedia.org /wiki/ambrosia, 2010; SANU, 1975). It gets dispersed by humans or by wind (http://bs.Wikipedia.org /wiki/ambrosia, 2010). The growing season starts mid-April, blooms from mid-July until cooler weather arrives. It is not used for nutrition due to its bitter taste (http://sr.wikipedia.org/sr, 2010).

Common ragweed grows the best on untreated soil. It may usually be found near roads and railway lines, on the banks of streams and rivers, on building sites, near landfills, or along garden plots (Konstantinović et al., 2009). However, this weed plant may be found with the crop species (sunflower, soybean, corn) and has an adverse effects on crop yield. The experiments with common ragweed showed that this plant is able to use the plant extracts as a source of nutrients. The observed stimulatory effects of the donor plant extract on the *A. artemisiifolia* may have a potential to facilitate and cause the dominance of the ragweed in a field (Kazinczi et al., 2008a).

A. artemisiifolia L. has adverse effect on humans. According to some authors (Makra et al., 2004) it is generally considered the greatest allergen of all pollens. It's pollen causes 50-60% of all cases of pollen allergy (http://sr. Wikipedia.org / sr /, 2010). According to data from literature 25% of the population in Hungary has the symptoms of allergy caused by common ragweed pollen (Kazinczi et al., 2008b).

A. artemisiifolia L. contains powerful allergens, which are found in the whole grain of pollen, but also in the submicron fractions below 5μm. Out of 52 proteins that are present in the polen extract, 22 are identified as allergens on the basis of their reactions to human IgE (Bagarozzi et al., 1998).

In a humid environment, such as the mucous of the respiratory system, causes pollen allergy or polynosis with the symptoms ranging from allergic sneezing to the attacks of severe bronchial asthma. Only 20-30 pollen grains per m^3 of air are sufficient to cause alergy (Samardžija, 2003). Besides allergen activity of polen, the plant itself can cause contact dermatitis.

According to reports the highest concentration of pollen in the air in Europe is in the Carpathian Basin, Serbia and Hungary. In the reports it is stated that highest concentration of pollen particles (3247/m^3 of air) was over Novi Sad in 2001. This value has never been overcome in other parts of Europe (Kazinczi et al., 2008b). In contrast to the harmful effects, that are quite well known and researched, the useful effects of this plant are not known or investigated. In the literature, however, some beneficial characteristics of ragweed are mentioned. It is known that the Indians used the leaves of this plant against insect bites and to prevent inflammatory processes from various injuries. Ragweed seeds are used as an important complementary food for game birds (pheasants and partridges), especially during the winter. These seeds are rich in oils,

and nutritional value is similar to soybean. Therefore, singing birds prefer to eat ragweed seed (Kazinczi et al., 2008b).

Given all these facts related to this plant, regarding the problem of its expansion and knowing that destruction of common ragweed has not been resolved, our aim is to determine basic nutrients and minerals in the samples of ragweed. Depending on the results a conclusion will be given for the following steps in research of this plant.

MATERIAL AND METHODS

The sampling was conducted from June to October 2007, at various stages of vegetation. The samples of ragweed (*Ambrosia artemisiifolia L.*) were taken from seven different sites:

Temerin – Ilanča area, the land cultivated and planted with alfalfa. Common ragweed was in the stage of growth prior to flowering (period of early vegetation), the plant height was up to 50 cm.

Zmajevo - the area of arable land, after wheat harvest (stubble). Common ragweed was in the stage of growth prior to flowering (the period of early vegetation), height 50-80 cm.

Odžaci - from the area near the road and canal. Common ragweed was in the stage of growth before flowering (secondary vegetation), height 80-100 cm.

Novi Sad **-** the area of Sajlovo, beside the road. Common ragweed was in the stage of growth before flowering (secondary vegetation), height 80-100 cm.

Šabac **-** the area of arable land after wheat harvest (stubble), abandoned field. Common ragweed was in the stage of growth before flowering (secondary vegetation), height 50 - 80 cm.

Mali Idjoš **-** along the road, on the road Srbobran-Feketić. Common ragweed was in the flowering stage (late vegetation), woody stems, hight over 100 cm.

Palić **–** from picnic Palić area, near the road. Common ragweed was in the flowering stage (late vegetation), woody stems, hight over 100 cm.

The samples were pulled out of soil, together with the root, dried in air, and then cut, ground and mineralized by dry ashing at a temperature of 550±10^{o}C. The content of Ca, Na and K was determined by flame emission spectrometry on Jenway-PFP7. The content of Mg, Fe, Cu, Mn, Zn and Co was determined by atomic absorption spectrophotometry using a Varian Spectr AA-10 instrument, and P content was determined by spectrophotometric method.

Determination of fat, crude fiber, ash and moisture in the samples was performed by standard methods, while the protein was determined by measuring total nitrogen by total combustion (according to Dumas), as the standard method (AOAC 990.03) on the instrument „Elementar Rapid N cube“. The content of nutrients and macroelements was expressed in percentages, while the content of microelements was given in milligrams per kilogram of air-dry matter. The results were statistically analyzed.

RESULTS AND DISCUSSION

The values of basic nutrients, macro and microelements are presented in Tables 1, 2 and 3. In Table 1 the variability between the values within a certain parameter is not

big, which also means that the differences in values of certain parameters in ragweed samples, which originated from different sites, are not great. A slightly higher variability was found for crude fat content (29.33%) and crude ash (31.55%), but this is the result of higher values of these parameters in the samples from only one site.

The average content of crude fat in the ragweed samples (1.50±0.44%) was lower than the fat content in dehydrated alfalfa hay (with 15% prot.) or in unshelled oats: 2.3% and 4.5% respectively (Sinovec and Ševković, 1995). In available literature data it is stated that alfalfa and oats are used as a standard in comparing nutritional values of weeds (Marten and Andersen, 1975).

Table 1. Content of the basic nutrients in the *Ambrosia artemisiifolia L*. samples
Tabela 1. Sadržaj osnovnih hranljivih materija u uzorcima Ambrosia artemisiifolia L.

Location *Lokalitet*	Investigated parameter (%) / *Ispitivani parametar (%)*					
	Moisture *Vlaga*	Crude protein *Sirovi protein*	Crude fat *Sirova mast*	*Crude fiber* S. *Celuloza*	Crude ash *Sirovi pepeo*	NFE[1] *BEM*[1]
Zmajevo	5.36	13.95	1.44	23.66	12.38	43.21
Mali Iđoš	8.24	10.80	1.50	34.08	9.10	36.28
Novi Sad	5.13	11.65	1.26	31.81	10.60	29.92
Temerin	5.18	13.15	1.01	28.91	18.77	32.98
Odžaci	4.90	12.70	2.42	31.46	8.23	40.17
Palić	7.18	10.80	1.41	33.70	8.97	37.94
Šabac	5.40	11.10	1.49	28.55	11.59	41.87
$\bar{X} \pm Sd$	5.91 ±1.27	12.02 ±1.25	1.50 ±0.44	30.30 ±3.62	11.38 ±3.59	37.48 ±4.80
$C_{v\%}$	21.49	10.40	29.33	11.95	31.55	12.81
Min–Max	4.90-8.24	10.80-13.95	1.01-2.42	23.66-34.08	8.23-18.77	29.92-43.21

[1]NFE – nitrogen free extract; *BEM – extractive substances without nitrogen.*

Our chemical studies have shown that *A. artemisiifolia* has high protein content. The average value in the analyzed samples was 12.02±1.25%, what is higher than the protein content in unshelled oats, but less than in dehydrated alfalfa hay: 11.6% and 15.2% respectively (Sinovec and Ševković, 1995). The values of protein content in the samples at some sites are quite consistent, with a low coefficient of variability. Slightly higher content was measured in the samples from Zmajevo and Temerin area, which can be attributed to soil type and agrotechnical measures, i.e. to the content of nitrogen in the soil. Hubbard and Boe, during 1984 and 1985, analyzed 27 plant species characteristic for swamp area that are located in the eastern parts of South Dakota. Among these plants were three samples of ragweed (*Ambrosia spp.*). The content of crude ash, crude protein content and *in vitro* digestible dry matter (IVDDM) were determined. The protein content in samples of ragweed (*Ambrosia spp.*) was 15.7% in relation to dry matter at 100°C. If this is applied on our dry weight, the protein content would be 14.77%, what is slightly higher from our value.This difference can be explained by another kind of ragweed, as well as the features of the soil from which the samples were taken. Marten and Andersen (1975) determined the content of crude protein, macro and micronutrients in 12 weed species, among which was *A. artemisiifolia*, from Minnesota state. The sampling and measurement was done in 3 years (1971, 1972, 1973). Over this period in the

samples of *A.artemisiifolia* the average value of protein content was 26%. Such a high protein content in the samples of *A. artemisiifolia* from Minnesota was addressed by the above mentioned authors Hubbard and Boe (1988). They explain this difference in protein content by another kind of ragweed and, primarily, by application of fertilizers. This fact was also indicated by Marten and Andersen (1975), who point out that the whole field, that was under the study, was treated with 37 kg N/ha in early spring, every year before the outbreak of weeds sprout.

The tests show a high content of cellulose fibers in common ragweed. The average value in our sample was 30.30±3.62%. The values in the samples from different sites were quite close, what was indicated also by the coefficient of variation (11.95%). Crude fiber content of this weed plant was higher than in dehydrated alfalfa hay (with 15% prot.) and unshelled oats: 26.4 and 11.0% respectively (Sinovec and Ševković, 1995). High level of ash in the samples (11.38±3.59%) indicate the presence of minerals (macro and microelements). The values of ash content in samples from some sites were not so close, hence the coefficient of variation was high (31.55%). The sample from the area of Temerin have largely contributed this situation since the crude ash value was 18.77%. The variability in the crude ash content was expected because the soils on different sites differ in their mineral status. Hubbard and Boe (1988), in the aforementioned study, examined the samples of ragweed (Ambrosia spp.) and the crude ash value was 12.7% of dry matter at 100°C, which, when calculated on our dry matter was 11.95% and agrees with our data of 11.38%, although we tested another type of weed plants.

The values of macroelements: Ca, P, Mg, Na and K in the samples of *A. artemisiifolia* from different locations are given in Table 2. Greater variability in the values of macroelements, comparing to the fundamental nutrients is obvious, which was expected.These differences are a result of various factors such as the content of minerals in soil, pH value, the use of mineral and organic fertilizers, plant protection and other. This variability in the values has been particularly emphasized in the case of magnesium, calcium and sodium, and slightly lower in phosphorus and potassium.

The average calcium (Ca) value in the samples of *A.artemisiifolia* was 1.12±0.47% what is almost equal to the content of Ca in alfalfa hay (with 15% prot.) and ten times higher then of calcium in unshelled oats: 1.21% and 0.10% respectively (Sinovec and Ševković, 1995). Low calcium content in the samples from Mali Iđoš area can be explained by the place where the sample was taken (besides the road).

Table 2. Content of the macroelements in the Ambrosia artemisiifolia L. samples
Tabela 2. Sadržaj makroelemenata u uzorcima Ambrosia artemisiifolia L.

Location *Lokalitet*	Investigated parameter (%) / *Ispitivani parametar (%)*				
	Ca	P	Na	K	Mg
Zmajevo	1.43	0.12	0.02	3.08	0.59
Mali Iđoš	0.62	0.22	0.03	3.34	0.26
Novi Sad	0.88	0.26	0.04	3.44	0.23
Temerin	1.80	0.23	0.04	3.21	0.78
Odžaci	0.83	0.24	0.02	2.67	0.20
Palić	0.70	0.24	0.02	2.69	0.16
Šabac	1.58	0.28	0.02	3.42	0.35

$\bar{X} \pm Sd$	1.12 ±0.47	0.23 ±0.05	0.03 ±0.009	3.12 ±0.33	0.37 ±0.23
$C_{v\%}$	42.17	21.74	33.33	10.58	62.16
Min – Max	0.62 – 1.80	0.12 – 0.28	0.02 – 0.04	2.67 – 3.44	0.16 – 0.78

Marten and Andersen (1975), in the aforementioned paper, measured significant differences of minerals in grassy weeds and broad leaf weeds. Grassy weeds contained half less calcium comparing to the broad leaf weeds. The average value of calcium (in three year period), in the samples of *A.artemisiifolia* amounted to 2.6% of dry matter which is higher than in our case, due to fertilizers, what has already been mentioned. Bosworth et al., (1985), report that in 20 weed species, that have been studied in England, had similar calcium content as well as legumes. Franco-Hernandez et al., (2010), measured the concentrations of metals in different plants, among which was the *A. artemisiifiolia*, which were grown on waste field (tailings) in the vicinity of the mine in central Mexico. The calcium concentration measured in *A.artemisiifolia L*. from the area of Villa de la Paz, was 9209 mg/kg in root and 14244 mg/kg in shoots (branches) of ragweed. The percentage is equal to 0.92% and 1.42%. Calcium content in dry matter of plants moves in a wide range of 0.2 to 3% (Petrović and Kastori, 1992), and the land it has about 1.37% (Ubavić and Bogdanović, 2001).

The mean value of phosphorus (P) in the samples was 0.23±0.05%, what is in agreement with the content of phosphorus in dehydrated alfalfa hay (with 15% prot.), but lower than in unshelled oats: 0.21% and 0.31% respectively (Sinovec and Ševković, 1995). The sample from Zmajevo should be pointed out, in which the phosphorus content was half than the average, although the sample was taken from arable land, after wheat harvest, what may indicate that the fertilizers were not sufficently applied on the given field.

Marten and Andersen (1975) measured phosphorus content in *A.artemisiifolia* and it was 0.4%, which is two times higher than in our case and which is explained in the same way as in the case of calcium. The phosphorus content in plants usually ranges from 0.1 to 1.0% of dry matter (Petrović and Kastori, 1992), and in the soil varies between 0.03 and 0.3% (Ubavić and Bogdanović, 2001).

The value of magnesium (Mg) was 0.37±0.23% in average, with the highest coefficient of variation 62.16%. This was particularly the case with the sample from Temerin area (0.78%), and the sample from Zmajevo area (0.59%). Magnesium content was higher than in the unshelled oats and slightly higher than in dehydrated alfalfa hay (with 15% prot.): 0.16% and 0.28% respectively (Sinovec and Ševković, 1995). In three years work Marten and Andersen (1975), measured 0.8% in *A.artemisiifolia* samples comparing to dry matter. Franco Hernandez et al., (2010), in the aforementioned paper, measured the value of magnesium in the samples of *A.artemisiifolia* from the same area and it was 985 mg/kg in roots and 2494 mg/kg in the branches, calculated on dry matter. In percentage it is equivalent to 0.0985% and 0.25%. If these values are added up, the result is 0.3485% which is in agreement with the average value for this mineral in our investigation. Magnesium content in dry matter of plants usually ranges from 0.15 to 0.6%, with an average of 0.2% magnesium in feed (Petrović and Kastori, 1992). The content of this mineral in soil varies between 0.1 and 1.5% (Ubavić and Bogdanović, 2001; Džamić and Stevanović, 2000).

Potassium (K) in *A.artemisiifolia* samples showed the least variation (10.58%), with an average 3.12±0.33%. The amount of potassium in the tested samples was higher

than in oats and dehydrated alfalfa hay (with 17% prot.): 0.40% and 2.40% respectively (Glamočić, 2002).

Marten and Andersen (1975), in the aforementioned paper, in *A.artemisiifolia* samples measured the value of 3.9% potassium of dry matter, which is in agreement with our results. It can be concluded that potassium content is high in this weed plant. The total content in dry matter of plants varies in a wide range, from 0.3 to 6% (Petrović and Kastori, 1992), and the variability in soil is between 0.5 and 3.0% (Ubavić and Bogdanović, 2001).

Unlike the contents of potassium, sodium (Na) shows a higher variability of 33.33% in the samples of ragweed. The average value of sodium is 0.03±0.009% and was lower than in the hay dehidrated alfalfa (with 15% prot.) and oats: 0.07% and 0.06% respectively (Glamočić, 2002). Sodium content in dry matter of plants ranges from 0.01 to 2.0% (Džamić and Stevanović, 2000).

In Table 3 the values of microelements: Co, Mn, Fe, Zn and Cu are given, measured in the *A.artemisiifolia* samples taken from different sites. The contents of microelements shows greater variability than the macro elements content in the samples of ragweed taken from different sites, which largely provides an information on the presence of microelements in the soil on some locations.

The average content of cobalt (Co) in the samples of ragweed was 0.55 ± 0.48 mg/kg, with a high coefficient of variation of 87.27%. We should note the locations in Odžaci and Novi Sad. In the samples from these localities the lowest cobalt content was measured: 0.07 and 0.17% respectively, which can be explained by the position where the samples were found (near the road). The average value of cobalt was greater than in oats and alfalfa hay (with 17% prot.): 0.06 and 0.10 mg/kg air-dry matter, respectively (Glamočić, 2002). Otherwise, the content of cobalt in dry matter of plants ranges from 0.12 to 0.4 ppm (Petrović and Kastori, 1992), while the amount of easily accessible cobalt in the soil of wine growing regions of Vojvodina is from 0.24 to 0.47 ppm (Ubavić and Bogdanović, 2001).

Table 3. Content of the microelements in the Ambrosia artemisiifolia L. samples
Tabela 3. Sadržaj mikroelemenata u uzorcima Ambrosia artemisiifolia L.

Location *Lokalitet*	Investigated parameter (mg/kg) / *Ispitivani parametar (mg/kg)*				
	Co	Mn	Fe	Zn	Cu
Zmajevo	0.48	61.25	351.67	166.67	7.43
Mali Iđoš	1.35	45.83	277.92	460.42	14.52
Novi Sad	0.17	37.50	376.67	260.83	14.23
Temerin	1.07	110.83	2.122.50	78.33	9.92
Odžaci	0.07	41.67	201.25	47.92	11.53
Palić	0.32	58.33	120.00	43.33	27.22
Šabac	0.42	123.33	309.17	46.67	9.47
$\bar{X} \pm Sd$	0.55 ±0.48	68.39 ±34.52	537.02 ±704.69	157.74 ±156.05	13.47 ±6.58
$C_{v\%}$	87.27	50.47	131.22	98.93	48.85
Min – Max	0.07 – 1.35	37.50 – 123.33	120.00 – 2.122.50	43.33 – 460.42	7.43 – 27.22

Another important microelement, which was measured in the samples of common ragweed was manganese (Mn). The average value of manganese in examined samples was 68.39±34.52 mg/kg, with a slightly smaller coefficient of variation (50.47%) compared to cobalt. Two sites (Temerin, Šabac) should be pointed out as the localities where the content of manganese was higher comparing to the samples from other locations. In both cases it was arable land where the amount of easily-accessible manganese was obviously high. Otherwise, in fruit and wine-growing regions of Vojvodina, the amount of easily-accessible manganese ranges from 37 to 91 ppm (Ubavić and Bogdanović, 2001). The average value of manganese in ragweed samples was higher than the value of manganese in oats and alfalfa hay (with 17% prot.): 40 mg/kg and 30 mg/kg respectively (Glamočić, 2002). In the study of Marten and Andersen (1975), in *A.artemisiifolia* samples the values for manganese were 78 mg/kg, which is in agreement with our measurements. The content of manganese in dry matter of plants ranges from 50 to 250 ppm (Petrović and Kastori, 1992).

The greatest variability of microelements was found in iron (Fe), as a result of the measured values in the ragweed sample from Temerin area (2122.50 mg/kg). This high value could be explained by the presence of alfalfa on the given lot, which is known for its high content of this element. The average value of iron, measured in the ragweed samples was 537.02 ± 704.69 mg/kg, with a coefficient of variation of 131.22%.

The measured iron content in ragweed was higher than in oats and alfalfa hay (with 17% prot.): 70 mg/kg and 300 mg/kg respectively (Glamočić, 2002). Determining the nutritional value of 12 weed species in the three-year investigation in *A.artemisiifolia* samples Martin and Anderson (1975) were given a value of 387 mg/kg of dry matter. Franco-Hernandez et al. (2010), in the aforementioned paper, measured the iron content in roots and shoots of *A.artemisiifolia* and got values of 516 and 312 mg/kg, respectively. The amount of this microelement in plants depends on its content in the soil, which is variable and ranges from 1.0 to 10.0% and the average value is about 3.8% (Ubavić and Bogdanović, 2001). Iron concentration in plant dry matter ranges from 50 to 200 ppm (Petrović and Kastori, 1992).

