

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



# CONTEMPORARY AGRICULTURE SAVREMENA POLJOPRIVREDA

The Serbian Journal of Agricultural Sciences Srpski časopis za poljoprivredne nauke





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# AGRICULTURAL BUDGET AS A FORM OF FINANCING AGRICULTURE IN THE REPUBLIC OF SERBIA

AGRARNI BUDŽET KAO OBLIK FINANSIRANJA POLJOPRIVREDE REPUBLIKE SRBIJE

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# EFFECT OF IRRIGATION AND NITROGEN RATES ON YIELD AND WATER PRODUCTIVITY OF SUGAR BEET

# BORIVOJ PEJIĆ, LIVIJA MAKSIMOVIĆ, STANKO MILIĆ, MILORAD RAJIĆ<sup>1</sup>

SUMMARY: Experiments were conducted at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops, on a calcareous chernozem soil on the loess terrace, from 2004 to 2006. The experiments included a variant of irrigation (T1) and a nonirrigated control (T0) and four variants of nitrogen fertilization, N1 90, N2 120, N3 150 and N4 180 kg ha-1. Coefficients of irrigation water use efficiency (I/WUE, t ha-1/mm) and evapotranspiration water use eficiency (ETm/WUE and ETa/WUE, t ha-1/mm) were used to assess the effect of applied irrigation schedule on the yield of sugar beet root and water productivity both in irrigation and rainfed condition under different variants of nitrogen fertilization. As there were no statistically significant differences in sugar beet root yield either among the variants with high nitrogen doses or among the values of I/WUE, ETm/ WUE and ETa/WUE, the variant of nitrogen fertilization with 120 kg ha-1 is adequate for both irrigation and rainfed conditions. Higher values of ETa/WUE in relation to ETm/ WUE indicated that water consumption by sugar beet was more productive under rainfed than under conditions of irrigation. Low values of I/WUE in some years and higher values of ETa/WUE than ETm/WUE in the period of study indicate that irrigation in the climate of Vojvodina is supplementary in character.

*Key words: sugar beet, irrigation, nitrogen rates, yield,water productiv-ity* 

#### **INTRODUCTION**

Sugar beet takes an important place among the field crops grown in the Vojvodina Province, not considering its acreage, but considering its economic importance as the

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raw material for the production of sugar. To meet the needs of all operative sugarbeet refineries, a certain amount of sugar beet must be produced. A required sugar beet acreage is difficult to plan because yields vary in dependence of weather conditions, soil properties and applied cultural practices. In the variable climatic conditions of the Vojvodina Province, in which summers are semi-arid to semi-humid high and stable yields of sugar beet roots can be obtained only in irrigation (Pejić et al., 2006).

Besides climatic factors, a decisive role in determining sugar beet yield performance and quality is played by fertilization practice. Among the nutritive elements required by sugar beet, nitrogen is most important. Nitrogen deficit in sugar beet nutrition leads to reduction of root yield which in turn considerably reduces total sugar production per unit area. On the other hand, excessive and untimely fertilizer application increases the root yield slightly while reducing sugar content in the root and the production of white sugar (Marinković et al., 1997).

Taking in consideration that irrigation in Vojvodina has a supplementary character, sugar beet irrigation scheduling gains additional importance. The soil moisture technical minimum for this crop is 70% of the field water capacity (FWC), i.e., irrigation should be performed when about two thirds of available water in the soil layer to 0.6 m are spent (Dragović, 1976, Mahmoodi et al., 2008). If irrigation schedule is not harmonized with crop requirements and water-physical soil properties, effect of irrigation may be negligible (Pejić et al., 2006).

Percentage of yield increase of sugar beet roots need not always be a reliable indicator of irrigation efficiency. Calculation of irrigation water use eficiency (I/WUE) and evapotranspiration water use eficiency coefficients (ETm/WUE and ETa/WUE) may give a more realistic assessment of the effect of irrigation, i.e., the effect of the irrigation schedule applied. If calculated values of I/WUE, ETm/WUE and ETa/WUE are below those previously established for that region, a failure must have occurred in the technology of crop, primarily in the application of inadequate irrigation schedule.

The objective of the research was to determine the most optimal dose of nitrogen fertilizer for sugar beets grown under irrigation and rainfed conditions, and also, based on the calculated values of I/WUE, ETm/WUE and ETa/WUE coefficients, to analyse the effectiveness of the applied irrigation schedules, i.e., the productivity of water applied in irrigation and rainfed conditions, and so obtain more information that could improve the production of sugar beet in the Vojvodina Province.