The average value of zinc (Zn), measured in the samples of ragweed was 157.74±156.05 mg/kg, with a high coefficient of variation of 98.93%. A sample from the area of Mali Iđoš should be pointed out. In this sample the measured value was 460.42 mg Zn/kg air-dry matter. Also, a group of samples from four locations (Temerin, Odžaci, Palić and Šabac) can be noted for their quite uniform values of zinc. The average value of zinc in the samples of ragweed, and individually measured values were higher than the amount of zinc in oats and dehydrated alfalfa hay (with 17% prot): 20 mg Zn/kg in both nutrients (Glamočić, 2002). In *A. artemisiifolia* samples Marten and Andersen (1975), measured the value of zinc of 32 mg/kg dry matter, during their three-year investigation. Franco-Hernandez et al., (2010), measured the highest concentration of zinc in *A. artemisiifolia* samples from the area of Villa de la Paz, which amounted 405.7 mg/kg in the plant shoots, and in roots 54.9 mg/kg of dry matter. Based on this it can be conclude that *A. artemisiifolia* could be used for recovery of the land contaminated with zinc. Tóth et al., (2005), analyzed three different soil types regarding their composition, metal content and the origin of soil, the content of cadmium, copper, nickel and zinc in roots, leaves and flowers of ragweed (*Ambrosia elatior L.*) from these sites. They concluded that the increase of these elements in the soil increases the content in plants. Common ragweed accumulated analyzed metals mainly in roots. The measured

zinc content in the soil outside the ruderal area was 40 mg Zn/kg, and zinc content in the root of ragweed from this soil was 30.5 mg/kg. The content of zinc in ragweed samples from ruderal land, which was not contaminated with analyzed metals, amounted to 73.3 mg/kg in the leaves and 71.70 mg/kg in the inflorescence. Zinc content in dry matter of plant on average ranges from 20 to 50 ppm (Petrović and Kastori, 1992).

Table 3 displayes measured values of copper (Cu) in ragweed samples with less variability comparing to other microelements. The sample from Palić area should be pointed as the sample with slightly higher value of 27.22 mg/kg. The average copper content in samples was 13.47 ± 6.58 mg/kg of dry matter, which is higher than the copper content in oats and alfalfa hay (with 17% prot.): 5 mg/kg and 10 mg/kg, respectively (Glamočić, 2002).

In their three-year investigation Marten and Andersen (1975), in the samples of *A. artemisiifolia* measured copper and the value was 8 mg/kg of dry matter. Franco-Hernandez et al., (2010), in their work with *A. artemisiifolia* samples, measured copper from 14.6 mg/kg in the root and 30.4 mg/kg in the shoots of the plant. In the root of ragweed (*Ambrosia elatior L.*), taken from outside the ruderal area, Tóth et al., (2005), measured the value of 11.0 mg Cu/kg. In samples from ruderal areas that were not contaminated with the metals, they got the value of 8.96 mg Cu/kg in the inflorescence. The values for copper presented in the literature are in the agreement with our measurements. The average copper content in dry matter of plants ranges from 2 to 20 ppm (Petrović and Kastori, 1992).

CONCLUSION

Our study shows that common ragweed has good nutritive values regarding to the content of basic nutrients and minerals. The average crude protein content (12.02 ± 1.25%) is higher than in unshelled oats, which is used as one of the main nutrients in feed, and crude fiber content (30.30 ± 3.62%) is higher than in unshelled oats and dehydrated alfalfa hay (with 15% protein). The average fat content is lower than in oats and dehydrated alfalfa hay. When it comes to minerals, it can be stated that common ragweed is not behind almost all most frequently used nutrients, such as oats or alfalfa hay. The average content of Ca and P was similar to that in dehydrated alfalfa hay (with 15% prot.), but the amount of Ca is ten times higher than in unshelled oats. The content of Mg and K is higher than in oats and dehydrated alfalfa hay (with 17 % prot.). This also applies to microelements. The average contents of Co, Mn, Fe, Zn and Cu in ragweed samples are higher than in oats and dehydrated alfalfa hay (with 17% protein). Large variability in minerals was observed, especially for microelements, as a consequence of that location, i.e. features of the land from which the samples were taken.

Based on the results of measurements, there is a need for further investigation of this weed, especially in terms of its impact on the health of small ruminants (sheep and goats) when used in animal nutrition. It is necessary to determine whether residua may be found in the products obtained from animals fed with *A. artemisiifolia L.* , what can cause adverse affects on human health. Animals usually refuse to eat this plant. The question is whether the cause is its distinguished bitterness, allergenicity action or something else. Answer to these questions should be found in future investigations.

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SADRŽAJ OSNOVNIH HRANLJIVIH I MINERALNIH MATERIJA U UZORCIMA AMBROZIJE (*AMBROSIA ARTEMISIIFOLIA L.)* SA RAZLIČITIH LOKALITETA

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Izvod

Ispitivan je sadržaj osnovnih hranljivih i mineralnih materija u uzorcima ambrozije (*A.artemisiifolia L.*), sa sedam različitih lokaliteta. Ispitivanje je izvršeno standardnim hemijskim metodama. Rezultati ispitivanja ukazuju na to da ova korovska biljka ima dobra nutritivna svojstva, kako u sadržaju osnovnih hranljivih materija tako i u sadržaju makro i mikroelemenata. Najveću varijabilnost u sadržaju nalazimo kod mikroelemenata i to kod gvožđa, kobalta i cinka. Prosečna vrednost sadržaja proteina (12,02 ±1,25%) je veća od sadržaja proteina u neoljuštenom ovsu i manja od sadržaja u senu dehidrirane lucerke, a prosečna vrednost sirove celuloze (30,30 ±3,62%), u uzorcima ambrozije je veća od njenog sadržaja u neoljuštenom ovsu i senu dehidrirane lucerke (sa 15% proteina). Prosečne vrednosti sadržaja ispitivanih mikroelemenata su veće od njihovih vrednosti u ovsu i senu dehidrirane lucerke (sa 17% proteina). Isti odnos imamo i kod makroelemenata: magnezijuma i kalijuma. U slučaju kalcijuma i fosfora, njihove prosečne vrednosti u uzorcima ambrozije su slične vrednostima ovih elemenata u senu dehidrirane lucerke (sa 15% proteina). Dobijeni rezultati ukazuju na potrebu daljeg istraživanja ove korovske biljke.

Ključne reči: *Ambrosia artemisiifolia L.,* hranljive materije, makroelementi, mikroelementi.

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REFERENCE VALUES AND FREQUENCY DISTRIBUTION OF METABOLIC PARAMETERS IN COWS DURING LACTATION AND IN PREGNANCY*

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BOJANA VIDOVIĆ, LJUBICA KRČMAR[1]

SUMMARY: Biochemical parameters in 40 Holstein-Friesian cows have been studied. Cows were in the second and third lactation. Blood was sampled during four productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2-3 weeks before the start of drying). Concentrations of glucose, protein, cholesterol, bilirubin, urea, NEFA, BHB, ALT, AST, Ca, P and Mg were testes by using standard biochemical methods. The parameters of the metabolic profile of Holstein-Friesian cows correspond to the reference values in cattle. Parameters are within the normal (Gaussian) distribution. Periparturient changes in the concentrations of metabolites are characterized by negative energy balance, with the development of liver failure and reducing the concentration of calcium. Frequency distribution of values of metabolic profile suggests that clinically healthy animals during the entire period of lactation may show signs of metabolic changes, which is probably caused by high demand for milk production, due to load stress and inadequate nutrition.

Key words: *metabolic profile, milk cows, the reference values, frequency distribution of parameters.*

INTRODUCTION

Forty years ago in England it was recommended that the purpose of evaluation of metabolic status in dairy cows is examination of the metabolic profile. Then the so-called Compton test was adopted which includes the determination of hematocrit,

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Original scientific paper */ Originalni naučni rad*

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the concentration of glucose, urea, phosphorus, copper, zinc, magnesium, total protein, albumin, globulin, beta-carotene and vitamin A in the blood. Development of clinical medicine introduced other important parameters such as bilirubin, nonesterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), triglycerides, cholesterol, and many others. Determination of values for these parameters in the blood is of great importance in terms of evaluation of nutrition, productivity and potential susceptibility to development of various diseases (Payne et al., 1970).

Each of these parameters has a specific meaning in clinical practice. The concentration of glucose, NEFA, BHB, cholesterol and triglycerides indicates the energy status of the organism. The concentration of bilirubin and protein with the previous parameters can indicate the degree of fattening of the liver and liver failure and the risk of ketosis. Lipolysis is a characteristic response of cows to different, in particular acute stressor (Cincović et al., 2010). The concentration of urea suggests the supply of the body protein, but also points to the health of the kidneys or liver. Calcemia indicates the risk of puerperal paresis and phosphoremia the possibility of developing locomotor disorders (Kaneko et al., 2008). It is known that at the beginning of lactation leads to important changes in the metabolism of cows. Periparturient period in dairy cows leads to reduced intake of food dry matter (Grummer et al., 2004), while the other grows daily milk production. Such physiological changes have led cows in a state of negative energy balance and lipolysis. Negative energy balance leads to numerous changes in the movement of values of metabolic parameters. On the other hand, most diseases occur in the periparturient period and are the consequences of that negative energy balance (James et al., 2005). The parameters of metabolic profile are important in assessing the conditions for feeding and care and its impact on the health of cows (Krnić et al., 2006).

The aim of this paper is to examine the reference values for selected parameters of metabolic profile, and to present the frequency distribution of values of parameters depending on the period of lactation.

MATERIAL AND METHODS

Biochemical parameters were examined in 40 Holstein-Friesian cows. Cows were in the second and third lactation and grown under the same conditions of diet (standard recipe meals based on corn silage, TMR) and care (free rearing system on deep litter). Blood was collected in four main productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2 - 3 weeks before the start of drying). Blood was taken from the jugular vein after the morning milking. After sampling the blood was immediately centrifuged (2000 rpm) and serum disposed in handy fridge, while the laboratory analysis was carried out during the day. Concentrations of glucose, protein, cholesterol, bilirubin, urea, NEFA, BHB, ALT, AST, Ca, P and Mg were tested by using standard biochemical methods. Biochemical analysis was performed by using standard methods of biochemical tests on blood analyzer Rayto RT1904C, with production of standardized kits RANDOX UK (NEFA) and POINT SCIETIFIC USA (other parameters).

RESULTS

The results presented in Table 1 show the average values of metabolic parameters in four product categories of cows. The results show that cows after delivery showed a number of adaptive mechanisms, which are reflected in the concentrations of certain metabolites. Due to metabolic stress concentration of glucose was lower and concentrations of NEFA and BHB higher in cows after birth compared to the other three studied groups. The concentration of calcium was significantly lower in the post-parturition. These parameters are outside the normal range after delivery. The concentrations of protein, bilirubin and urea were also significantly different in cows after delivery, but do not exceed physiological values. Table 2 shows the number of cows which were established by these changes. In 10 cows NEFA concentration was high above the physiological data, while the higher concentration of BHB in 34 cows. The concentration of glucose was lower in 17 cows, and calcium concentrations in 20 cows. Charts 1-12 show the frequency distribution of cows with different values of parameters, in order to get the impression of the situation in the population.

Table 1. The average values of blood biochemical parameters according to stage of lactation
Tabela 1. Prosečne vrednosti biohemijskih parametara krvi shodno stadijumu laktacije

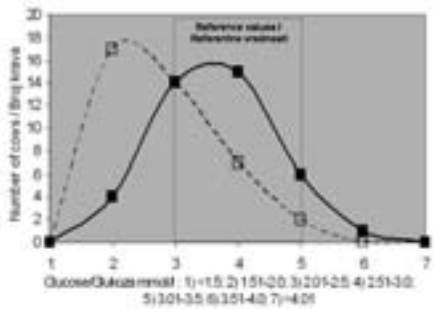
	Precalving *Zasušene*	Fresh cows *Početak laktacije*	Mid lactation *Sredina laktacije*	End lactat. *Kraj laktacije*	Ref.value *Ref.vredn. literatura*
Glucose *Glukoza*, mmol/l	2.55	1.91*	2.46	2.31	*2.2-3.3*
Proteins *Proteini*, g/l	78.44	77.13*	80.16	80.43	*60-80*
Hoelsterol mmol/l	4.21	4.13	3.72	3.62	*1.3-3.6*
Bilirubin μmol/l	4.23	5.71*	5.55	4.82	*0.9-6.9*
Urea mmol/l	3.22	2.36*	3.01	3.06	*1.7-6.7*
NEFA mmol/l	0.22	0.47**	0.25	0.15	*0.1-0.35*
BHB mmol/l	0.41	0.98**	0.43	0.35	*0.3-0.5*
ALT U/I	26.8	28.5	27.74	28.03	*10-40*
AST U/I	83.31	88.97	79.56	82.27	*78-132*
Ca mmol/l	2.5	1.98*	2.4	2.37	*2-3*
P mmol/l	1.93	1.91	1.84	1.82	*1.6-2.3*
Mg mmol/l	0.94	1.01	1.07	1.12	*0.7-1.2*

*p<0.05, ** p<0.01.

Table 2.The number of cows whose metabolic parameter values are outside the reference (+ turn to the right, - turn left)
Tabela 2. Broj krava čije su vrednosti metaboličkog parametra van referentnih (+ skretanje u desno, - skretanje u levo)

	Precalving *Zasušene*		Fresh cows *Početak laktacije*		Mid lactation *Sredina laktacije*		End lactat. *Kraj laktacije*	
Vrednost izvan referentnih	+	-	+	-	+	-	+	-
Glukoza mmol/l	0	1	0	17	1	1	0	1
Proteini g/l	0	1	1	5	2	0	1	2

Hoelsterol mmol/l	0	0	2	0	3	0	5	0
Bilirubin µmol/l	3	0	9	0	4	0	3	0
Urea mmol/l	0	1	0	1	1	1	0	0
NEFA mmol/l	2	0	10	0	2	0	1	0
BHB mmol/l	0	0	34	0	1	0	0	0
ALT U/I	/	/	/	/	/	/	/	/
AST U/I	/	/	/	/	/	/	/	/
Ca mmol/l	1	2	3	20	1	3	4	3
P mmol/l	0	0	0	3	0	3	0	0
Mg mmol/l	1	0	1	2	1	3	2	1



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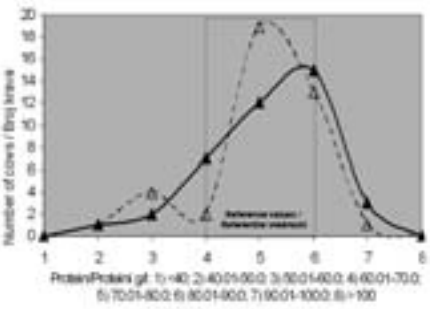

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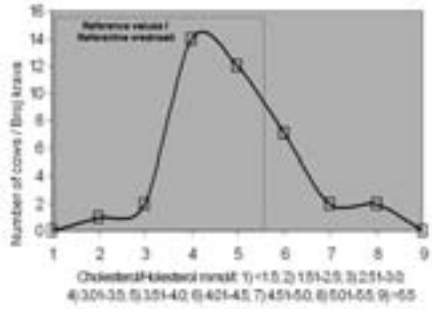

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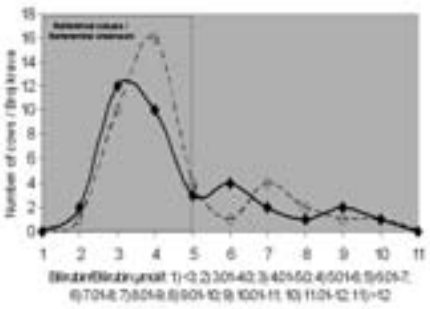

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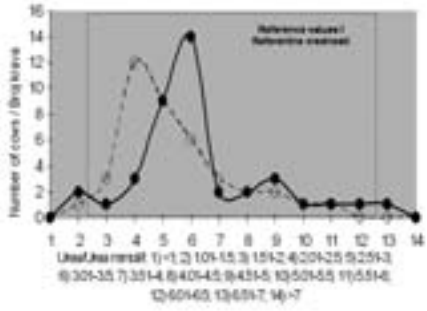

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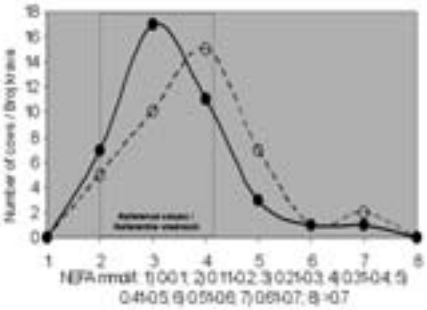

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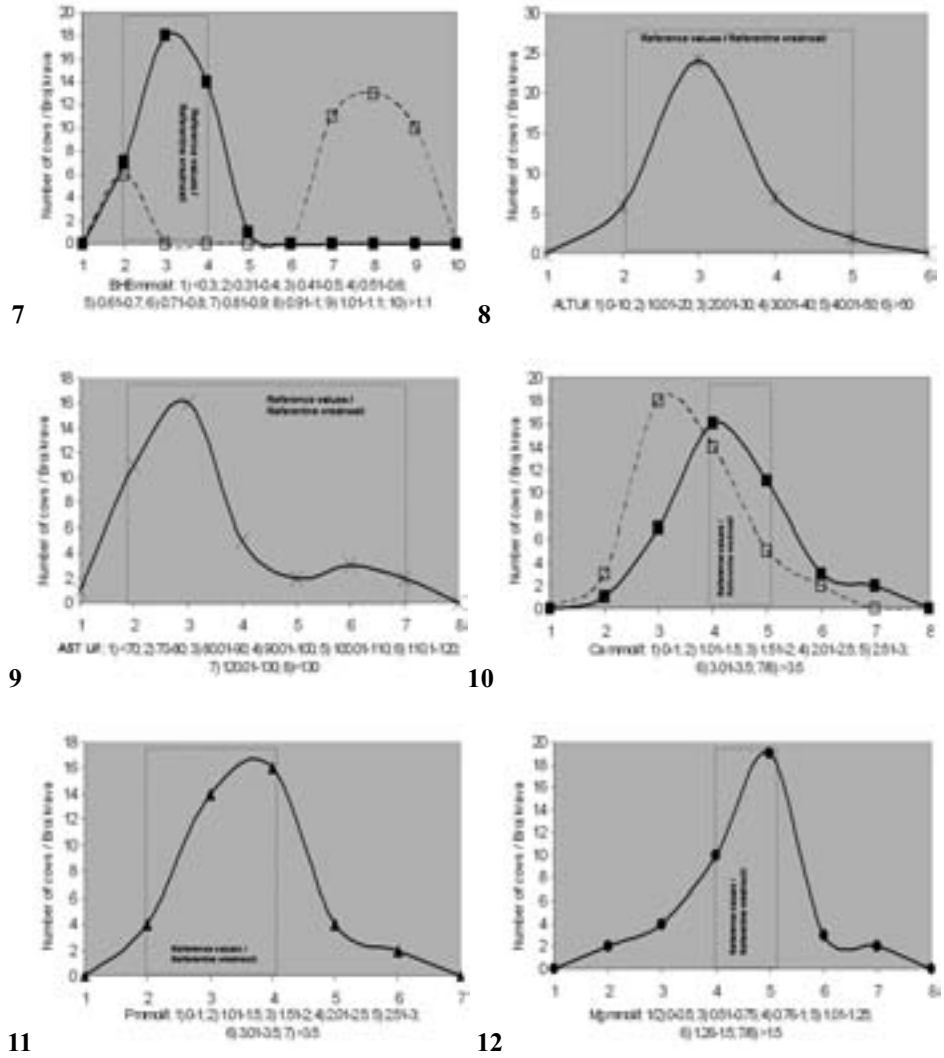

Graph 1-12: Frequency distribution of average values of the parameters examined in 40 cows (solid line), with a distinctive distribution of trends in the periparturient period for the parameters whose values differ significantly from the average population (dashed line), a rectangle shows the reference values obtained by analysis of the literature.

Grafici 1-12: Distribucija frekvencije prosečnih vrednosti parametara kod 40 ispitivanih krava (puna linija), sa naglašenom distribucijom kretanja u peripartalnom periodu za parametre čije se vrednosti signifikantno razlikuju za ovaj period (isprekidana linija), pravougaonik pokazuje referentne vrednosti dobijene anlizom literature.

DISCUSSION

Our results are in agreement with the results of most researchers in our region and beyond. Thus, Jovanović et al. (1987) found that 10-15 days before parturition the

concentration of glucose is about 2.71 mmol / l, 10 days after calving 2.4 mmol / l, the second month of lactation 2.6 in the fifth month of lactation 2.7 mmol / l. Reduced blood glucose in the periparturient period (Table 1, Graph 1) resulted from the negative energy balance, due to reduced food intake and very intense lactation (Table 1). Because of the negative energy balance volume of lipid for energy purposes increased, which is reflected in increased lipolysis and ketogenesis. So the periparturient period, especially in the days after calving increases the concentration of NEFA and BHB, and the obtained values are in accordance with the results of previous studies (Kovačević et al., 2002; Hachenberg et al., 2007, Park et al., 2010). Bilirubin concentration was slightly higher in the puerperal period compared to the rest of lactation, and in the range of previously obtained results (Šamanc, 1992). Bilirubin concentration correlated with the degree of fatness of liver. It is important to note (Graph 4) that, looking at the average level of the tested cows, a number of animals still show a slightly higher concentration of bilirubin in relation to the reference value. This situation is due to the stress, inadequate nutrition and energy load of cows in our population. Cholesterol (Table 1; Graph 3) agrees with previously obtained results (Cincović et al., 2010), and the relationship of cholesterol and NEFA can be used to assess fatty liver in periparturient period. Hypoalbuminemia, hypocholesterolemia and hypoglycemia with ketosis cows show reduced biosynthetic function of liver (Đokovic et al., 2007).

Protein concentration (Table 1, Graph 2) belongs to the low variable indicators, because disorder occurs only in the decompensated stages of various diseases. The concentration of proteins in different studies on dairy cows ranged from 60-80 g / l (Jovanovic et al., 1987; Kupezinski and Chudoba-Drozdovski, 2002). The concentration of urea (Table 1, Graph 5) was slightly lower in the periparturient period compared to other parts of lactation, possibly due to the decrease of dry matter intake in food and altered liver metabolism during this period, which agrees with the result in lower proteinemia. Similar results were obtained by Važić et al. (2010). Transaminases up (Table 1, Graph 8 / 9) are in the expected range, and period of lactation has no significant effect on their value in healthy cows.

Stojević et al. (2005) found low values of these enzymes in serum. The concentration of ions was within the expected values and a somewhat wider range of distribution than the current reference value. Similar values can be found in recent studies obtained from Đoković et al. (2010). In the early puerperium Calcemia is smaller than in other periods of lactation. Calcemia less than 2 is common in periparalnom period. Decrease of calcemia below 1.8 mmol / l may be a predisposing factor towards the development of puerperal paresis, abomasal dislocation and other (Goff et al., 2000).

CONCLUSION

The parameters of the metabolic profile of Holstein-Friesian cows correspond to the reference values for a given type of animal. Periparturient changes in the concentrations of metabolites are characterized by negative energy balance, with the development of liver dysfunction and reduced Calcemia. Frequency distribution of values of metabolic profile suggests that clinically healthy animals may show signs of metabolic changes, which is probably caused by high demand for milk production, load stress and inadequate nutrition.

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DIURNALNA VARIJACIJA METABOLITA KRVI KOD KRAVA TOKOM TOPLOTNOG STRESA

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Izvod

Ispitivani su biohemijski parametri kod 40 krava Holstajn-Frizijske rase. Krave su bile u drugoj i trecoj laktaciji. Krv je uzorkovana u okviru četiri osnovna produktivna perioda i to: u zasušenju (2-3 nedelje pred porođaj), u postpartalnom periodu (0-2 nedelje postpartum), u sredini laktacije (15-17 nedelja postpartum) i u kasnoj laktaciji (2-3 nedelje pred početak zasušenja). Standardnim biohemijskim metodama ispitana je koncetracija: glukoze, proteina, holesterola, bilirubina, uree, NEFA, BHB, ALT i AST, Ca, P i Mg. Parametri metaboličkog profila Holštaj-frizijskih krava odgovara referentnm vrednostima za datu vrstu, a parametri se nalaze u okviru normalne (Gausove) distribucije. Peripartalne promene u koncentraciji metabolita odlikuju se negativnim energetskim bilansom, sa razvojem insuficijencije jetre i smanjenjem kalcemije. Distribucija frekvencija vrednosti metaboličkog profila ukazuje da klinički zdrave životinje tokom kopletnog perioda laktacije mogu pokazivati znake metaboličke izmene, koja je najverovatnije posledica velikih zahteva za proizvodnjom mleka, opterećenosti stresom i neadekvatne ishrane.

Ključne reči: metabolički profil, mlečne krave, referentne vrednosti, distribucija frekvencije parametara.

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INFLUENCE OF RESPIRATION RATE AND RECTAL TEMPERATURE IN HOLSTEIN COWS TO MILK PRODUCTION DURING HEAT STRESS*

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SUMMARY: The aim of this paper is to examine the relationship between rectal temperature and respiration rate and the production of milk during exposure to heat stress. The results show that increased values of temperature humidity index (THI) cause increases of respiration rate, so that for every unit of THI over 64, respiration rate increases by 0.6. The correlation coefficient between the rate of respiration and THI was 0.55 ($p < 0.05$). Rectal temperature in cows increases by 0.09 ° C for each unit of THI over 64. The correlation between the THI and the mean rectal temperature was highly significant and amounts to 0.92 ($p < 0.01$). The results show a very high negative correlation between the respiration and the production of milk (-0.82, $p < 0.01$) i.e. rectal temperature and milk production (-0.92, $p < 0.01$). For each unit of respiration (breath / min) over 45, the amount of milk decreases by about 0.3 L, while for every degree over for the rectal temperature, the milk production decreases by about 3.5 L (0.35l/0.1 ° C). The respiration rate and THI showed a slight linear dependence, probably because during the trial there was mild to moderate thermal load of cows.

Key words: *heat stress, dairy cows, THI, respiration rate, rectal temperature, milk production.*

INTRODUCTION

Global warming and extreme climate changes led dairy cows to the state of thermal stress. As an index of heat stress the index THI (temperature-humidity index) is used. Numerous results show the negative impact of THI index on the health and pro-

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Original scientific paper / *Originalni naučni rad*

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ductivity of cows (Kadzere, 2002, Hristov et al., 2007, Bernabucci et al., 2010). Although the THI index of quality parameters is used for estimating the heat load of the organism of cows (Dikman and Hansen, 2009), due to various formulas and methods for calculating this index, it is necessary to explore the parameters of adaptation to heat stress indicating thermal overload.

Conducted measurements have given the high correlation between the THI and rectal temperature, rumen contractions and milk production (Cincović and Belić 2009, Belić et al., 2010). During exposure to heat stress there was a diurnal variation in body temperature and respiratory rate, and in the value of various metabolites (glucose, NEFA, urea, cholesterol) (Cincović et al., 2010; Cincović et al., 2010b), which indicates the sensitivity of the indicators to stress.

The aim of this paper is to examine the relationship between rectal temperature and respiration rate and the production of milk during exposure to heat stress.

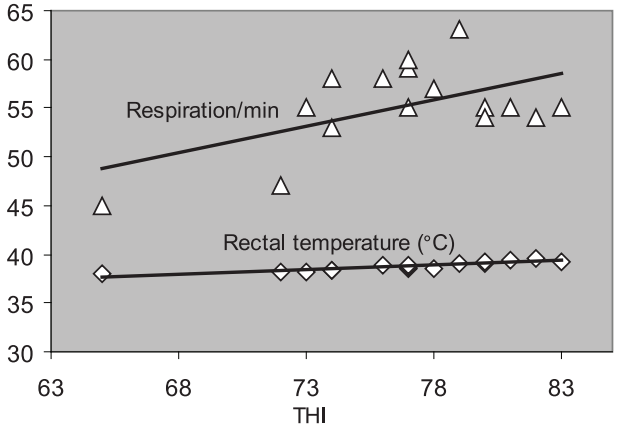
MATERIAL AND METHODS

The experiment included 12 Holstein-Friesian cows in the second and third lactation, which were grown under the same conditions, nutrition and care. The experiment was carried out during July and August 2010. THI value was calculated by formula mean THI = 0.8 x mean AT + [RC mean x (mean BP-14.4)] + 46.4, where AT is the ambient air temperature and relative humidity RH expressed in decimals (McDowell, 1979). Rectal temperature and respiratory frequency were measured every third day, four times a day (at 8, 12, 16, 20 h). Milk production was monitored daily.

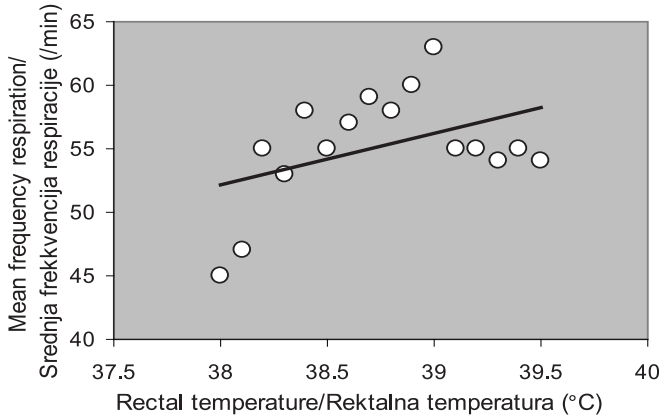
The parameters are compared through linear regression and correlation was calculated for the relationships: THI and rectal temperature, THI and respiration rate and rectal temperature and milk production and respiration rate and milk production.

RESULTS

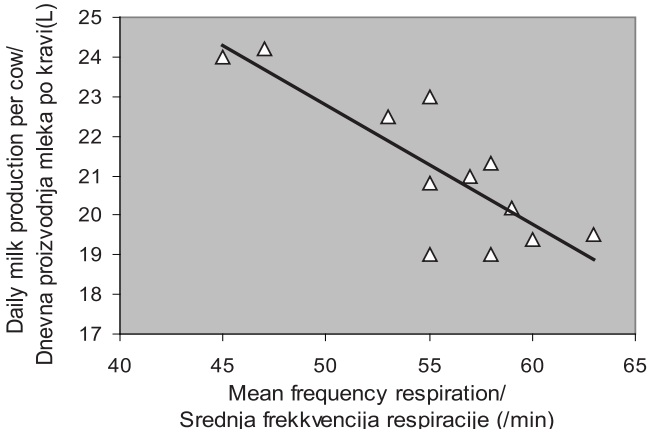
Graph 1 shows the relationship between rectal temperature / respiration rate and the value of THI. The obtained linear equation that links the rectal temperature and THI (Y = 31.4 +0.09 x) and respiration rate and THI (Y = 13.46 +0.6 x) determine that the respiratory organs are more sensitive to higher values of THI (significantly higher b parameter equation). However, by the analysis of the Pearson moment (0.55 for respiration rate and rectal temperature for 0.9), we see that it is significantly higher when it comes to rectal temperature. In addition to the sensitivity of the respiratory tract to higher values of THI, there is their sensitivity to temperature. Respiratory rate was positively correlated with body temperature, but their correlation was insignificant. Chart 3 and 4 show that the rectal temperature and respiration levels negatively correlated with milk production. Both indicators can be used effectively in the future assessment of milk production because the parameter b of linear equations does not differ significantly, and there is a very high correlation with milk production in both cases, which will be further analyzed in the discussion.



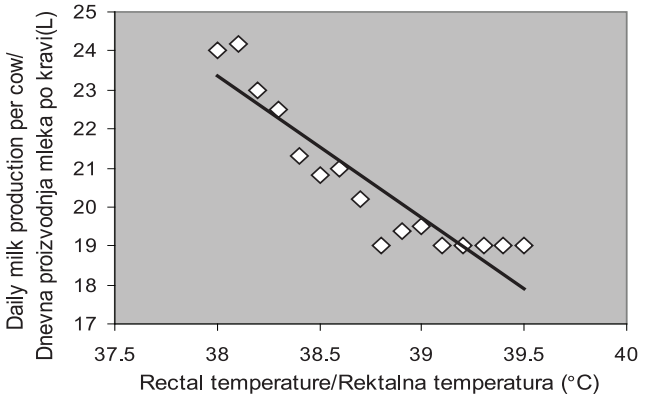
Graph 1. The linear relationship between THI and rectal temperature / respiration rate
Grafik 1. Linearni odnos THI i rektalne temperature i THI i frekvence respiracije



Graph 2. The linear relationship between mean rectal temperature and respiration rate
Grafik 2. Linearni odnos srednje rektalne temperature i vrednosti respiracije



Graph 3. The linear relationship between respiration rate and milk production
Grafik 3. Linearni odnos frekvence respiracije i proizvodnje mleka



Graph 4. A linear relationship between rectal temperature and milk production
Grafik 4. Linearni odnos vrednosti rektalne tempearture i proizvodnje mleka

DISCUSSION

THI values in this study ranged from 63 to 81 (unrepresented results). This range of values THI indicates the presence of heat stress. Initial studies have shown that the critical value of THI above which developing of adaptation of about 72, while later results suggest that the lower critical value is about 64 (Igono et al., 1992).

The results presented in Graph 1 show that with increasing values of THI respiration rate increases, so that for every unit of THI over 64, respiration rate increases by 0.6. The correlation coefficient between the respiration rate and THI is at the level of 0.55 ($p < 0.05$). Also, rectal temperature (Graph 1) in cows increases by 0.09 ° C for each unit of THI over the 64. The correlation between the THI and mean rectal temperature

was highly significant and amounts to 0.92 ($p < 0.01$). For each °C over 38, respiration rate increases by 6 (Graph 2). However, this value is much lower compared to the real one, because they take into account all-day measurements and body temperature in thermal comfort part of the day. Heat stress rapidly leads to more significant growth of respiration rate per unit of body temperature. Thus, Belic et al. (2010) found that the diurnal difference of minimum and maximum rectal temperature during exposure to heat stress is 0.9, and the value of the difference in the respiration rate is even 25. The correlation coefficient between the rectal temperature and respiration level is about 0.4. Elevated rectal temperature during exposure to heat stress caused inability of adequate cooling of the body. The skin and respiratory tract of cattle have great capacity to dissipate heat by evaporation, through sweating and panting, respectively. Increased respiration results from a tendency of the organism that by evaporation through the lungs went to excess heat (Gaughan et al, 2000).

Heat stress significantly decreases milk production. This phenomenon pathophysiologically explains the fact that increased body temperature during exposure to heat stress leads to reduced food intake and reduction of milk production. In a strictly controlled laboratory conditions, Spiers et al. (2004) showed that the food intake (kg) and milk production are of significant correlation, while the importance of food intake is negatively correlated with average daily rectal temperatures. The results show a very high negative correlation between the respiration and the production of milk (-0.82, $p < 0.01$) (Graph 3) and rectal temperature and milk production (-0.92, $p < 0.01$) (Graph 4). For each unit of respiration (breath / min) over 45, the amount of milk decreases by about 0.3 l. The increase in rectal temperature by one degree will lead to a drop in milk production by 3.5 liter (0.35l/0.1 °C).

The results show that the respiration rate is important in assessing the response of cows to the values of THI index, while the rectal temperature is more important to evaluate the possible reduction of milk production during exposure to heat stress.

CONCLUSION

For estimation of milk production in cows during heat stress, the values of rectal temperature and respiration per minute can be used as predictive factors. Rectal temperature was in a stronger correlation with milk production, while the respiration rate per minute was in a stronger correlation with the value of THI. The value of respiration and THI showed a slight linear dependence, probably because during the trial there was mild to moderate thermal load of cows.

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UTICAJ NIVOA RESPIRACIJE I REKTALNE TEMPERATURE HOLŠTAJN KRAVA NA PROIZVODNJU MLEKA TOKOM TOPLOTNOG STRESA

MARKO R.CINCOVIĆ, BRANISLAVA BELIĆ, SINIŠA GATARIĆ

Izvod

Cilj ovoga rada jeste da se ispita odnos rektalne temperature i frekvencije respiracije prema proizvodnji mleka tokom delovanja toplotnog stresa. Rezultati prikazani u Graph. 1 pokazuju da sa porastom vrednosti THI raste frekvenca respiracije, tako da za svaku jedinicu THI preko 64 frekvenca respiracije raste za 0.6. Koeficijent korelacije između frekvence respiracije i THI je na nivou 0.55 ($p<0.05$). Rektalna temperatura kod krava raste za 0.09°C za svaku jedinicu THI preko 64. Korelacija između vrednosti THI i prosečne rektalne temperature je visoko značajna i iznosi 0.92 ($p<0.01$). Rezultati našeg istraživanja pokazuju vrlo visoku negativnu korelaciju između vrednosti respiracije i proizvodnje mleka (-0.82; $p<0.01$), odnosno rektalne temperature i proiz-

vodnje mleka (-0.92, $p<0.01$). Za svaku jedinicu respiracije (udisaj/min) preko 45 opada količina mleka za oko 0.3 L, dok za svaki stepen više kada je rektalna temperaura u pitanju proizvodnja mleka opada za oko 3,5 L (0.35L/0.1°C). Vrednost respiracije i THI pokazuju blagu linearnu zavisnost, verovatno zbog toga što je tokom ogleda postojala blaga do srednja termalna opterećenost krava.

Ključne reči: toplotni stres, mlečne krave, THI, frekvenca respiracije, rektalna temperatura, proizvodnja mleka.

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COMPARISON OF TWO METHODS IN THE DETERMINATION OF HEART'S MEAN ELECTRICAL AXIS IN DOGS

LJUBICA SPASOJEVIĆ KOSIĆ[1]

SUMMARY: In the process of interpreting an electrocardiogram (ECG) the mean electrical axis of the heart needs to be determined. In veterinary electrocardiography the mean electrical axis (MEA) is figured in the frontal plane and can be calculated by using several different ways. In this study, values of the MEA, calculated by two methods (the isoelectric lead method and the vector method), were compared. The aim of this study is to establish whether different methods give different values for MEA. German shepherd dogs of different age, presenting in sinus rhythm, were used in the study. Electrocardiograms (ECGs) were recorded while the dogs were in standard position (right lateral recumbency). The determination of mean electrical axis were performed by examining the QRS complexes in each of the peripheral leads (I, II, III, avR, aVL, aVF). Mean values of the MEA were calculated by two methods were $\bar{x}1 = 76,50^o \pm 19,83$ *i* $\bar{x}2 = 80,17^o \pm 11,44$*. Values of the MEA determined by two methods were not statistically different (p = 0,384). These results show that value of the MEA in healthy dogs is not under significant influence of the method chosen for calculation.*

Key words: *mean electrical axis, calculation, German Shepherd dogs.*

INTRODUCTION

An interpretation of electrocardiogram implies the assessment of heart rate and rhythm, and determination of heart's mean electrical axis (MEA) and ECG's parameters. Determination of the MEA means the determination of the average (mean) direction that the wavefront of depolarization moves in the heart. The MEA can be applied to atrial depolarization (P wave), ventricular depolarization (QRS) or ventricular repolarization (T wave), but most commonly determined only for ventricular depolarization. The direction of net vector of electrical activity is determined by examining the QRS complexes in each of the six basic leads of Bailey's hexaxial lead system. In veterinary electrocardiography the mean electrical axis is usually figured in the frontal plane. According to this only the six peripheral leads of ECG recording are used to calculate the MEA (Edwards, 1987).

Original scientific paper / *Originalni naučni rad*

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Calculation of the MEA with an ECG is non - invasive diagnostic method, easy and chip, which evaluates how the heart is oriented in the chest, what is the size of cardiac chambers (increase or loss of ventricular myocardium), and are there conduction defects in bundle branches. These changes could be the result of variety of heart diseases (congenital and acquired heart diseases, myocardial infarct, heart base tumors). Normally, the MEA for the dogs is between +40º do +100º. Shiftings of the MEA direction can be left axis deviation (myocardial hypertrophy, conduction defects of left bundle branch) and right axis deviation (right ventricle enlargement, right bundle brunch block) (Kittleson, 1998).

The former studies have evaluated values of MEA due to different positioning of the patients (Detweiler, 1993; Rishniw et al., 2002; Harvey et al., 2005; Ghită et al., 2007). Studies have shown that position of the heart in thorax and changes of electrodes placement influence the MEA value. In dogs MEA shifts to the left when dog is placed in standing (Rishniw et al., 2002) or sitting position (Coleman et al., 2005), because of the changes of heart position in thorax. Due to all these a posibility of incorrectly diagnosing left heart enlargement in a normal dog is increased. There are different methods for calculating MEA (Edwards, 1987; Kittleson 1998; Rishniw et al., 2002; Harvey et al., 2005; Farasin et al., 2006; Singh, Athar, 2003; Singh, Athar, 2006). In this work two methods, commonly used in clinical practice, are compared: the isoelectric lead method and the vector method. The aim of this study is to establish is there any difference of MEA value if different methods of MEA calculation are used.

MATHERIALS AND METHODS

In this study 31 German Shepherd dogs were used. Dogs' age were from 1 – 13 years. All dogs considered healthy based on history of no cardiac disease and normal cardiovascular physical examinations. The dogs enrolled in this study were in sinus rrhythm. The assessment of the MEA is performed with examination of standard 6-lead electrocardiogram (I, II, III, aVR, aVL, aVF). During ECG recordings all dogs were placed in standard recording position (right lateral recumbency). For calculation of the MEA two methods are used: the isoelectric lead method and the vector method.