#### MATERIAL AND METHODS

The experiments were conducted at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops, on the calcareous chernozem soil on the loess terrace, from 2004 to 2006. The experimental setup was the random block system adapted to the conditions of sprinkler irrigation. The experiment included a variant with irrigation ( $T_1$  - 70% of the field water capacity - FWC) and a nonirrigated control variant ( $T_0$ ). Irrigation schedule was determined by monitoring the dynamics of soil moisture.

The study included four variants of nitrogen fertilization:

- N<sub>1</sub> 90 kg N ha<sup>-1</sup> (applied before primary tillage);
- $N_2 120 \text{ kg N ha}^{-1} (90 + 30 \text{ kg N ha}^{-1} \text{ before primary tillage + preplanting});$
- N<sub>3</sub> 150 kg N ha<sup>-1</sup> (90 + 30 + 30 kg N ha<sup>-1</sup> before primary tillage + preplanting + top dressing);

•  $N_4$  - 180 kg N ha<sup>-1</sup> (90 + 30 + 30 + 30 kg N ha<sup>-1</sup> before primary tillage + preplanting + top dressing).

All variants of nitrogen fertilization had received 90 kg ha<sup>-1</sup> of both  $P_2O_5$  and  $K_2O_5$  before the primary tillage. The nitrogen applied before planting and for top dressing was added in the form of KAN that contained 27 % of active ingredient. Consumption of water for sugar beet potential evapotranspiraciju (ETm) during growing season was calculated using the procedure of hydrophytothermic bioclimatic indexes (Dragović, 2000). After calculating ETm, actual evapotranspiration (ETa) was calculated on the basis of rainfall data and water reserve accumulated in the soil before the beginning of the growing season. Rainfall (P) and temperature (T) data were taken from Rimski Šančevi Meteorological Station. The coefficient of irrigation water use efficiency (I/WUE, t ha<sup>-1</sup>/ mm) was calculated on the basis of the differences in sugar beet root yields obtained in variants T<sub>1</sub> and T<sub>0</sub> in relation to irrigation water applied (Wirr) (Bos, 1985):

I/WUE = Yirr – Ydry/Wirr

- Yirr = yield in irrigation conditions (t ha<sup>-1</sup>/mm)
- Ydry = yield in nonirrigated rainfed conditions (t ha<sup>-1</sup>/mm)
- Wirr = irrigation water applied (mm)

Evapotranspiration water use efficiency coefficients (ET/WUE, t ha<sup>-1</sup>/mm) were calculated on the basis of yields obtained in variants  $T_1$  and  $T_0$  and evapotranspiration rates in variants  $T_1$  (ETm) and  $T_0$  (ETa), (Bos, 1985):

ET/WUE = Y/ET

- $Y = yield (t ha^{-1}/mm)$
- ETm = evapotranspiration rate under conditions of irrigation (mm)
- ETa = evapotranspiration rate under nonirrigated rainfed conditions (mm)

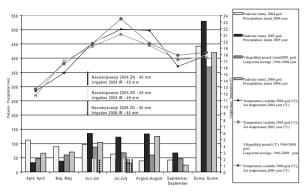
The experimental sugar beet plots received conventional cultivarion practices adjusted to the conditions of irrigation. Sugar beet was harvested at technological maturity and root yield (Y) was calculated in t ha<sup>-1</sup>. Statistical processing of data was done by the analysis of variance, testing the obtained results by the LSD test.

#### **RESULTS AND DISCUSSION**

In the Vojvodina Province, sugar beet is considered to be an irrigation-requiring crop, because it rarely meets its water requirement from rainfall received during the growing season. The situation is especially critical in the summer months of June, July and August. Dragović (1976) stated for the Vojvodina Province that the sugar beet water requirements were 555 mm for the growing season or 60 mm in April, 90 mm in May, 110 mm in June, 120 mm in July, 125 mm in August, and 50 mm in September. In the study period, evapotranspiration rate in irrigation conditions (ETm) ranged from 534 to 602 mm, and in rainfed conditions (ETa) in the interval from 417 to 534 mm (Table 2).

In the course of this study, the growing season of 2004, 2005 and 2006 had the rainfalls of 442, 530 and 420 mm (Graph 1). A comparison of monthly precipitation amounts and monthly sugar beet water requirements indicated a deficit in readily available water and the need for irrigation. In the 2004 growing season, four irrigations were performed in July and August, each with the irrigation water of 45 mm. In 2005, although the amount of rainfall matched the level of sugar beet water requirement, three irrigation had to be performed in June and July, each with 45 mm of water, because the rainfall distribution was in disagreement with the dynamics of sugar beet requirements

for water. In 2006, four irrigations were performed in June and July, each with 45 mm of water (Graph 1).