The isoelectric lead method: first, the isoelectric lead is found. The isoelectic lead is one in which the sum of all the positive and negative deflection of the QRS complex equals zero. The six- axis system is used to find the lead which is perpendicular to the isoelectric lead. The MEA is oriented toward the direction of QRS of the perpendicular lead. If QRS is positive the MEA is oriented toward the positive pole of that lead and if it is negative, the MEA is oriented toward the negative pole of that lead.

The vector method for determining the MEA involves selecting any two limb leads (I, II or III). The algebraic sum of positive and negative deflection of the QRS complex is calculated for each of the two selected leads. Values are plotted on graphical display of each of the two selected leads. Afterwards, perpendiculars are drown from each lead. The MEA is represented by the line drown from the center of the axis chart to the point at which the two perpendicular lines meet.

The data are reported as the mean ± SD. Student t- test for independent samples is used to compare values of the MEA calculated by two different methods. Difference in the incidence of MEA deviation from normal reference values was assessed by Fisher probability test. Values of $p < 0,05$ were considered significant.

RESULTS AND DISCUSSION

In one dog, axis could not be determined in frontal plane due to fact that QRS complexes was isoelectric in all peripheral leads (Figure 1). This dog is excluded from the study.

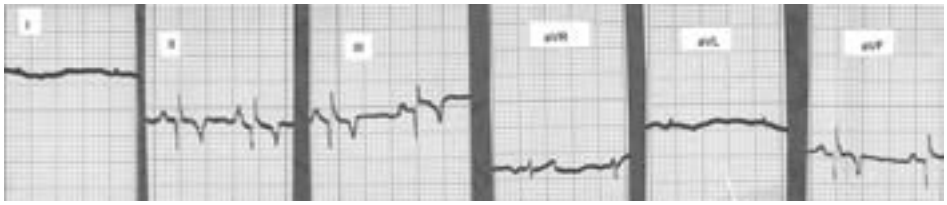

Figure 1. Electrically vertical heart
Slika 1. Električno vertikalno srce

Analysis of MEA value of each dog calculated by the isoelectric lead method, has shown the devitation from the reference values in two dogs. If MEA was calculated by the vector method, deviation from the reference values has existed in one dog. The incidence of MEA deviation from normal reference values assessed by different method of the MEA calculation, were not statistically significant.

Interpretation of ECGs of many dogs' breeds shows that differences among them could exist. The studies performed in German Shepherd dogs (Rezakhani et al., 1990; Rouholamin et al., 2000; Spasojević Kosić, 2009) points some specificity of this breed. The mean heart rate is significantly different in dogs less than 12 months of age compered with dogs older than 12 months (Rouholamin et al., 2000). The most common arrhythmias in German Shepherd dogs are either sinus arrhythmia and wandering pacemaker (Rouholamin et al., 2000) or sinus arrhythmia and sinus pause (Spasojević Kosić, 2009). The mean duration of the P wave, and of the PR interval differed significantly between dogs younger and older than 12 months (Rouholamin et al., 2000). In the former study in German Shepherd dogs similar values of ECG's parameters are obtained, exept in the amplitudes of P and R waves in lead II and in the duration of PR interval in lead II (Rezakhani et al., 1990; Spasojević Kosić, 2009). These differences can be explained by different avarage age of dogs included in the studies. Although no significant incidence of phisiologic or benign arrhythmias is shown, the apperance of AV block 1º is registered in one third of dogs older than 8 years (Spasojević Kosić, 2009).

The normal MEA in the dog is 40 degrees to 100 degrees, or 40 degrees to 90 degrees for toy breeds (Edwards, 1987). The values of MEA obtained in the former studies of German Shepherd dogs are 81,19 ± 7,59º (Rezakhani et al., 1990), 81,97 ± 1,87º (Rouholamin et al., 2000) and 80,34 ± 2,80° (Spasojević Kosić, 2009). In this study, similar values of MEA are obtained irrespective of the method of calculation. The mean values of MEA calculated by the isoelectric lead method and the vector method were 76,50 ± 19,83 º and $\bar{x}2$ = 80,17 ± 11,44 º respectively. There was no significant difference among values of MEA calculated by these two methods (p = 0,384). Since patient's position during ECG recording affects the obtained values of MEA, all dogs during ECG recordings were in standard position. Application of different methods of calculating MEA in healthy dogs does not result in significant differences in MEA.

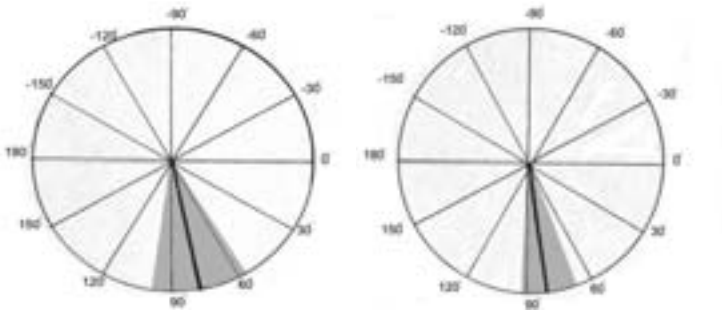

Figure 2. Mean (dark line) ± SD (shaded area) mean electrical axis (MEA) recorded from 30 German Shepherd dogs according to (A) the isoelectric lead method and (B) the vector method.
Slika 2. Srednja vrednost (tamna linija) ± SD (osenčena površina) srednje električne osovine srca snimnjene od 30 nemačkih ovčara prema (A) metodu izoelektričnog odvoda i (B) metodu vektora.

In humans, in instances of the left axis deviation of about -30°, differentiation between left ventricular enlargement and left incomplete hemi block and of minimal right axis deviation, accurate assessment of MEA is of paramount importance considering the management of the case (Singh, Athar, 2003).

The main limitation of this study is the fact that it is performed in healthy dogs. ECG changes could be more prominent in the dogs with heart disease, so it is necessary to further verify the results of this research among dogs with cardiac diseases.

CONCLUSION

Calculation of the MEA should be seen as an integral part of ECG's interpretation. In healthy dogs, calculation can be done by any of proposed methods, because the chosen method does not significantly affect MEA value. Additional studies are needed to evaluate the accuracy of calculating the MEA in dogs with heart disease.

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POREĐENJE DVA METODA ODREĐIVANJA SREDNJE ELEKTRIČNE OSOVINE SRCA KOD PASA

LJUBICA SPASOJEVIĆ KOSIĆ

Izvod

Pri interpretaciji elektrokardiograma (EKG) potrebno je odrediti vrednost srednje električne osovine srca (SEO). U veterinarskoj elektrokardiografiji SEO srca se prikazuje u frontalnoj ravni, a može se izračunati korišćenjem različitih metoda. U ovom radu poredili smo vrednosti SEO kod pasa koje su dobijene pomoću dva metoda izračunavanja: metod izoelektričnog odvoda i metod vektora. Cilj ovog ispitivanja je da utvrdi da li postoji razlika u vrednosti SEO srca ukoliko se za njeno određivanje koriste različite metode. Psi rase nemački ovčar različite starosti, koji su imali sinusni srčani ritam su obuhvaćeni ovim ispitivanjem (n = 31). Svi psi su prilikom snimanja elektrokardiograma postavljeni u standardni položaj za snimanje (desni lateralni ležeći položaj). Poređenje dve metode izračunavanja SEO obavljeno je korišćenjem perifernih odvoda snimnjenih elektrokardiograma (I, II, III, avR, aVL, aVF). Prosečne vrednosti SEO bile su $\bar{x}1 = 76,50 \pm 19,83$ º i $\bar{x}2 = 80,17 \pm 11,44$º. Nije postojala statistički značajna razlika u vrednosti SEO srca koje su dobijene korišćenjem dve metode izračunavanja ($p = 0,384$). Dobijeni rezultati ukazuju da kod zdravih pasa na vrednost SEO ne utiče značajno odabrani metod njenog izračunavanja.

Ključne reči: srednja električna osovina, izračunavanje, nemački ovčari.

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SOW SEASONAL INFERTILITY*

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IVAN STANČIĆ, IVAN RADOVIĆ, SAŠA DRAGIN[1]

SUMMARY: The level of reproductive efficiency is predominantly related to the values of the basic parameters of sow fertility, such as weaning-to-estrus interval, farrowing rate (%) and litter size. These values, however, were frequently reduced, in the warmer part of the year. This phenomenon is defined as seasonal sows infertility. This paper review the basic factors of sows seasonal infertility, their mechanismsof action, as well as the possibilities of practical solutions of this significant problem in pig reproduction.

Key words: *season, infertility, factors, sow.*

INTRODUCTION

The most significant parameters of sows reproductive efficiency are: duration of weaning-to-estrus interval, farrowing rate (%) and the number of live-born pigs per litter (Nielsen, 1981a; Tomes et al., 1982; Stančić, 1994). Significant reduction of fertility is evident during the warm summer months. This phenomenon is known as "seasonal infertility in pigs" (Vanroose et al., 2000). Namely, for as long as 40 years, considerably lower values of sow fertility parameters have been evident in the warmer part of the year (Almond, 1992). Thus, Aumaitre et al. (1976) determined significantly lower farrowing rates, extended weaning-to-estrus interval, increased number of irregular rebreeding and the reduced number of live-born pigs per litter in the warm part of the year. This phenomenon is related to the negative effect of increased ambient temperature on sow reproductive functions (Gordon, 1997).

Although the majority of seasonal infertility factors are well-known, the interaction and its mechanisms are not entirely clarified and, therefore, there is no single adequate technology for solving this problem. Therefore, examining this problem is always important. Accordingly, the aim of this paper is to review some of the recent findings regarding the phenomenon of seasonal infertility in sows.

Scientific review paper / *Pregledni naučni rad*

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SEASONAL INFERTILITY

Seasonal infertility is defined as a phenomenon of occurrence of lower values of the basic parameters of sow fertility during the summer months. Some of these parameters are: extended weaning-to-estrus interval, lower values of conception rate, increased number of rebreeding, abortions and pseudopregnancy, as well as the lowered number of vital live-born pigs (Love, 1978; Rozeboom et al., 2000). The decrease of the stated fertility parameters during warm summer months was perceived as an important factor causing economic losses in intensive herds of domestic pig breeds in as early as 1970s (Gordon, 1997; Peltoniemi et al., 1999). Accordingly, Aumaitre et al. (1976) in France determined that the weaning-to-estrus interval is longer for averagely 10 days in the sows farrowed in the warm period of the year (June-September), compared with the other, colder period of the year. Likewise, the USA researchers determined the considerably extended weaning-to-estrus interval (Hurtgen et al., 1980), as well as the period of successive farrowing during the warm period of the year (Hurtgen i Leman, 1981).

The research conducted by Peltoniemi et al. (1999), on 1080 breeding herds in Finland, showed that the decrease of sow fertility during the summer period was reflected in the following: (1) significantly increased number of sows weaned due to prolonged post-lactation anestrus, (2) considerable increase of rebreeding, (3) considerable increase of sows whose weaning-to-estrus interval was significantly longer than 10 days, (4) significantly increased number of pseudopregnant sows (inseminated, they do not rebreed, but are not pregnant), and (5) substantially increased number of sows with inactive ovaries, determined by examination of sexual organs on dead animals. The results of the research conducted in the Eastern Europe, on a great number of breeding herds, during a number of years, also indicate significant decrease of weaning-to-estrus interval, farrowing rate and live born piglets per litter, as well as the regular and iregular returns in the warm season (Almond and Bilkei, 2005).

Summer infertility was considerably more frequent in gilts, compared with sows (Britt et al., 1983). Research conducted by a number of authors, according to Gordon (1997), indicated to the general conclusion that the parameters of sow fertility during the warm season are reduced 15 to 20% compared with the cold period of the year.

Summarizing the results of the mentioned authors, as well as the results of certain other authors, analysed by Stančić et al. (2002) review paper, it can be concluded that the seasonal infertility is manifested most in considerably extended weaning-to-estrus interval, decrease of farrowing rate (%), as well as lowering of the number of vital live-born pigs per litter.

Weaning-to-estrus interval (WEI). In good herds, over 85% of sows manifest the first estrus within 7 days after weaning (Tubbs, 1990). The intervals longer than this are considered to be extended (abnormal) and they are an indication of disturbed activity of re-establishing post-lactation ovary activity, as well as of the possibility of lower reproductive efficiency of such sows in the following reproductive cycle (Napel et al., 1998). Postponing of re-establishing post-lactation ovary activity (follicular growth, ovulation and estrus manifestation) is, most frequently, the consequence of prolonged daily photoperiod and the increase of ambient temperature during warm months (Peltoniemi et al., 1999). It appears that the extended WEI during the summer season is the consequence of reduced capability of hypothalamus to re-establish normal pulsatile secretion of Gn-RH. This inhibits the release of hypophyseal gonadotropins (FSH and LH), which

results in postponement of the first post-lactation ovulation and manifestation of estrus (Prunier et al., 1996). The effect of the increased ambient temperature on the extension of WEI can be explained indirectly. Namely, it is well-known that the appetite of sows is significantly lowered during the summer months (Aherne and Kirkwood, 1985), and that reduced intake of energy in the organism reduces or inhibits secretion of LH (Prunier et al., 1996). This results in postponement of maturing and follicle ovulation after weaning and, consequently, extension of WEI (Kirkwood, 2009). On the other hand, the extended daily photoperiod has direct effects, through the influence of the reduced pineal melatonin secretion on the inhibition of Gn-RH secretion in hypothalamus and, consequently, omission of hypophyseal gonadotropin secretion (Tast, 2002).

Farrowing rate. Considerably lower sows farrowing rate, during the summer months, significantly lowers economic efficiency of production. The most frequent reason for farrowing failure, i.e. unsuccessful conception, in summer months is irregular rebreeding, that is, re-establishment of estrus 25 to 35 days after insemination. Such early pregnancy loss is a consequence of embryo mortality (Xue et al., 1994) or the regression of pregnancy corpora lutea (Wrathall et al., 1986). Namely, recent research indicates that high ambiental temperature leads to the increased embryo mortality, and consequently, to pregnancy loss (Stančić et al., 2004). Besides, it seems that the increased temperature inhibits prolactin (LTH) release from hypophysis, which is necessary for enhancement of secretory activity of corpora lutea after 16th day of gestation, which also causes pregnancy loss and irregular rebreeding (Tast et al., 2002). Certain research indicates that seasonal pregnancy loss, however, is the consequence of oversensitivity of the corpus luteum of pregnancy to increased ambient temperature, which inhibits secretion of progesterone *(Kirkwood, 2009).*

Litter size. The results on the effects of a season on litter size at farrowing are rather contradictory. This can be attributed to the fact that, on one hand, litter size is influenced by the interaction of a numerous genetic and paragenetic factors, and of different conditions and research methods, on the other hand (Stančić et al., 2002). Even if there is the influence of the season on the number of live-born pigs per litter, it can be indirect. Namely, it is well known that sows with extended weaning-to-estrus interval, which occurs frequently in summer, have substantially lower number of piglets in the resulting litter (Borchardt Netto, 1998; Wettemann and Bazer, 1985; Stančić, 1997a and 1997b; Stančić et al., 2000). Furthermore, in the warm period of the year, the piglets mortality during lactation period is increased, as well, which also significantly decreases the total number of produced piglets per sow on the annual basis (Gagrčin et al., 2007). The presented results show that the phenomenon of seasonal infertility in sows is very complex, and that its physiological basis has not been entirely clarified. However, the results of all the research consistently indicate that the lowered fertility in sows is the consequence of the interaction of high ambient temperature and extended daily photoperiod in the warm period of the year. These factors take effect through neuroendocrine activity at the level of central nervous system – hypothalamus – hypophysis – ovaries (Tast, 2002). Such effects of a season on domestic pig breeds can be based on the fact that domestic pig breeds originate from European wild boar, which is especially seasonally sexually active, as their mating season is in the cold period of the year. This accounts for the seasonal effect of the extended daily photoperiod on neuroendocrine mechanisms regulating cyclic ovarian activity and estrous cycle manifestation (Mauget, 1982).

Seasonal infertility control. The phenomenon of seasonal infertility in sows is very complex as it includes both direct and indirect interactions of a great number of genetic and paragenetic factors, whose mechanisms of physiological performance is not entirely clarified (Kirkwood, 2009). Therefore, it is impossible to define a single simple technology which would entirely eliminate the effects of this phenomenon on productive efficiency of breeding herds in intensive pig production today. Nevertheless, on the basis of the results of numerous research, as well as practical experience, during the last 40 years, it is possible to point out certain zoo-technological and sanitary-veterinary measures, which can significantly contribute to the increase of sow fertility in the warm period of the year.

According to the results of a number of authors, summarized by Rozeboom et al. (2000) and Stančić et al. (2002), these measures are the following:

- Provide effective ventilation and cooling system of the facilities.
- Build eaves above pens for sows and gilts.
- Provide cold and clean drinking water *ad libidum*.
- Distribute daily meals in a number of small meals during the day.
- Reduce the quantity and increase the energy value of daily meals.
- Make sure that food contains no mycotoxins.
- Detect estrus at least 2 times a day, every 12 hours, with the direct boar contact.
- Control the quality of every single boars ejaculate, and make a smaller number of inseminating doses, with the increasing number of progressive motile spermatozoides.
- Apply the third insemination in sows which exhibit the "standing heat" 12 hours after the second insemination.
- Perform insemination with adequate catheters, with cervix stimulation, and maximal hygiene of the entire process of artificial insemination.
- Eliminate the physical activity of sows as much as possible
- Avoid transport and/or relocating sows
- Reduce the number of sows per group
- Increase the number of inseminated gilts during the period July-September.
- Ensure high level of hygiene of the animals and facilities
- Maintain good health and stamina of animals
- Treat the breeding sows, especially gilts, with gonadotropin preparations (PMSG+HCG) around 24 hours after weaning, to increase the level of estrus reaction within the first 7 days after weaning, in order to ensure better synchronisation of ovulation and the increase of ovulation rate, well as conception rate.

DISCUSSION

The decreasing fertility during summer, known as "summer or seasonal infertility syndrome" (Love, 1978; Rozeboom et al., 2000), casues considerable zoo-technological and sanitary-veterinary problems, as well as economic losses in the intensive pig production. Although the domestic pig breeds are reproductively active during the whole year (manifesting ovulatory estrus and being capable for fertilization) (Mauget, 1982), there is the evident difference in values of all the parameters of fertility (duration of weaning-to-estrus interval, conception rate, rebreeding rate and abortions, as well as the average litter size) between the cold and warm periods of the year (Rozeboom et

al., 2000; Stančić et al., 2002). Namely, substantially lower values of the mentioned parameters of fertility during warm summer months were recognized as a significant factor of economic losses in intensive breeding herds of domestic pig breeds in as late as 1970s (Aumaitre et al., 1976). Summer infertility is much more frequent in gilts, than in sows (Britt et al., 1983). The research of a number of authors, according to Gordon (1997), point to the general conclusion that the parameters of sow fertility, during the warm summer period of the year, decrease for 15-20% compared with the cold period of the year.

Such highly significant differences in the duration of WEI in the cold and warm periods of the year have been recognized by Aumaitre et al.(1976), Hurtgen et al. (1980), Peltoniemi et al.(1999), Stančić et al. (2002) and Almond and Bilkei (2005). In Eastern European countries, this interval lasts 5.9 days on average in the cold and 7.8 days in the warm period of the year (Almond and Bilkei, 2005). The extended duration of WEI reduces reproductive efficiency of breeding herds both directly and indirectly. Firstly, the reproductive efficiency is reduced directly, as sows with extended WEI achieve lower farrowing rates after insemination in the first post-lactation estrus and have a significantly lower number of pigs per litter (Stančić, 1994; Kemp and Soede, 1996; Stančić, 1997a and 1997b; Borchardt Netto, 1998; Stančić, 2000; Stančić et al., 2002; Timotijević et al., 2003). Secondly, the reproductive efficiency is reduced indirectly, as the extended WEI prolongs the interval between successive farrowing and, consequently, reduces the farrowing index, resulting in the reduced yearly pig production and the increased number of non-productive feeding days (Tomes et al., 1982; Tubbs, 1990; Stančić, 2005). According to the research conducted by Prunier et al. (1996), the extended WEI during the warm period of the year is a consequence of the decreased capability of hypothalamus to re-establish the normal pulsatile secretion of Gn-RH. This inhibits the release of hypophyseal gonadotropin (FSH and LH), which results in postponement of the first post-lactation ovulation and estrus manifestation.

The farrowing rate is also statistically lower in the warm than in the cold period of the year. Thus, Almond and Bilkei (2005) determined that this value reaches 91% in the cold and 78% in the warm periods of the year. The most frequent reason for farrowing failure, i.e. unsuccessful conception, in summer months is irregular rebreeding, that is, re-establishment of estrus 25 to 35 days after insemination. Our previous research (Stančić, 2002) also determined the statistically significant ($P>0.01$) increase in irregular rebreeding in the warm (53.4%) compared with the cold period of the year (27.5%). Such early pregnancy loss is a consequence of embryo mortality (Xue et al., 1994) or the regression of corpora lutea of pregnancy (Wrathall et al., 1986). Namely, recent research indicate that high ambiental temperature leads to the increased embryo mortality, and consequently, to pregnancy loss (Stančić et al., 2004). Besides, it seems that the increased temperature inhibits prolactin release from hypophysis, which is necessary for enhancement of secretory activity of corpus luteum (CL) after 16^{th} day of gestation, which also causes pregnancy loss and irregular rebreeding (Tast et al., 2002; Kirkwood, 2009). According to the research of certain authors (Christianson, 1992), abortions were most frequently caused by infectious factors, and less frequently by stress induced by increased ambient temperature.

The average number of live-born and the total number of pigs per litter is lower, while the number of avital, mummified and stillborn pigs increases during the warmer period of the year (Almond and Bilkei, 2005). However, the views on the effects of a

season on litter size are quite contradictory (Stančić et al., 2002). Even if there is the effect of a season on the number of live-born pigs per litter, it can be indirect. Namely, it is well-known that sows with extended weaning-to-estrus interval, which is frequent in the summer period, have significantly lower number of pigs per litter (Borchardt Netto, 1998; Wettemann and Bazer, 1985; Stančić, 1997a and 1997b; Stančić et al., 2000). Moreover, during the summer months, embryo mortality is increased, and consequently, the number of live-born pigs per litter decreases (Stančić, 1991; Christianson, 1992; Xue et al., 1994; Stančić, 1995; Tast et al., 2002; Stančić et al. 2004). Certain authors point out that the stress induced by increased ambient temperature reduces sows immunity to infectious diseases which causes increased mortality and/or fetal mummification (Yeske, 2007; Givens and Marley, 2008).

Quick (within 7 days) re-establishment of ovary activity (follicular growth and ovulation) and estrus manifestation after lactation are the basic factor of the efficient reproductive activity of sow. However, during the warm days the weaning-to-estrus interval is significantly extended. Therefore, the PMSG treatment is used, in order to increase the synchronization of the occurrence of estrus in as many sows as possible within the first 7 days after weaning (Bracken et al., 2006). Franek and Bilkei (2008) determined that within 7 days after weaning in the warm period, 94% of sows treated with PMSG and 75% of control ones (without treatment) manifested estrus. The average duration of the weaning-to-estrus interval was 3.8 days in the treated sows, and 6.2 days in the control sows. Better synchronization of estrus, shortened weaning-to-estrus interval and increased fertility rate in PMSG treated sows during the warm period was also recognized by other authors (Stančić and Šahinović, 1991; Grafenau et al., 1997; Taker et al., 2008).

The results of the presented research clearly indicate that the values of the examined parameters of fertility in sows are significantly lower during the warm period compared with the cold period of the year. Furthermore, these parameters indicate considerably higher decrease in gilts than in sows.

The phenomenon of the sows seasonal infertility is very complex. The precise mechanisms of physiological basis of this phenomenon have not been entirely clarified (Kirkwood, 2009). However, the results of all the research consistently indicate that the lowered fertility in sows is affected by the interaction of high ambient temperatures and extended daily photoperiod in the warm period of the year. These factors take effect through neuroendocrine mechanisms at the level of central nervous system – hypothalamus – hypophysis – ovaries (Tast, 2002). Such effects of a season on domestic pig breeds can be based on the fact that domestic pig breeds originate from European wild boar, which is especially seasonally sexually active, as their mating season is in the cold period of the year. This accounts for the seasonal effect of the extended daily photoperiod in the warm period of the year, as the seasonal sexual activity is the consequence of the direct effect of photoperiod on neuroendocrine mechanisms regulating cyclic ovarian activity and estrous manifestation (Mauget, 1982).

Although all the factors of seasonal infertility, as well as the mechanisms of their physiological effects are not crearly elucidet, it is possible to decrease the negative effects of the warm period of the year on sow fertility to a certain extent by using adequate technologies of housing, insemination, health care and by treating sows with gonadotropin preparations (Rozeboom et al., 2000; Stančić et al., 2002).

CONCLUSION

On the basis of the presented results, regarding the infective and non-infective etiology of sows, it is possible to conclude the following:

1) Non-infectious factors which cause pig infertility are numerous, the most significant of which would be the effects of increased ambient temperature and the extended duration of daily photoperiod during the warmer period of the year. In this period, there is a substantially extended weaning-to-estrus interval, the reduced number of estrous sows within 7 days after weaning, significantly lower farrowing rate (%), significantly increased number of sows with irregular rebreeding (embryo mortality), and abortion, substantially higher number of stillborn avital and mummified pigs per litter.
2) Negative effects of the warm period of the year are more evident in gilts than in sows.
3) Although neither all the factors of seasonal infertility nor all of the mechanisms of their physiological effects are known, it is possible to reduce the negative effects of the warm period of the year on sow fertility to a certain extent by adequate technologies of housing, nutrition, insemination, health protection and gonadotropin preparation treatment for sows.

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SEZONSKI INFERTILITET KRMAČA

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Izvod

Nivo reproduktivne efikasnosti krmača primarno zavisi od parametara fertiliteta, kao što su interval zalučenje-estrus, vrednost (%) prašenja i veličina legla. Vrednosti ovih parametara su značajno redukovane tokom toplijeg perioda godine. Ovaj fenomen je poznat pod nazivom sezonski ili letnji infertilitet krmača. U ovom preglednom radu su opisani osnovni faktori sezonskog infertiliteta krmača, mehanizmi njihovog fiziološkog delovanja, kao i mogućnosti praktičnog rešavanja ovog značajnog problema u reprodikciji svinja.

Ključne reči: sezona, infertilitet, faktori, krmača.

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MODERN TECHNOLOGY OF ARTIFICIAL INSEMINATION IN DOMESTIC ANIMALS

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SUMMARY: By applying the technology of artificial insemination (AI) remarkable progress has been achieved in livestock production over the past 50 years. The basic requirement of modern livestock production is obtaining the maximum number of insemination doses per ejaculate, the possibility of long-term storage of the inseminated doses, as well as achieving the maximum fertility rate of inseminated females. Therefore, the aim of this paper is to present new findings on the modern technology of artificial insemination of domestic animals.

Key words: *AI, technology, fertility, domestic animals.*

INTRODUCTION

Artificial insemination (AI) is the basic and the most important biotechnological method, which has been used in intensive livestock production for more than 60 years (Foote, 2002). Application of artificial insemination led to great improvements in livestock production due to numerous advantages of artificial insemination compared with natural insemination. The most important advantages of artificial insemination are: producing considerably more offspring from genetically superior males, the possibility of effective prevention of spreading of contagious diseases, the possibility of long-term storage of the sperm from males with genetically desirable traits, easy transport of sperm over long distances, etc (Stančić and Veselinović, 2002). The significance and the scope of AI application is best illustrated by the fact that the world's annual production today is over 300 million insemination doses of bull sperm, around 90 million of sperm doses of boars and around 8 million doses of ram sperm (Thibier and Wagner, 2002).

The technology of AI is constantly evolving with the main aims to: (a) achieve the maximum degree of fertility of inseminated females, (b) maximise the number of insemination doses per ejaculate, (c) ensure the maximum hygiene of AI application, and (d) achieve maximum economic efficiency of AI technology (Ponsart et al., 2004). In this regard, contemporary research has been particularly focused on finding efficient methods for evaluation of sperm quality, methods for efficient dilution and long-term storage of insemination doses of sperm of different animal species, techniques of in-

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seminating females, determining the optimal moment for insemination, efficient methods of estrus detection and synchronisation, etc (Foote, 2002).

BRIEF HISTORY OF ARTIFICIAL INSEMINATION

The first written documents related to artificial insemination date from the seventeenth century, when Leeuwenhoek (1678), using microscope magnification of 270 times, saw movements of spermatozoa, which he called "animalcules". More than 100 years later, the Italian scientist Lazzaro Spallanzani (1784) artificially inseminated a bitch, which whelped three pups 62 days later. However, it was more than 100 years afterwards that Heape et al. (1897) reported that in different countries artificial insemination of rabbits, dogs and horses was carried out successfully. Practical application of AI in animal husbandry was initiated in Russia by the academician E.I. Ivanov in 1899. Ivanov was the first to develop the methodology of sperm quality control, and formulate diluent composition for sperm and the methods of selecting genetically superior males for the use in AI technology (Ivanov, 1922). His work was continued also in Russia by Milanov (1938), who developed the basic principles of today's AI technology (taking sperm by artificial vagina, semen dilution, insemination technique, etc), especially in sheep and cattle (Milovanov, 1964). Milovanov's results were subsequently developed by Japanese scientists (Ishikawa, Ito, Niwa, Sato, Yamane and others), whose results were described in detail by Niwa (1958) and Nishikawa (1964). Sudden development of AI application in cattle began in the USA in early 40s of the twentieth century (Salisbury et al., 1978), while AI in pigs started in Japan (Ito et al., 1948). Significant contribution in development of the basic principles and methods of modern AI technology was made by the scientists from Cambridge University (UK), C. Polge, A.U. Smith and A.S. Parkers (Polge, 1956). AI technology has been developed also in other countries (Denmark, Germany, France, the USA, and others). In our country (former Yugoslavia), AI was significantly developed in the second half of the twentieth century, on large industrial cattle and pig farms, especially in the Federal Republic of Slovenia, the Federal Republic of Croatia, and the Federal Republic of Serbia (Perkučin et al., 1965; Miljković, 1969; Mišković et al., 1987).

Starting from the second half of the twentieth century, artificial insemination has been more and more massively used in livestock production, and today it represents the most important biotechnological method of improving livestock production (Ponsart et al., 2004).

AI IN CATTLE

Artificial insemination of cattle began with fresh sperm doses, diluted with yolk-citrate diluent (Salisbury et al., 1941). However, the technology of long-term storage of insemination doses by deep freezing in liquid nitrogen and at the temperature -196^{o}C was shortly developed. Today over 90% of AI in cows is performed with the doses thawed after storage in deep-frozen state.

Modern technology of AI in cattle involves applying methods of native semen quality control, sperm dilution, long-term storage of insemination doses, insemination techniques, determination of the optimal moment for insemination and estrus synchro-

nization in a larger number of cows intended for simultaneous insemination (DuPonte, 2007; Alm-Packalen, 2009; Foote, 2002, Troxel, 2010; Zeron et al., 2010).

Today, a dose for AI in cows is 0.25 ml (i.e. a mini straw), containing 12 to 15 x 10^6 of progressively motile spermatozoa (Johnson et al., 1995; Parish and Larson, 2010). Insemination is performed intracervically, with a special catheter, and with a dose which was thawed at the temperature 37 to 38°C immediately prior to insemination (Parish and Larson, 2010). For the successful insemination it is very important to determine the optimal time for insemination. Namely, the maximum conception rate is achieved if insemination is performed around 10 to 12 hours before ovulation. Therefore, it is necessary to apply an efficient methodology of estrus detection in cows, which is often a problem on farms since estrus lasts rather short (often less than 12 hours). It is thus the best to detect estrus by observation of external signals of estrus at least 4 times a day at equal time intervals, combined with rectal examination. It is becoming more common to use vasectomised bulls which detect estrous cows most efficiently (Foote, 2002), as well as various models of electronic monitoring of cows' behaviour (Nebel et al., 2000). Today the methods of efficient synchronization of estrus and ovulation are well developed, so a high level of successful conception rate can be achieved also by fixed-time insemination without detection of the estrus signals (Nebel et al., 2000; Grafenau et al., 2008).

The development of sperm-sexing technology (sexing – the separation of sexes) of spermatozoa, and DNA quantification with the method of flow cytometry (Johnson and Seidel, 1999) enable even wider application of AI in the development of livestock production. Namely, by insemination of cows with the doses which contain "male" or "female" spermatozoa, it is possible to obtain calves of a particular sex, as desired by the producer.

AI IN PIGS

In industrial conditions today, more than 80% of sows and gilts are artificially inseminated by classical intracervical insemination (Gadea, 2003). This technology involves the use of insemination doses of fresh diluted semen, which contain 3 to 5 x 10^9 of progressively motile spermatozoa (Alm et al., 2006; Stančić et al., 2006; Stančić et al., 2010). Doses of liquid diluted sperm are stored at the temperature of +17 °C, and they are most commonly used within 1 to 2 days after taking the sperm from boars (Johnson et al., 2000). On the average, around 1,300 insemination doses are obtained annually from one boar (21 doses per ejaculate), which is sufficient for successful insemination of only 300 sows per year (Singleton, 2001). In our country, the number (%) of artificially inseminated breeding sows is considerably lower compared with the developed European countries. However, there is a need for increasing the number of obtained insemination doses on the annual basis, especially of genetically superior boars (Stančić et al., 2007; Stančić et al., 2008; Stančić et al., 2009).

Consequently, there is the growing need for increasing reproductive exploitation of genetically superior boars. Therefore, the possibilities of obtaining a considerably larger number of insemination doses per boar on the annual basis have been researched (Glossop, 2000; Stančić, 2000; Stančić et al., 2006; Stančić et al., 2008). Such increase can be achieved by a more significant reduction of the number of spermatozoa in one insemination dose, from the current 3 to 5 billion, to 1 to 2 billion (Belstra, 2002). The use

of insemination doses with significantly reduced number of spermatozoa, and without significant loss of sow fertility, is enabled by new technology of shallow intrauterine insemination (Vansickle, 2002; Watson et al. 2002; Belstra, 2004; Roseboom et al., 2004; Mezalira et al., 2005; Stančić et al., 2006; Stančić et al., 2007; Radović et al., 2006; Radović et al., 2007; Stančić et al., 2010). For the shallow intrauterine insemination (in the body of the uterus) modified catheters for classical intracervical insemination are used. Namely, a thin, more flexible catheter, through which sperm is deposited in the body of uterus, is inserted through the classical catheter. The insemination procedure is as follows: the top of the classical catheter is inserted through the cervical canal, in the same way as in the classical insemination. Then, the thinner catheter is slowly inserted all the way to the body of the uterus (around 20 cm in length), where the deposition of the sperm dose is made (Grafenau et al. 2004).

Further reduction of the volume and number of spermatozoa per an insemination dose can be achieved by using deep intrauterine insemination. This insemination is performed by transcervical insertion of a special catheter deep into the uterine horn. The aim is to deposit the insemination doses as close as possible to the top of the uterine horn, i.e. uterotubal junction (Rath, 2002; Grafenau et al., 2004). The results so far have shown that it is sufficient to insert a flexible catheter of 30 to 40 cm into the horn, cranially from the bifurcation of the uterus, in order to achieve good results of fertility in sows inseminated with the doses of 10 to 20 ml, containing 1 to 5 x 10^8 spermatozoa (Wolken et al., 2002; Roca et al., 2003; Wongtawan et al., 2005; Vasquez et al., 2005). The satisfactory conception rate is achieved also by the doses of 0.5 ml with 1 x 10^6 of progressively motile sperm, if such a dose is directly deposited into the top of the uterine horn surgically (laparotomy or laparoscopy) (Krüger et al., 2000; Wolken, 2001).

Apart from the quality of the insemination dose and technique of the conducted insemination, in order to achieve a high degree of fertility of inseminated sows, it is necessary that insemination should be at the optimal time in relation to the beginning of the standing reflex, i.e. the moment of ovulation. In this regard, the application of efficient estrus detection technology is very important (Spronk et al., 1997; Kaeoket et al., 2005; Radović et al., 2006; Stančić et al., 2007; Stančić et al., 2007; Stančić et al., 2008; Stančić et al., 2009; Stančić et al., 2010).

AI IN SHEEP AND GOATS

Artificial insemination of sheep and goats is applied in industrial production in order to get a considerably higher number of lambs and genetically superior rams, and to increase the efficiency of reproductive exploitation of rams (Donovan et al., 2001; Sohnrey and Holtz, 2005). The technique used is vaginal, cervical, transcervical and intrauterine (via laparoscopy) deposition of insemination doses. The doses of fresh diluted sperm or deep frozen doses are used (Donovan et al., 2001; Leethongdee, 2009). The conception rate is much lower in sheep inseminated with the doses which were deep frozen, both when insemination was carried out in a spontaneous and synchronised estrus (Donovan et al., 2004; Dogan et al., 2004). Recently, the technique of laparoscopic intrauterine insemination has been applied with the doses of 0.25 ml, which contain 80 x 10^6 spermatozoa. With this technique, 60 to 70 % of successful conception is achieved (Luther, 2008), which is similar to the conception achieved with the classical cervical

insemination with the doses of fresh diluted semen, containing 200 x 10^6 spermatozoa in the dose of 0.25 ml (Leethongdee, 2009).

AI IN HORSES

For artificial insemination of mares, the most commonly used doses are liquid diluted sperm of 10 to 15 ml, containing 500 to 600 x 10^6 of progressively motile spermatozoa. Native ejaculates should have at least 60 % of progressive motility (Heckenbichler et al., 2010). These doses can be stored at +20°C or +5°C, not longer than 24 to 48 hours. After this time, the achieved conception rate of inseminated mares decreases significantly by 7 to 10% with the insemination doses kept at +20°C, and about 40% with the insemination doses kept at +5°C (Newkombe et al., 2005).

In recent years, artificial insemination of mares with the doses having had long-term deep-freeze storage has been used more frequently. The results so far have shown that that the number of progressively motile spermatozoa in these doses ranges from 100 to 600 x 10^6, while it is considered that the optimal number of spermatozoa in the dose is around 250 x 10^6. The achieved conception rate significantly varies from 50 to 90%, depending on the applied technology of deep freezing, the content of sperm diluent, duration of storing of insemination doses, technology of the doses thawing, insemination technology (place of sperm deposition in female sexual organs and the optimal time of insemination of mares), the quality of sperm of certain stallions, as well as the season (mating season or anestric season) in which the native ejaculates were obtained (Metcalf, 2007; Barbacini and Loomis, 2007).

Significant reduction of the volume of insemination doses and the number of progressively motile sperm in doses can be achieved by deep intrauterine insemination of mares. With this technology, using endoscopy, a special catheter is transcervically inserted close to uterotubal junction of a mare's uterine horn. In this way, after visualisation of the papilla of caudal isthmus of oviduct, the dose of 5 ml of sperm, containing 50 to 100 x 10^6 of progressively motile spermatozoa, is deposited in the surroundings of the papilla (Pycock, 2005).

AI IN DOGS

Intravaginal insemination with doses of fresh diluted sperm is an efficient classical method of artificial insemination of bitches, and this method gives good results of fertility (Rota et al., 1999). However, the use of insemination doses which had a long-term deep-freeze storage gives highly variable results, depending on the sperm quality, the deposition site of insemination doses (intravaginal or intrauterine), type of insemination catheter, as well as the breed of the inseminated bitches. Thus, Linde-Forsberg et al. (1999) found that the successful conception rate is 84% after intrauterine and 59% after intravaginal insemination with the doses kept deep frozen. Intrauterine insemination doses of fresh diluted sperm give considerably better conception rate (86.7%) compared with the insemination doses kept deep frozen (60.7%). Litter size is also higher (6 pups) after insemination with fresh sperm, compared with the insemination with deep frozen sperm (4 pups) (Nižanski, 2006).

In the modern technology of artificial insemination of bitches with the doses stored deep frozen, the doses of 2 to 2.5 ml, containing 2 x 10^8 of progressively motile sperm, are used. To dilute the sperm, tris-fructose-citrate diluent with 8% (v/v) glycerol and 20% (v/v) egg yolk is used. Thawing of the frozen doses is performed at 37 °C for 30 seconds. Intrauterine insemination is applied exclusively and it is performed transcervically or with the method of laparoscopy. By using this method 62 to 91% of successful conception is achieved (Thomasen et al., 2006).

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SAVREMENA TEHNOLOGIJA VEŠTAČKOG OSEMENJAVANJA DOMAĆIH ŽIVOTINJA

IVAN B. STANČIĆ, SAŠA B. DRAGIN

Izvod

Primenom tehnologije veštačkog osemenjavanja (VO) je postgnut ogrman napredak u stočarskoj proizvodnji, tokom poslednjih 50 godina. Osnovni zahtev savremene stočarske proizvodnje je dobijanje maksimalnog broja inseminacionih doza po ejakulatu, mogućnost dugotrajnog ćuvanja inseminacionih doza, kao i postizanje maksimalne vrednosti fertiliteta osemenjenih ženki. Zbog toga je cilj ovog rada da se prikažu novija saznanja u vezi sa savremenom tehnologijom veštačkog osemenavanja domaćih životinja.

Ključne reči: VO, tehnologija, fertilitet, domaće životinje.

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OVERVIEW OF THE WEED FLORA IN THE SERBIA*

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SUMMARY: Weed flora in Serbia is characterized by high diversity, comprising a total of 741 species classified into 322 genera and 63 families. Many weed species are not strictly specialized and occur in two or more weed categories. Out of the total number of species, class Liliopsida (Monocotyledones) includes 85 species (11.48%) and the class Magnoliopsida (Dicotyledones) 655 species (88.51%). The Equisetopsida is represented by 3 species. The most common families include 618 species altogether. According to the number of species, the most important are: Asteraceae (112), Poaceae (78), Fabaceae (52), Lamiaceae (47), Scrophulariaceae (46), Brassicaceae (44), Caryophyllaceae (40), Apiaceae (28), Chenopodiaceae (25), Ranunculaceae (23), Rosaceae (22), Boraginaceae (18), Cyperaceae (11), etc. The following genera are prominent by their number of species: Veronica (19), Chenopodium (16), Ranunculus (12), Rumex (13), Vicia (12), Bromus (11), Euphorbia (11), Centaurea (9), Galium (7), Trifolium (6), etc.

***Key words**: Weeds, Weed flora, Check-list , Serbia.*

INTRODUCTION

This paper presents the literature and herbarium data on appearance and distribution of weed flora within the territory of Serbia. Most data used in this paper were collected from literature, including various local and regional floras, floristics and phytocoenological papers, chorological contributions, proceedings and presentations, separates from the bulletins, journals, manuscripts, original scientific papers etc. The data collected by study of herbarium material have originated in the Collection of Weeds at the Natural History Museum in Belgrade. This paper represents the highest-quality compilation of numerous (available) literature, herbarium and field data on distribution

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Scientific review paper / *Pregledni naučni rad*

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2. Diversity fossil and recent flora and fauna of Serbia - evaluation of the degree of diversity and vulnerability assessment as indicators of the protection of natural values (No 146023) funded by 01.01.2006. to 31.12.2007.

of weed plants at the territory of Serbia, as well as the resulting synthesis and revision of data on distribution of weeds. These data were used to prepare a complete and unique database on weed flora of Serbia, as one of the most important results of this project.

RESULTS AND DISCUSSION

The survey of relevant literature (Boža et al., 1997, 2003; Čanak et al., 1978; Janjić and Kojić, 2003; Josifović ed. 1970-1986; Kojić and Pejčinović, 1982; Kojić and Šinžar, 1985; Kojić et al., 1975; Kojić and Vrbničanin, 1998; Konstantinović, 1993, 1996, 1998, 1999; Konstantinović et al., 1993, 1994, 1996a, 1996b, 2001, 2004; Konstantinović and Hunyadi, 1989; Konstantinović and Arsenović, 1992; Konstantinović and Milošević, 1995; Konstantinović and Đukić, 1996; Konstantinović and Dražić, 1997; Konstantinović and Marković, 2000; Konstantinović and Meseldžija, 2003a, 2003b, 2004; Nestorović, 2001, 2002, 2003, 2004a, 2004b, 2004c, 2005a, 2005b, 2005c, 2008, 2009; Nestorović and Jovanović, 2002, 2003; Nestorović et al., 2005; Parabućski et al., 1983; Stepić, 1995; Stojanović et al., 1990; Stojanović and Nikolić, 2003; Šinžar et al., 1997) of sources from the region of Serbia showed that there are 741 species of weeds (Table 1) from 322 genera and 63 families, inhabiting agrophytocenoses, ruderal habitats, meadows and pastures, and aquatic systems. Weed species comprise about 1/4 of all vascular plants in this area, as there are 3272 existing species and 390 subspecies (Stevanović et al., 1995) of vascular plants in Serbia. Altogether, there is a high degree of floristical diversity of weed species, among the highest in Europe (Nestorović, 2004v, 2008).

Table 1. – List of weed species growing in Serbia.

Weed species

Fam. *AMARANTHACEAE*
Amaranthus albus L.
Amaranthus blitoides Wats.
Amaranthus caudatus L.
Amaranthus cruentus L.
Amaranthus deflexus L.
Amaranthus graecizans L.
Amaranthus hybridus L.
Amaranthus lividus L.
Amaranthus retroflexus L.
Celosia argentea L.
Fam. *APIACEAE*
Aethusa cynapium L.
Angelica archangelica L.
Anthriscus cerefolium (L.) Hoffm.
Anthriscus sylvestris (L.) Hoffm.
Bifora radians Bieb.
Bupleurum affine Sadl.
Bupleurum apiculatum Friv.
Caucalis latifolia L.
Caucalis platycarpos L.
Chaerophyllum aureum L.
Chaerophyllum temulentum L.
Conium maculatum L.
Daucus carota L.
Eryngium campestre L.
Falcaria vulgaris L.
Foeniculum vulgare Mill.
Heracleum sphondylium L.
Oenanthe aquatica (L.) Poir.
Oenanthe fistulosa L.
Oenanthe silaifolia Bieb.
Orlaya grandiflora (L.) Hoffm.
Pastinaca sativa L.
Peucedanum alsaticum L.
Pimpinella major (L.) Huds.
Pimpinella saxifraga L.
Tordylium maximum L.
Torilis anthriscus (L.) Gmel.
Torilis arvensis (Huds.) Link.
Fam**.** *APOCYNACEAE*
Vinca major L.
Vinca minor L.
Fam. *ARISTOLOCHIACEAE*
Aristolochia clematitis L.
Fam. *ASCLEPIADACEAE*
Asclepias syriaca (L.)
Fam. *ASPLENIACEAE*

Asplenium ruta-muraria L.
Fam. *ASTERACEAE*
Achillea millefolium L.
Ambrosia artemisiifolia L.
Anthemis arvensis L.
Anthemis cotula L.
Anthemis ruthenica Willd.
Anthemis tinctoria L.
Arctium lappa L.
Arctium minus (Hill.) Bernh.
Arctium tomentosum Mill.
Artemisia absinthium L.
Artemisia annua L.
Artemisia campestris L.
Artemisia scoparia W. et K.
Artemisia vulgaris L.
Aster lanceolatus Willd.
Aster salignus Willd.
Aster tradescantii L.
Bellis perennis L.
Bidens cernuus L.
Bidens frondosa L.
Bidens tripartita L.
Carduus acanthoides L.
Carduus candicans Waldst. and Kit. subsp. *candicans*
Carduus crispus L.
Carduus nutans L.
Carlina acaulis L.
Carlina vulgaris L.
Carthamus lanatus L.
Centaurea arenaria Bieb. ex Willd. subsp. *borysthenica* (Gruner) Dostál
Centaurea cyanus L.
Centaurea jacea L.
Centaurea pannonica (Heuff.) Simonk.
Centaurea rhenana Bor.
Centaurea scabiosa L.
Centaurea solstitialis L.
Centaurea stoebe L.
Centaurea tauscheri Kern.
Chondrilla juncea L.
Chrysanthemum indicum L.
Cichorium intybus L.
Cirsium arvense (L.) Scop.
Cirsium canum (L.) All.
Cirsium eriophorum (L.) Scop.
Cirsium lanceolatum (L.) Scop.
Cirsium pannonicum (L.) Link.
Conyza canadensis (L.) Cron.
Crepis biennis L.
Crepis capillaris (L.) Wallr.
Crepis foetida L.
Crepis foetida L. subsp. *rhoeadifolia* (Bieb.) Čelak.
Crepis setosa Hall.
Crupina vulgaris Cass.
Echinops sphaerocephalus L.
Erigeron acer L.
Eupatorium cannabinum L.
Filago arvensis L.
Filago germanica L.
Galinsoga ciliata (Raf.) Black.
Galinsoga parviflora Cav.
Gnaphalium uliginosum L.
Helmintia echioides L.
Hieracium bauhinii Bess.
Hieracium cymosum L.
Hieracium echioides Lumn.
Hieracium hoppeanum Schult.
Hieracium murorum L.
Hieracium pavichii Heuff.
Hieracium pilosella L.
Hieracium piloselloides Vill.
Hieracium umbellatum L.
Hypochoeris radicata L.
Inula bifrons (Gou.) L.
Inula britannica L.
Inula conyza DC.
Inula salicina L.
Iva xanthifolia Nutt.
Lactuca saligna L.
Lactuca serriola L.
Lactuca viminea (L.) et Presl.
Lapsana communis L.
Leontodon autumnale L.
Leontodon hispidus L.
Leucanthemum vulgare Lam.
Matricaria inodora L.
Matricaria recutita L.
Matricaria tenuifolia (Kit.) Simk.
Onopordum acanthium L.
Picris hieracioides L.
Pulicaria dysenterica (L.) Gaertn.
Pulicaria vulgaris Gaertn.
Senecio erucifolius L.
Senecio jacobaea L.
Senecio vernalis W. et K.
Senecio vulgaris L.
Solidago canadensis L.
Solidago gigantea Ait.
Solidago virgaurea L.
Sonchus arvensis L.
Sonchus asper (L.) Mill.

Sonchus oleraceus L.
Stenactis annua (L.) Nees.
Tanacetum corymbosum (L.) Sch.-Bip.
Tanacetum vulgare L.
Taraxacum officinale Webb.
Tragopogon dubius Scop.
Tragopogon pratensis L.
Tussilago farfara L.
Xanthium italicum Morr.
Xanthium spinosum L.
Xanthium strumarium L.
Xeranthemum annuum L.
Xeranthemum foetidum Mnch.
Fam. *BORAGINACEAE*
Anchusa arvensis (L.) M. B.
Anchusa barrelieri (All.) Witm.
Anchusa italica Retz.
Anchusa officinalis L.
Cerinthe minor L.
Cynoglossum officinale L.
Echium italicum L.
Echium vulgare L.
Heliotropium europaeum L.
Lappula echinata Gilib.
Lithospermum arvense L.
Myosotis arvensis (L.) Hill.
Myosotis collina Hoffm.
Myosotis palustris (L.) Nath.
Myosotis sylvatica (Ehrh.) Hoffm.
Nonea pulla (L.) Lam. et DC.
Symphytum officinale L.
Symphytum tuberosum L.
Fam. *BRASSICACEAE*
Alliaria officinalis Andrz.
Alyssum alyssoides L.
Alyssum montanum L.
Arabidopsis thaliana (L.) Heyn.
Arabis hirsuta (L.) Scop.
Arabis turrita L.
Armoracia lapathifolia Gilib.
Barbarea vulgaris R. Br.
Berteroa incana (L.) DC.
Brassica campestris L.
Brassica napus L.
Brassica nigra (L.) Koch
Calepina irregularis Thell.
Camelina microcarpa Andrz.
Capsella bursa-pastoris (L.) Med.
Capsella rubella Reut.
Cardamine pratensis L.
Cheiranthus cheiri L.
Descurainia sophia (L.) Webb.
Diplotaxis muralis (L.) DC.
Diplotaxis tenuifolia (Jusl.) DC.
Draba verna L.
Erysimum diffusum Ehrh.
Iberis amara L.
Lepidium campestre (L.) R. Br.
Lepidium draba L.
Lepidium graminifolium L.
Lepidium ruderale L.
Myagrum perfoliatum L.
Raphanus raphanistrum L.
Rorippa amphibia (L.) Bess.
Rorippa austriaca (Cr.) Bess.
Rorippa islandica (Oed.) Sch. – Thell.
Rorippa pyrenaica (L.) Rchb.
Rorippa sylvestris (L.) Bess.
Sinapis arvensis L.
Sisymbrium altissimum L.
Sisymbrium loeselii L.
Sisymbrium officinale (L.) Scop.
Sisymbrium orientale L.
Thlaspi alliaceum L.
Thlaspi arvense L.
Thlaspi perfoliatum L.
Turritis glabra L.
Fam. *CAMPANULACEAE*
Campanula patula L.
Fam. *CANNABACEAE*
Humulus lupulus L.
Fam. *CARYOPHYLLACEAE*
Agrostemma githago L.
Arenaria serpyllifolia L.
Cerastium arvense L.
Cerastium caespitosum Gilib.
Cerastium dubium (Host.) Schw.
Cerastium glomeratum Thuill.
Cerastium moesiacum Friv.
Cerastium pumilum Curt.
Cerastium semidecandrum L.
Cucubalus baccifer L.
Dianthus armeria L.
Dianthus barbatus L.
Dianthus cruentus Gris.
Dianthus deltoides L.
Dianthus pallens S. et S.
Dianthus pontederae Kern.
Gypsophila muralis L.
Holosteum umbellatum L.
Lychnis coronaria (L.) Desr.
Lychnis flos-cuculi L.
Minuartia glomerata (M. B.) Deg.
Minuartia verna (L.) Hiern.

Moenchia mantica (L.) Bartol.
Myosoton aquaticum (L.) Mnch.
Petrorhagia illyrica Ball. – Heyw.
Petrorhagia prolifera Ball. – Heyw.
Petrorhagia saxifraga (L.) Link.
Sagina procumbens L.
Saponaria officinalis L.
Scleranthus annuus L.
Scleranthus dichotomus Schur.
Scleranthus perennis L.
Silene alba (Mill.) Krause
Silene vulgaris (Mnch.) Gracke
Spergula arvensis L.
Stellaria graminea L.
Stellaria media (L.) Vill.
Stellaria nemorum L.
Vaccaria pyramidata Med.
Viscaria vulgaris Rochl.
Fam. *CERATOPHYLLACEAE*
Ceratophyllum demersum L.
Ceratophyllum submersum L.
Fam. *CHENOPODIACEAE*
Atriplex hastata L.
Atriplex oblongifolia W. et K.
Atriplex patula L.
Atriplex tatarica L.
Chenopodium album L.
Chenopodium ambrosioides L.
Chenopodium botrys L.
Chenopodium capitatum (L.) Aschers.
Chenopodium ficifolium Sm.
Chenopodium foliosum Aschers.
Chenopodium giganteum D. Don
Chenopodium glaucum L.
Chenopodium hybridum L.
Chenopodium murale L.
Chenopodium opulifolium Schrad.
Chenopodium polyspermum L.
Chenopodium rubrum L.
Chenopodium striatum (Kras.) Murr.
Chenopodium urbicum L.
Chenopodium vulvaria L.
Corispermum nitidum Kit.
Kochia scoparia (L.) Schrad.
Polycnemum arvense L.
Polycnemum majus A. Br.
Salsola ruthenica Iljin
Fam**.** *CONVOLVULACEAE*
Calystegia sepium L.
Convolvulus arvensis L.
Fam. *CUCURBITACEAE*
Bryonia alba L.
Bryonia dioica Jacq.
Fam. *CUSCUTACEAE*
Cuscuta australis R. Br.
Cuscuta campestris Yunck.
Cuscuta epithymum L.
Cuscuta europaea L.
Fam. *CYPERACEAE*
Bolboschoenus maritimus (L.) Palla
Carex distans L.
Carex divisa Huds.
Carex divulsa Good.
Carex hirta L.
Carex leporina L.
Carex montana L.
Carex praecox Schreb.
Carex pseudocyperus L.
Carex riparia Curt.
Eleocharis palustris (L.) Roem. – Sch.
Fam. *HYPOLEPIDACEAE*
Pteridium aquilinum (L.) Kuhn
Fam. *DIPSACACEAE*
Cephalaria transylvanica (L.) Schr.
Dipsacus laciniatus L.
Dipsacus fullonum L.
Knautia arvensis (L.) Coult.
Knautia drymeja Heuff.
Scabiosa ochroleuca L.
Scabiosa columbaria L. subsp. *portae* (A. Kern. ex Huter) Hayek
Fam. *EQUISETACEAE*
Equisetum arvense L.
Equisetum palustre L.
Equisetum telmateia Ehrh.
Fam. *EUPHORBIACEAE*
Euphorbia chamaesyce L.
Euphorbia cyparissias L.
Euphorbia esula L.
Euphorbia falcata L.
Euphorbia helioscopia L.
Euphorbia lucida W. et K.
Euphorbia palustris L.
Euphorbia peplus L.
Euphorbia platyphyllos L.
Euphorbia salicifolia Host.
Euphorbia virgata W. et K.
Mercurialis annua L.
Fam. *FABACEAE*
Amorpha fruticosa L.
Astragalus cicer L.
Astragalus glycyphyllos L.
Astragalus onobrychis L.
Chamaecytisus supinus (L.) Link

Coronilla varia L.
Dorycnium germanicum (Gr.) Rouy
Dorycnium herbaceum Vill.
Galega officinalis L.
Genista tinctoria L.
Glycyrrhiza echinata L.
Lathyrus aphaca L.
Lathyrus hirsutus L.
Lathyrus latifolius L.
Lathyrus nissolia L.
Lathyrus pratensis L.
Lathyrus sativus L.
Lathyrus sphaericus Retz.
Lathyrus tuberosus L.
Lotus corniculatus L.
Medicago arabica (L.) All.
Medicago falcata L.
Medicago lupulina L.
Medicago minima (L.) Bart.
Medicago orbicularis (L.) All.
Medicago rigidula (L.) Desr.
Melilotus albus Medic.
Melilotus officinalis (L.) Pall.
Onobrychis viciifolia Scop.
Ononis arvensis L.
Ononis spinosa L.
Robinia pseudacacia L.
Trifolium arvense L.
Trifolium campestre Schreb.
Trifolium fragiferum L.
Trifolium hybridum L.
Trifolium pratense L.
Trifolium repens L.
Trigonella coerulea (L.) Ser.
Trigonella monspeliaca L.
Vicia angustifolia Grupf.
Vicia cracca L.
Vicia dasycarpa Ten.
Vicia grandiflora Scop.
Vicia hirsuta (L.) Gray.
Vicia lutea L.
Vicia pannonica Cr.
Vicia peregrina L.
Vicia sativa L.
Vicia striata M. B.
Vicia tetrasperma (L.) Schreb.
Vicia villosa Roth.
Fam. *FUMARIACEAE*
Fumaria officinalis L.
Fam. *GERANIACEAE*
Erodium ciconium (Jusl.) Ait.
Erodium cicutarium (L.) L'Herit.
Geranium columbinum L.
Geranium dissectum Jusl.
Geranium molle L.
Geranium pusillum L.
Geranium pyrenaicum Burn.
Geranium sanguineum L.
Fam. *HALORAGACEAE*
Myriophyllum spicatum L.
Myriophyllum verticillatum L.
Fam. *HYDROCHARITACEAE*
Hydrocharis morsus-ranae L.
Fam. *HYPERICACEAE*
Hypericum perforatum L.
Fam. *JUNCACEAE*
Juncus compressus (L.) Schrad.
Juncus inflexus L.
Luzula campestris (L.) Lam. et D. C.
Fam. *LAMIACEAE*
Ajuga chamaepitys (L.) Schreb.
Ajuga genevensis L.
Ajuga reptans L.
Ballota nigra L.
Calamintha acinos (L.) Clairv.
Calamintha alpina (L.) Lam.
Clinopodium vulgare L.
Galeopsis ladanum L.
Galeopsis speciosa Mill.
Galeopsis tetrahit L.
Glechoma hederacea L.
Glechoma hirsuta W. et K.
Lamium album L.
Lamium amplexicaule L.
Lamium galeobdolon (L.) Nath.
Lamium maculatum L.
Lamium purpureum L.
Leonurus cardiaca L.
Leonurus marrubiastrum L.
Lycopus europaeus L.
Lycopus exaltatus L.
Marrubium peregrinum L.
Mentha aquatica L.
Mentha arvensis L.
Mentha longifolia (L.) Huds.
Mentha microphylla Koch.
Mentha piperita L.
Mentha pulegium L.
Nepeta nuda L.
Origanum vulgare L.
Prunella laciniata L.
Prunella vulgaris L.
Salvia nemorosa L.
Salvia pratensis L.

Salvia verticillata L.
Scutellaria galericulata L.
Scutellaria hastifolia L.
Sideritis montana L.
Stachys annua L.
Stachys germanica L.
Stachys officinalis (L.) Trev.
Stachys palustris L.
Stachys recta L.
Stachys scardica Griseb.
Teucrium chamaedrys L.
Teucrium scordium L.
Thymus serpyllum L.
Fam. *LEMNACEAE*
Lemna gibba L.
Lemna minor L.
Lemna trisulca L.
Spirodela polyrhiza (L.) Schl.
Fam**.** *LILIACEAE*
Colchicum autumnale L.
Leopoldia comosa (L.) Parl.
Ornithogalum gussonii Ten.
Ornithogalum pyramidale L.
Ornithogalum umbellatum L.
Veratrum album L.
Veratrum nigrum L.
Fam. *LINACEAE*
Linum austriacum L.
Linum catharticum L.
Linum tenuifolium L.
Fam**.** *LYTHRACEAE*
Lythrum hyssopifolium L.
Lythrum salicaria L.
Lythrum virgatum L.
Fam**.** *MALVACEAE*
Abutilon theophrasti Med.
Althaea cannabina L.
Althaea hirsuta L.
Althaea officinalis L.
Althaea pallida L.
Althaea rosea (L.) Cav.
Hibiscus trionum L.
Malva moschata L.
Malva neglecta Wallr.
Malva pusilla Sm.
Malva sylvestris L.
Fam. MENYANTHACEAE
Nymphoides peltata (S. G. Gmel.) Kuntze L.
Fam. *NAJADACEAE*
Najas marina L.
Najas minor L.
Fam. *OENOTHERACEAE*
Epilobium adnatum Gris.
Epilobium hirsutum L.
Epilobium palustre L.
Epilobium parviflorum Schreb.
Oenothera biennis L.
Fam. *OROBANCHACEAE*
Orobanche purpurea Jacq.
Fam. *OXALIDACEAE*
Oxalis acetosella L.
Oxalis corniculata L.
Oxalis stricta L.
Fam. *PAPAVERACEAE*
Chelidonium majus L.
Papaver dubium L.
Papaver hybridum L.
Papaver rhoeas L.
Fam. *PLANTAGINACEAE*
Plantago altissima L.
Plantago indica L.
Plantago lanceolata L.
Plantago major L.
Plantago media L.
Fam. *POACEAE*
Aegilops cylindrica Host.
Agropyron repens (L.) P. B.
Agrostis alba L.
Agrostis rupestris All.
Agrostis vulgaris With.
Aira capillaris Host.
Alopecurus myosuroides Huds.
Alopecurus pratensis L.
Alopecurus utriculatus Pers.
Andropogon ischaemum L.
Anthoxanthum odoratum L.
Apera spica-venti (L.) P. B.
Arrhenatherum elatius (L.) M. K.
Avena fatua L.
Brachypodium pinnatum (L.) P. B.
Brachypodium sylvaticum (L.) P. B.
Briza media L.
Bromus arvensis L.
Bromus commutatus Schr.
Bromus hordeaceus L. subsp. *hordeaceus*
Bromus inermis Leyk.
Bromus japonicus Thunb.
Bromus pannonicus Kumm. and Sendtn.
Bromus racemosus L.
Bromus secalinus L.
Bromus squarrosus L.
Bromus sterilis L.
Bromus tectorum L.
Calamagrostis epigejos (L.) Roth.

Chrysopogon gryllus (L.) Trin.
Cynodon dactylon (L.) Pers.
Cynosurus cristatus L.
Dactylis glomerata L.
Digitaria ciliaris (Retz.) Koel.
Digitaria sanguinalis (L.) Scop.
Echinochloa crus-galli (L.) Beauv.
Eleusine indica (L.) Gaertn.
Eragrostis megastachya (Koel.) Lk.
Eragrostis minor Host.
Eragrostis pilosa (L.) P. B.
Festuca arundinacea Schreb.
Festuca gigantea (L.) Vill.
Festuca ovina L.
Festuca pratensis Huds.
Festuca rubra L.
Glyceria aquatica (L.) Wahl.
Glyceria fluitans R. Br.
Holcus lanatus L.
Holcus mollis L.
Hordeum bulbosum L.
Hordeum murinum L.
Koeleria glauca DC.
Koeleria gracilis Pers.
Koeleria pyramidata (Lam.) Dom.
Lolium multiflorum Lam.
Lolium perenne L.
Melica ciliata L.
Melica transsilvanica Schur.
Nardus stricta L.
Panicum capillare L.
Phalaris arundinacea L.
Phleum pratense L.
Phragmites communis Trin.
Poa annua L.
Poa bulbosa L.
Poa compressa L.
Poa pratensis L.
Poa trivialis L.
Psilurus aristatus (L.) Duv.-Jouve
Sclerochloa dura (L.) P. B.
Sesleria tenuifolia L.
Setaria glauca (L.) P. B.
Setaria verticillata (L.) P. B.
Setaria viridis (L.) P. B.
Sorghum halepense (L.) Pers.
Tragus racemosus (L.) Desf.
Vulpia ciliata (Dant.) Lk.
Vulpia myuros (L.) Gmel.
Fam. *POLYGALACEAE*
Polygala comosa Schk.
Polygala major Jacq.
Polygala vulgaris L.
Fam. *POLYGONACEAE*
Bilderdykia aubertii (L. H.) Mold.
Bilderdykia convolvulus L.
Bilderdykia dumetorum (L.) Dum.
Polygonum amphibium L.
Polygonum aviculare L.
Polygonum bistorta L.
Polygonum hydropiper L.
Polygonum lapathifolium L.
Polygonum mite Schr.
Polygonum orientale L.
Polygonum persicaria L.
Rumex acetosa L.
Rumex acetosella L.
Rumex aquaticus L.
Rumex conglomeratus Murr.
Rumex crispus L.
Rumex hydrolapathum Huds.
Rumex maritimus L.
Rumex obtusifolius L.
Rumex palustris Sm.
Rumex pulcher L.
Rumex sanguineus L.
Rumex stenophyllus Led.
Rumex thyrsiflorus Fing.
Fam. *PORTULACACEAE*
Portulaca oleracea L.
Fam**.** *POTAMOGETONACEAE*
Potamogeton crispus L.
Potamogeton fluitans Roth.
Potamogeton gramineus L.
Potamogeton lucens L.
Potamogeton perfoliatus L.
Potamogeton pusillus L.
Potamogeton trichoides Cham. et Schl.
Zannichellia palustris L.
Fam. *PRIMULACEAE*
Anagallis arvensis L.
Anagallis foemina Mill.
Lysimachia nummularia L.
Lysimachia punctata L.
Lysimachia vulgaris L.
Fam. *RANUNCULACEAE*
Adonis aestivalis L.
Adonis flammea Jacq.
Adonis vernalis L.
Clematis recta L.
Clematis vitalba L.
Consolida orientalis Schr.
Consolida regalis Gray.
Helleborus odorus W. et K.

Nigella arvensis L.
Ranunculus acer L.
Ranunculus aquaticus L.
Ranunculus arvensis L.
Ranunculus bulbosus L.
Ranunculus circinnatus Sibth.
Ranunculus polyanthemos L.
Ranunculus repens L.
Ranunculus sardous Cr.
Ranunculus sceleratus L.
Ranunculus stevenii Andrz.
Ranunculus trichophyllus Chaix
Ranunculus velutinus Ten.
Thalictrum foetidum L.
Thalictrum minus L.
Fam. *RESEDACEAE*
Reseda lutea L.
Reseda phyteuma L.
Fam. *ROSACEAE*
Agrimonia eupatoria L.
Crataegus monogyna Jacq.
Filipendula hexapetala Gilib.
Fragaria vesca L.
Geum urbanum L.
Potentilla anserina L.
Potentilla arenaria Borkh.
Potentilla argentea L.
Potentilla erecta (L.) Rausch.
Potentilla hirta L.
Potentilla recta L.
Potentilla reptans L.
Potentilla supina L.
Prunus spinosa L.
Rosa arvensis Huds.
Rosa canina L.
Rosa gallica L.
Rosa pendulina L.
Rubus caesius L.
Rubus canescens D. C.
Rubus idaeus L.
Sanguisorba minor Scop.
Fam. *RUBIACEAE*
Asperula arvensis L.
Galium album Mill.
Galium aparine L.
Galium cruciata (L.) Scop.
Galium mollugo L.
Galium palustre L.
Galium tricornutum With.
Galium verum L.
Sherardia arvensis L.
Fam. *SALVINIACEAE*
Salvinia natans (L.) All.
Fam. *SAMBUCACEAE*
Sambucus ebulus L.
Fam. *SIMARUBACEAE*
Ailanthus altissima (Mill.) Swingle
Fam. *SCROPHULARIACEAE*
Antirrhinum majus L.
Cymbalaria muralis Gaertn.
Digitalis laevigata W. et K.
Digitalis lanata Ehrh.
Gratiola officinalis L.
Kickxia elatine (L.) Dum.
Kickxia spuria (L.) Dum.
Linaria genistifolia (L.) Mill.
Linaria vulgaris Mill.
Melampyrum arvense L.
Melampyrum pratense L.
Rhinanthus alectorolophus (Scop.) Rol.
Rhinanthus angustifolius Gmel.
Rhinanthus minor L.
Rhinanthus rumelicus Vel.
Scrophularia nodosa L.
Verbascum banaticum Roch.
Verbascum blattaria L.
Verbascum chaixii Vill.
Verbascum densiflorum Bert.
Verbascum glabratum Friv.
Verbascum longifolium Ten.
Verbascum lychnitis L.
Verbascum nigrum L.
Verbascum phlomoides L.
Verbascum phoeniceum L.
Verbascum pulverulentum Vill.
Veronica agrestis L.
Veronica anagallis–aquatica L.
Veronica arvensis L.
Veronica beccabunga L.
Veronica chamaedrys L.
Veronica spicata L. subsp. *barrelieri*
Veronica hederifolia L.
Veronica austriaca L. subsp. *austriaca* .
Veronica longifolia L.
Veronica officinalis L.
Veronica peregrina L.
Veronica persica Poir.
Veronica polita Fries.
Veronica prostrata L.
Veronica serpyllifolia L.
Veronica spicata L.
Veronica austriaca L. subsp. *teucrium* (L.) D. A. Webb
Veronica triphyllos L.

Veronica verna L.
Fam. *SOLANACEAE*
Datura stramonium L.
Hyoscyamus niger L.
Physalis alkekengi L.
Solanum dulcamara L.
Solanum luteum Mill.
Solanum nigrum L.
Fam. *SPARGANIACEAE*
Sparganium erectum L.
Fam. *TRAPACEAE*
Trapa natans L.
Fam. *THYMELAEACEAE*
Thymelaea passerina (L.) Coss. – Germ.
Fam. *TYPHACEAE*
Typha angustifolia L.
Typha latifolia L.
Fam. *URTICACEAE*
Parietaria officinalis L.
Urtica dioica L.
Urtica urens L.
Fam. *VALERIANACEAE*
Valerianella carinata Lois.
Valerianella dentata (L.) Pall.
Valerianella locusta (L.) Betcke
Fam. *VERBENACEAE*
Verbena officinalis L.
Fam. *VIOLACEAE*
Viola arvensis Murr
Viola canina L.
Viola hirta L.
Viola kitaibeliana R. et Schult.
Viola odorata L.
Viola tricolor L.

Out of the total number of species, the class *Liliopsida* (*Monocotyledones*) includes 85 species (11.48%) and the class *Magnoliopsida* (*Dicotyledones*) 655 species (88.51%). The class *Equisetopsida* is represented with 3 species. The most common families include 618 species altogether. According to the number of species, the most important are: *Asteraceae* (112), *Poaceae* (78), *Fabaceae* (52), *Lamiaceae* (47), *Scrophulariaceae* (46), *Brassicaceae* (44), *Caryophyllaceae* (40), *Apiaceae* (28), *Chenopodiaceae* (25), *Polygonaceae* (24), *Ranunculaceae* (23), *Rosaceae* (22), *Boraginaceae* (18), *Cyperaceae* (11), *Malvaceae* (11), *Amaranthaceae* (10) and so on. The following genera are prominent by their number of species: *Veronica* (19), *Chenopodium* (16), *Rumex* (13), *Ranunculus* (12), *Vicia* (12), *Bromus* (11), *Euphorbia* (11), *Centaurea* (9), *Galium* (7), *Trifolium* (6), and so on.

According to the relevant literature sources it may be concluded that in the last several decades there has been a significant reduction in the composition of weed flora in Serbia, due primarily to long-term use of herbicides and other chemical and agrotechnical measures. One illustrative example is that in former Yugoslavia there were 1409 weed species (Kojić et al., 1975), while the weed flora of Serbia included 1009 species (Kojić and Vrbničanin, 1998). Now the weed flora of Serbia includes only 741 species of weed plants (Table 1).

Table 2 – Plant species that used to be considered weeds.

Plant species

Fam. *APIACEAE*
Aegopodium podagraria L.
Angelica sylvestris L.
Berula erecta L.
Bupleurum falcatum L.
Bupleurum ranunculoides L.
Peucedanum arenarium W. et K.
Scandix pecten-veneris L.
Fam**.** *APOCYNACEAE*
Vinca herbacea W. et K.
Fam. *ASTERACEAE*
Achillea asplenifolia Vent.
Antennaria dioica Geartn.
Anthemis carpatica Willd.
Aster linosyris (L.) Bernh.
Carduus carduelis (L.) Kern.
Carlina acanthifolia All. subsp*. acanthifolia*
Centaurea affinis Friv.

Centaurea transalpina Schleich.
Centaurea kotschyana Heuff.
Centaurea phrygia L.
Centaurea alba L. subsp. *splendens* (L.) Arcang.
Centaurea triumfetti All.
Ptilostemon afer (Jacq.) Greuter
Crepis conyzifolia (Gou.) D. T.
Crepis nicaeensis Balb.
Crepis viscidula Froel.
Echinops banaticus Roch.
Senecio doria Nath.
Sonchus palustris L.
Fam. *BORAGINACEAE*
Echium russicum J. F. Gmel.
Fam. *BRASSICACEAE*
Barbarea stricta Andrz.
Brassica elongata Ehrh.
Bunias orientalis L.
Conringia orientalis (L.) Dum.
Draba aizoides L.
Erysimum crepidifolium Rchb.
Erysimum hieracifolium L.
Lobularia maritima (L.) Desv.
Neslia paniculata (L.) Desv.
Rapistrum perenne (L.) All.
Rorippa prolifera (Heuff.) Weilr.
Syrenia cana (Piller and Mitterp.) Neilr.
Fam. *CAMPANULACEAE*
Edraianthus graminifolius (L.) DC.
Jasione laevis Lam. subsp. *orbiculata* (Griseb. ex Velen.) Tutin.
Legousia speculum-veneris (L.) Chaix.
Fam. *CAPRIFOLIACEAE*
Lonicera caprifolium L.
Fam. *CARYOPHYLLACEAE*
Cerastium banaticum (Roch.) Heuff.
Cerastium brachypetalum Pers.
Cerastium decalvans Schl. – Vukot.
Dianthus carthusianorum L.
Dianthus giganteus D'Uvr.
Dianthus petraeus W. et K.
Dianthus pinifolius S. et S.
Dianthus scardicus Wettst.
Paronychia kapela (Hacq.) A. Kern.
Silene densiflora d'Uvr.
Silene dichotoma Ehrh.
Silene viridiflora L.
Fam. *CHENOPODIACEAE*
Bassia laniflora (S. G. Gmel.) A. J. Scott
Bassia prostrata (L.) A.J.Scott
Fam. *CISTACEAE*
Fumana procumbens (Dun.) Gr. et God.
Helianthemum canum (L.) Baumg.
Helianthemum nummularium (L.) Mill.
Helianthemum oelandicum (L.) DC.
Helianthemum salicifolium (L.) Mill.
Tuberaria guttata (L.) Fourr.
Fam. *CRASSULACEAE*
Pistorinia hispanica (L.) DC.
Sedum acre L.
Sedum album L.
Sedum annuum L.
Sedum atratum L.
Sedum ochroleucum Chaix
Sedum sexangulare L.
Sedum urvillei DC.
Sempervivum marmoreum Griseb.
Fam. *CYPERACEAE*
Carex caryophyllea Lat.
Carex gracilis Curt.
Carex michelii Host.
Carex nutans Host.
Carex panicea L.
Carex paniculata L.
Carex pilulifera L.
Carex rupestris Bell.
Carex spicata Huds.
Carex tomentosa L.
Carex vulpina L.
Cyperus fuscus L.
Cyperus glomeratus L.
Cyperus michelianus (L.) Link.
Eriophorum latifolium Hoppe
Schoenoplectus lacustris (L.) Palla
Scirpus sylvaticus L.
Fam. *DIPSACACEAE*
Knautia dinarica (Murb.) Borbas
Knautia integrifolia (L.) Bert.
Scabiosa argentea L.
Scabiosa columbaria L.
Fam. *EUPHORBIACEAE*
Euphorbia glabriflora Vis.
Euphorbia nicaeensis All. subsp. *glareosa* (Pall. ex M.Bieb.) Radcl.-Sm.
Euphorbia seguierana Neck
Fam. *FABACEAE*
Anthyllis vulneraria L. L.
Astragalus contortuplicatus L.
Astragalus dasyanthus Pall.
Chamaecytisus albus (Hacq.) Rothm.
Chamaecytisus austriacus (L.) Link.
Chamaecytisus heuffelii (Wierzb.) Rothm.
Chamaecytisus hirsutus (L.) Link.

Chamaecytisus ratisbonensis (L.) Rot.
Chamaecytisus rochelii (Wierz.) Rot.
Chamaespartium sagittale (L.) P. E. Gibbs.
Genista depressa M. B.
Genista radiata (L.) Scop.
Melilotus altissima Thuill.
Trifolium incarnatum L.
Trifolium pallidum W. et K.
Trifolium patens Schreb.
Trifolium resupinatum L.
Trifolium striatum L.
Trifolium subterraneum L.
Fam. *FUMARIACEAE*
Fumaria vaillantii Lois.
Fam. *GENTIANACEAE*
Centaurium erythraea Rafn subsp. *erythraea*
Gentiana asclepiadea L.
Gentianella austriaca (Kerner) Holub
Gentiana acaulis L.
Gentiana lutea L.
Gentiana pneumonanthe L.
Gentiana utriculosa L.
Fam. *GLOBULARIACEAE*
Globularia cordifolia L.
Fam. *HYPERICACEAE*
Hypericum alpinum W. et K.
Hypericum barbatum Jacq.
Hypericum maculatum Cr.
Hypericum richeri Vill.
Hypericum rumelicum Boiss.
Fam. *IRIDACEAE*
Crocus biflorus Mill.
Crocus veluchensis Herb.
Gladiolus imbricatus L.
Iris graminea L.
Iris pseudacorus L.
Iris pumila L.
Iris sibirica L.
Fam. *JUNCACEAE*
Juncus anceps Laharpe.
Juncus articulatus L.
Juncus conglomeratus L.
Juncus effusus L.
Juncus filiformis L.
Juncus thomasii Ten.
Juncus trifidus L.
Luzula luzuloides (Lam.) Dandy and Wilmott
Luzula spicata (L.) Lam. et D. C.
Fam. *LAMIACEAE*
Melissa officinalis L.
Thymus glabrescens Willd.
Thymus longicaulis C. Presl.
Thymus pannonicus All.
Thymus praecox Opiz subsp. *zygiformis* (Heinr. Braun) Jalas
Thymus praecox Opiz subsp. *skorpilii* (Velen.) Jalas.
Thymus pulegioides L.
Thymus serpyllum L.
Fam**.** *LILIACEAE*
Allium angulosum L.
Muscari botryoides (L.) Mill.
Ornithogalum pyrenaicum L.
Fam. *LINACEAE*
Linum bienne Mill.
Linum flavum L.
Linum trigynum L.
Linum hologynum Rchb.
Linum perenne L.
Fam**.** *MALVACEAE*
Lavatera thuringiaca L.
Fam. *NYMPHAEACEAE*
Nuphar luteum (L.) Sm.
Nymphaea alba L.
Fam. *ORCHIDACEAE*
Dactylorhiza incarnata (L.) Soó
Dactylorhiza maculata (L.) Soó
Dactylorhiza sambucina (L.) Soó
Gymnadenia conopsea (L.) R. Br.
Orchis coriophora L.
Orchis laxiflora Lam.
Orchis mascula L.
Orchis morio L.
Orchis papilionacea L.
Orchis ustulata L.
Traunsteinera globosa (L.) Rchb.
Fam. *PLANTAGINACEAE*
Plantago holosteum Scop.
Fam. *POACEAE*
Beckmannia eruciformis (L.) Host.
Crypsis alopecuroides Schr.
Danthonia decumbens (L.) DC.
Deschampsia cespitosa (L.) P. B.
Deschampsia flexuosa (L.) Trin.
Koeleria eriostachya Panč.
Koeleria nitidula Vel.
Koeleria splendens Presl.
Molinia coerulea (L.) Mnch.
Sesleria coerulans Friv.
Stipa capillata L.
Stipa joannis Čelak
Stipa borysthenica Klokov.
Fam. *POLYGONACEAE*
Polygonum arenarium W. et K.

Polygonum viviparum L.
Fam. *POLYPODIACEAE*
Thelypteris palustris Schott.
Fam. *PRIMULACEAE*
Primula elatior (L.) Hill
Primula veris Huds.
Fam. *RANUNCULACEAE*
Clematis integrifolia L.
Ranunculus auricomus L.
Ranunculus millefoliatus Valh.
Ranunculus montanus Willd.
Ranunculus oreophilus M. B.
Trollius europaeus L.
Fam. *ROSACEAE*
Dryas octopetala L.
Geum montanum L.
Potentilla aurea L.
Potentilla aurea L. subsp. *chrysocraspeda* (Lehm.) Nyman
Fam. *RUBIACEAE*
Asperula cynanchica L.
Asperula purpurea (L.) Ehrend.
Cruciata pedemontana (Bellardi) Ehrend.
Galium boreale L.
Galium debile Desv.
Galium rubioides L.
Fam. *SCROPHULARIACEAE*
Euphrasia rostkoviana Hayne
Euphrasia stricta Host.
Limosella aquatica L.
Pedicularis comosa L. subsp. *campestris* (Griseb.) Soó
Pedicularis comosa L.
Pedicularis heterodonta Panč.
Veronica austriaca L.
Veronica bellidioides L.
Fam. *TRAPACEAE*
Trapa annosa Jank.
Trapa brevicarpa Jank.
Trapa natans L.
Fam. *VACCINIACEAE*
Vaccinium myrtillus L.
Vaccinium uliginosum L.
Vaccinium vitis-idaea L.
Fam. *VALERIANACEAE*
Valeriana officinalis L.
Fam. *VIOLACEAE*
Viola dacica Borb.

There were some significant changes in the diversity of species of weed communities, mostly in the form of reduction in floristic composition (disappearance of vulnerable plants and increasing dominance of resistant ones), particularly as a consequence of the use of various herbicides on diverse resistances of weed plants. However, in order to present the historical timeline on the understanding of the term "weeds", Table 2 includes 227 species that used to be considered weeds. The species included in Table 2 are currently considered to be rare or threatened species. This all indicates that the activity of herbicides, as well as other chemical and agrotechnical measures, has led to impoverishment and large-scale quantitative and qualitative changes in weed flora of Serbia.

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PREGLED KOROVSKE FLORE SRBIJE

MARKO LJ. NESTOROVIĆ, BRANKO KONSTANTINOVIĆ

Izvod

Korovsku floru Srbije karakteriše visoko izražen diverzitet, utvrđeno je prisustvo 741 vrsta korova iz 322 roda i 63 porodice. Većina korovskih vrsta nije usko specijalizovana, već se sreće u okviru različitih korovskih kategorija. Većina vrsta ima široku ekološku valencu za veći broj ekoloških faktora. Od ukupnog broja vrsta, klasi *Liliopsida* (*Monocotyledones*) pripada 85 vrsta (11,48%), dok klasi *Magnoliopsida* (*Dicotyledones*) pripada 655 vrsta (88,51%). Klasa *Equisetopsida* je zastupljen sa 3 vrste. Najzastupljenije familije su: *Asteraceae* (112), *Poaceae* (78), *Fabaceae* (52), *Lamiaceae* (47), *Scrophulariaceae* (46), *Brassicaceae* (44), *Caryophyllaceae* (40), *Apiaceae* (28), *Chenopodiaceae* (25), *Ranunculaceae* (23), *Rosaceae* (22), *Boraginaceae* (18), *Cyperaceae* (11), itd. Po broju vrsta ističu se rodovi: *Veronica* (19), *Chenopodium* (16), *Rumex* (13), *Ranunculus* (12), *Vicia* (12), *Bromus* (11), *Euphorbia* (11), *Centaurea* (9), *Galium* (7), *Trifolium* (6), itd.

Ključne reči: korovi, korovska flora, ček lista, Srbija.

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ORGANIC AGRICULTURE AND RURAL DEVELOPMENT IN AP VOJVODINA

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SUMMARY: The authors discuss the link between the concept of organic food production and the concept of rural development. They are discussing the limiting factors and problems of implementation these concepts in the Republic of Serbia and Vojvodina. The problems of rural areas in Serbia are primarily reflected in the fact that in most villages the population is mainly elderly people and the young are trying to achieve employment in large urban centres. As a possibility of reviving the village, the authors propose the concept of rural development which develops not only agriculture but also activities related to agriculture. The authors are especially considering organic farming and its role in this concept of development.

Key words: *rural development, organic agriculture, farming, R. Serbia, Vojvodina.*

INTRODUCTION

Contemporary trends in the movement of population place an emphasis on the uneven regional distribution of people. As a consequence of industrialization, speedy urban development and neglect of rural areas, there is a problem of rural depopulation and land reclamation. In the EU countries, these problems have been manifested in the sixties. In response to the growing problems of rural areas EU countries developed a new concept of rural development which emphasizes the development of agriculture, as well as activities related to agriculture. In Serbia, this concept is relatively new where the problem is primarily in the fact that Serbia has long applied a uniform concept of development that favoured the cities and neglected rural areas, which led to disparities and uneven development of rural and urban area (Tomaš, 2010).

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The AP of Vojvodina, according to the OECD, classification is divided into seven regions. In each of these regions problems with the population have been observed in rural areas. Among the most significant issues are the problems of land fragmentation and the so-called problems of "nursing households" or senility of rural population (Njegovan and Pejanović, 2009).

The paper starts from the hypothesis that organic farming, due to its characteristics and level of development in Serbia, could be a possibility of development and revival of rural areas.

STATE AND PROBLEMS OF RURAL DEVELOPMENT IN VOJVODINA

The AP Vojvodina is a region of Serbia, in which agriculture is the dominant economic activity in most municipalities. Consequently, agricultural activity is the main source of income for many households in this region. However, the infrastructure is underdeveloped and existing capacity under-maintained and non-functional in terms of today's, let alone future needs. Some municipalities have developed differently due to different levels of development of formal and informal institutions that reflect the different level of development of rural and urban population. So, there are drastic structural imbalances, institutional problems, unfavourable demographic trends and material constraints in most municipalities (Njegovan and Pejanovic, 2009).

In the AP Vojvodina there are 2,031,992 inhabitants (which makes 27.1% of the total population of the Republic of Serbia) living in 464 settlements, of which 412 are villages. In spite of that, for quite a long period of time there has been a trend of a drastic reduction in the total and active population in rural areas (Njegovan et all., 2010) (Table 1).

Table 1: Changes in population of Vojvodina according to the censuses from 1961- 2002.
Tabela 1: Kretanje stanovništva AP Vojvodine prema popisima od 1961- 2002. godine

	1961.	**1971.**	**1981.**	**1991.**	**2002.**
Total population Ukupno stanovništvo	1.854.956	1.952.533	2.034.772	2.013.889	2.031.992
Agricultural population Poljoprivredno stanovništvo	961.000	761.000	391.426	269.438	215.147
% of total population % od ukupnog	52	39	19	13	11
Active agricultural population Aktivno poljopriivredno stanovništvo	457.400	385.100	213.307	149.583	125.506

Source: Statistical Yearbook of the Republic of Serbia, 2007., pg. 74.

Although the nominal number of inhabitants is permanently increasing, the agricultural population in the last 40 years recorded a drastic decline (from 52% of the total population in 1961 to 11% in 2001 or 4.47 times less).

Out of the total available arable land, 67.2% accounts for privately owned rural properties. Private farms, despite their majority of land, have an unfavourable property structure. Specifically, the average size of arable land used by private farms is 3.52 hectares. This area is usually divided into three parts, the average size totalling 125 acres. These parameters are not good for conventional agriculture but, due to the higher prices

of organic products in the market, and the fact that organic agriculture is labour intensive, they can be considered as an advantage in organic agricultural production.

Number of agricultural households by 2002 census amounted to 201,475. As significant production units, there are also cooperatives and agricultural companies. In the AP Vojvodina there is a total of 497 cooperatives and agricultural companies that have around 624,000 ha of agricultural land, of which about 511,000 ha is arable land. Most of the cooperatives and companies have a surface area of 1,000 to 2,500 ha. Since organic agriculture depends on small and family households, the aspect of cooperation among producers is highly important, and in Serbia and AP Vojvodina is a segment that needs to be more efficient and active.

One of the most important factors of rural development of Vojvodina is its` population and civil society. The first is the consciousness of the people about the necessity of proactive attitude in relation to the development of local communities and rural areas in general, and its participation through the groups of citizens, associations, activities and all other groups that have an interest to participate in these processes. So far, this important aspect of rural development - participation of citizens and work to raise their capacity limit has represented a fundamental obstacle for more intensive development.

Summarizing, it can be pointed out that the problems of rural development in Vojvodina are, in fact, that agriculture is still dominant economic activity in most municipalities, that infrastructure is underdeveloped and existing capacity under-maintained and non-functional in terms of today, let alone future needs. Also, a significant problem is the insufficient development of formal and informal institutions and civil society and, therefore, marked disproportion between the development levels of municipalities.

Increased regional disparities are often the result of inefficient use of the local development area. (Pejanović et all, 2009). The rapid establishment of institutional conditions for more consistent and therefore more creative and more effective rural policy should result in reducing rural issues and in promoting local development potential. Reference to this is, in our opinion, the concept of organic agriculture which can bring the development of local area on higher level, providing more chance for local population to earn income.

In this developmental orientation significant socio-economic problems escalated and in the period after the year of 2000 Serbia started the process of transition of the economy and society. This process has not bypassed the rural areas that predominantly occupied the total area of the APV. As a result, it is reflected in the position of the rural population and the impact on the overall situation in rural areas.

SOME ASPECTS OF ORGANIC AGRICULTURE IN THE REPUBLIC OF SERBIA

Since January 2011, in the Republic of Serbia, organic farming is regulated by the Law on Organic Production („Official Gazette of RS“No.30/10) which was enacted in the Republic Parliament in May 2010. This law and following legal acts had been prepared according to the, European Council Regulation (EC) No. 834/2007, Reg. (EC) No. 889/2008. and Reg. (EC) No. 1235/2008. The law is followed by the two rulebooks on organic production and import rules.

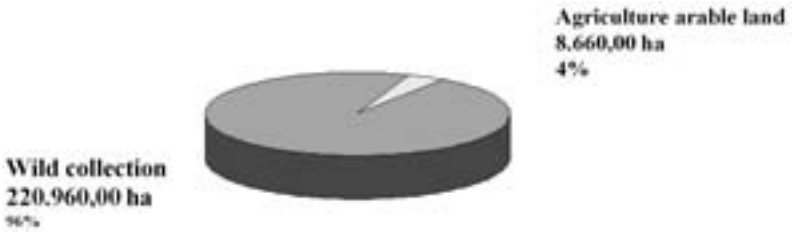
In order to achieve the standards set by world markets, support to the organic sector is necessary. The action plan for organic farming in Serbia reflects the political will

to establish strategic goals in the field as well as to engage all state administration capacities in achieving them. The overall objective of the action plan is to increase the total area of cultivated land as certified organic or in conversion to 50,000 ha until 2014.

After a number of activities aimed at supporting the organic sector, in May 2010, GTZ launched a project on organic sector analysis as well as opportunities for the development of different segments that can contribute to the improvement organic production in Vojvodina and Serbia, and indirectly, to the development of rural areas. For the purpose of research, GTZ engaged advisory consortium comprising German consulting houses AFC and the Swiss Research Institute of Organic Agriculture FIBL, with the support and cooperation of Serbian experts. The conducted research has so far included: site visits and scanning of the situation in the sector through interviews with various stakeholders in the sector; sector analysis with the collection of statistical data (area, species, regional distribution, number and type of actors, processing, etc.) in cooperation with relevant institutions and certification bodies whose activity is registered in the Republic of Serbia; farm survey with number of certified organic farmers from all over the country.

STRUCTURE OF ORGANIC AGRICULTURE IN THE REPUBLIC OF SERBIA

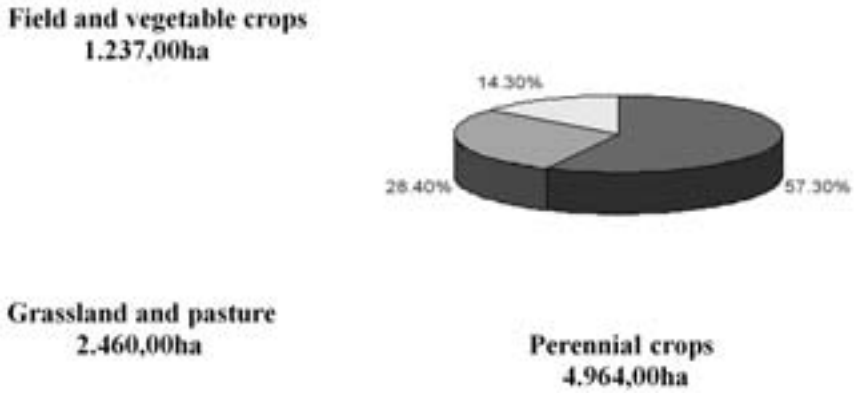
According to the GTZ survey data based on the information from active certification bodies, around 230.000 ha of land are currently either organically certified or in the certification process. This surface includes the land which is used for the collection of wild growing berries, mushrooms and herbals, representing 96% of the total organically certified national surface (almost 201'000 ha). The organic arable land is 3.5-4% of that area and accounts to about 8.660 ha. (Graph 1: source: Organic Agriculture in Serbia, At a glance, GTZ, 2010.)



Graph 1: Structure of organic land in Republic of Serbia
Grafikon 1: Struktura organskih površina u Republici Srbiji

Out of the total agricultural land under organic cultivation, perennial crops are planted on almost 60% and annual crops on 15% ha. The balance 25% goes to grassland and pasture. (Graph 2)

Within the category of perennials, apples dominate, followed by different berries, notably raspberries, and plums. Cereals, soybeans and vegetables are the main annual crops cultivated.



Graph 2: Structure of organic crops in Republic of Serbia
Grafikon 2: Struktura useva na organskim površinama u R. Srbiji

Source: Organic Agriculture in Serbia, At a glance, GTZ, 2010.

In terms of regional distribution and importance of organic farming in some regions, research has shown that 90% of field and vegetable crops are produced in Vojvodina, while perennials and pastures and meadows are mainly localized in the region of Southern and Western Serbia. In Vojvodina, the most important are soybeans, corn and wheat and most of these areas are in the period of conversion. In addition to cereals and industrial crops in Vojvodina in the system of organic farming are also fruits and various vegetables.

In South and West Serbia, the production of fruit species is significant, especially raspberries, strawberries, and blackberries, but also apples and plums. Almost all production is export driven.

The survey data estimate that at least 3,000 small-scale farmers are involved in organic production. In addition, farmers involved in wild collection in certified regions are not registered. Regarding market-near actors, most of them are involved in different activities at the same time (processors are likely to be also exporters, traders, input suppliers, and importers). The survey data relates to approx. 20 companies who are currently involved in organic processing and trading activities.

Additionally, retailers, certification bodies, and supporting government and non-governmental institutions are relevant for the sector.

MAIN CHARACTERISTICS OF ORGANIC PRODUCTION IN THE REPUBLIC OF SERBIA

Resent survey enable GTZ experts to define profile of typical organic farmer in Serbia, which is of great help to have a good overview over the sector and understand how it is evolving. On behalf of the GTZ, in August 2010 the AFC/FIBL Consortium and Serbian experts conducted an organic farm survey. The number of farms which cultivate land according to organic practise in 2010 is estimated at 3,000. The Organic Farm Survey aimed to collect more detailed data on the typical Serbian organic farmer. The survey encompasses 140 organic farms (GTZ, 2010).

The results of survey have shown:

- More than 60% of such farms operate on acreage of less than six hectares and 25% on 10-20 hectares. Such areas are worked typically by household members, and every second farm hires seasonal labour for harvesting. However, small farms with less than five hectares cultivate cereals on small plots and for home consumption only, growing fruit trees and berries on most of their land instead. Vegetables are grown mostly on farms whose ranges from 5 to 10 ha.
- The larger the farm, the bigger its acreage under organic certification, but it never accounts more than15-25% of total land available. It generally goes to berry cultivation, which is mostly certified, followed by fruits and vegetables. In the category of berries raspberries dominate, while plums and apples are most important crops among fruits.
- There has recently not been much investment in organic farming: plantations are usually old, machinery likewise (usually older than ten years), greenhouses and organized stores available only to every third farmer and leasing land or purchasing inputs or machinery on credits is practiced by just 5-20% of all farmers surveyed. Future investment plans are therefore very moderate. They concentrate on rehabilitating the irrigation infrastructure, deemed problematic mostly for fruit farmers.
- Obtaining organic inputs is considered by virtually all participants as a challenge. Certified seed is only rarely available; pesticides permitted under organic regimes practically not existing and even fertilizing is an issue: organic farming relies on manure and on compost. But considering that only every second farmer keeps animals and if so only a few, manure available to them is hardly enough to provide.
- Organically certified product is typically sold to wholesalers and to processing companies, with which almost 70% of the growers conclude contracts prior to the start of the season. Direct selling e.g. on the green market is practiced only by 20% of farmers. Due to such system, the mark-up in price they obtain for their organic produce is very moderate (with 10-20% on average) and confirms that value-addition is not generated on the farm level. However the products offered are usually also not ready for optimum marketing: since there is often lack of storage facilities, products are only available during peak periods, when the growers flood the market. Sorting is only carried out by every second farmer and usually according to size, rarely according to quality. Packaging and transport logistic are also mayor issue.
- To some extent, farmers are aware of these problems: while low yield (insufficient fertilization), diseases and pests (absence of appropriate pesticides), as well as irrigation are seen as mayor production constraints.
- Such economic situation, however, has not motivated farmers to form cooperatives or associations. Only 5% of them are organized in associations and only 30% in business associations, such as Serbia Organica, Terra's and Topas –these three being the most popular.

In Vojvodina, the most important crops produced in organic farming are grains, where dominates spelt and rye, followed by industrial plants (primarily soya), fruits (mostly apples) and various vegetables.

Farmers involved in organic production in Serbia and Vojvodina are faced with many problems. However, organic production is a new concept of agriculture production in our country and as such is still adapting to conditions at the macroeconomic

level. On the other hand, it is a concept that could partially solve the existing problems in rural areas (fragmentation properties and senility) because it relies on traditional production methods using modern scientific approaches. On the global scale, the organic market has shown continuously growth and resistant to negative economic trends, which proved its prosperity despite the global economic crisis.

CONCLUSION

Organic production has a significant role in the development of rural areas because it enables economic growth, diversification of activities, attraction of financial resources and it is also an integral part of the Strategy for agriculture and rural development (National Action Plan, 2010).

In contrast to conventional agriculture, organic production enable successful development of multifunctional agriculture, which includes food production as well as non-agricultural products (e.g. souvenirs, handicrafts) and services such as education, recreation, agro, eco, ethno and rural tourism, (Lazic, 2009). This is of great importance in rural areas in Vojvodina, where 65.5% of farms have less than 3 hectares of land. Multifunctional agriculture contributes to the conservation of soil, water, health of plants, animals and people, biodiversity and agrobiodiversity, and to preserving the values of rural environment, household farms, local ethnological, cultural values and traditions. (Branka Lazic, 2009). Overall ecological and economic importance of organic production is reflected in the revitalization of rural areas.

Organic production enables hiring of young people and active involving of women in agribusiness, which leads to decrease of the unemployment rate in Serbia and contributes to economic development of rural areas, creating added value to the product or service.

Vojvodina is mostly field and vegetable crop region and in the organic farming the most important are soybeans, wheat and corn and vegetable variety. Also great wealth in Vojvodina are the indigenous varieties of apples, pears, plums, with high resistance to pests and pathogens, so they can be grown without the use of chemical fertilizers and pesticides. The development of organic fruit production provides the basis for the development and organic beekeeping. In addition, in these areas, extending the natural meadows and pastures are suitable for raising livestock.

According to these facts, it is concluded that Vojvodina has a high agricultural potential for the development of organic production, and that there is an increasing interest in the private sector to invest in organic production. This would contribute mainly to human health and the health environment; enhance quality of life and economic development, while preserving the values of rural environment, cultural values and traditions of the region.

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ORGANSKA POLJOPRIVREDA I RURALNI RAZVOJ U AP VOJVODINI

RADOVAN PEJANOVIĆ, MIRELA TOMAŠ, JOVANA VUČKOVIĆ, EMILIJA STEFANOVIĆ, DANICA GLAVAŠ-TRBIĆ, MARIJA KALENTIĆ

Izvod

Autori razmatraju vezu između koncepta organske proizvodnje hrane i koncepta ruralnog razvoja. Pri tom ističu ograničavajuće faktore i probleme realizacije ovih koncepata u Republici Srbiji i AP Vojvodini. Problemi ruralnih područja u Republici Srbiji se pre svega ogledaju u činjenici da se u većini sela nalazi mahom starije stanovništvo i da mladi pretežno pokušavaju da svoje zaposlenje ostvare u većim urbanim centrima.

Kao mogućnost oživljavanja sela, autori predlažu koncept ruralnog razvoja koji razvija ne samo poljoprivredu već i delatnosti oko poljoprivrede. Posebno se razmatra organska poljoprivredna proizvodnja i njena uloga u ovom konceptu razvoja.

Ključne reči: ruralni razvoj, organska proizvodnja, poljoprivreda, R. Srbija, AP Vojvodina.

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