Graph 1. Mean monthly air temperatures (T °C), monthly precipitation sums (P mm), irrigation schedules and irrigation water applied (mm) in the sugar beet growing season (Rimski Šančevi, 2004-2006)

Grafikon 1. Srednja mesečna temperatura vazduha (T°C), mesečna suma padavina (P mm) u vegetacionom periodu šećerne repe, vreme zalivanja, norma navodnjavanja (mm) (Rimski Šančevi, 2004-2006)

In the study period, on average, there were no statistically significant differences in sugar beet root yield between the irrigated and rainfed variants. The irrigation increased the yield by 9.4% or 8.01 t ha<sup>-1</sup> (Table 1).

The effect of fertilization on the yield of sugar beet roots varied considerably in dependence of nitrogen doses and irrigation. In all three study years, highest sugar beet root yields were achieved in fertilization variants with high nitrogen doses (Table 1).

| Year             | T.             |                      | Fe                    | ertilization - I      | D <i>jubrenje</i> (kg | N ha-1)   |
|------------------|----------------|----------------------|-----------------------|-----------------------|-----------------------|---|
| Godina           | $T_0^1$        | 90 (N <sub>1</sub> ) | 120 (N <sub>2</sub> ) | 150 (N <sub>3</sub> ) | 180 (N <sub>4</sub> ) | Average<br>Prosek   |
| 2004             | T <sub>1</sub> | 91.95                | 99.95                 | 113.35                | 105.26                | 102.63  |
| 2004             | T <sub>0</sub> | 85.80                | 87.54                 | 95.16                 | 96.99                 | 91.37   |
| 2005             | T <sub>1</sub> | 78.49                | 88.09                 | 94.58                 | 91.57                 | 88.18   |
| 2003             | T <sub>0</sub> | 77.07                | 84.79                 | 86.38                 | 90.25                 | 84.62   |
| 2006             | T <sub>1</sub> | 67.20                | 94.00                 | 97.70                 | 93.27                 | 88.04   |
| 2008             | T <sub>0</sub> | 56.07                | 85.05                 | 90.32                 | 83.95                 | 78.85   |
| Average - Prosek |                | 76.10b               | 89.90a                | 96.25a                | 93.55a                | Average - $Prosek$<br>T <sub>1</sub> 92.95 ns - T <sub>0</sub> 84.94 ns |

| Table 1. Yield of sugar beet root (t ha <sup>-1</sup> ) |
|---|
| Tabela 1. Prinos korena šećerne repe (t ha-1)           |

Values with no common superscript are significantly different (P $\leq$  0.05)

Vrednosti koje nemaju isto slovo u superskriptu su statistički značajno različite ( $P \le 0.05$ )

Statistically significant differences in root yield were obtained between the fertilization variants with high nitrogen doses as compared with the fertilization variant with 90 kg N ha<sup>-1</sup> (Table 1). As no statistically significant differences in sugar beet root yield were established between variants with 120, 150 and 180 kg N ha<sup>-1</sup>, it was concluded that the fertilization variant with 120 kg N ha<sup>-1</sup> was acceptable for both irrigation and rainfed conditions, taking in consideration that favorable conditions for mineralization in soil can provide additional amounts of nitrogen (Marinković et al., 1997). Studying nitrogen fertilization of sugar beet suitable for conditions of Poland, Podlaska and Artysza (1995) found that the amount of 120 kg N ha<sup>-1</sup> is most acceptable from the aspects of yield performance and sugar content in roots.

On average for all fertilization variants, the value of the coefficient I/WUE for the investigated period was 0.048 t ha<sup>-1</sup>/mm, the actual values ranging in the interval from 0.033 t ha<sup>-1</sup>/mm in the fertilization variant with 180 kg N ha<sup>-1</sup> to 0.068 t ha<sup>-1</sup>/mm in the fertilization variant with 150 kg N ha<sup>-1</sup> (Table 2).

Table 2. Amounts of water used for potential (ETm) and actual (ETa) evapotranspiration, irrigation water applied, and water use efficiency coeficients of sugar beet (WUE) depending on different rates of nitrogen fertilizer

| Tabela 2. Utrošak vode na potencijalnu (ETm) i stvarnu (ETa) evapotranspiraciju, norna       |
|--|
| navodnjavanja, koeficijenti iskorišćenosti vode šećerne repe (WUE) u uslovima različite doze |
| djubrenja azotom   |

|                   | Irr.               |  |                                 | Fertiliz                | zation - <i>Dju</i>      | <i>brenje</i> (kg        | N ha <sup>-1</sup> )     |                   |
|-------------------|--------------------|--|---------------------------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------|
| Year<br>Godina    | ETm<br>ETa<br>(mm) | wat. app.<br><i>Norma</i><br><i>nav.</i><br>(mm) | WUE<br>(t ha <sup>-1</sup> /mm) | 90<br>(N <sub>1</sub> ) | 120<br>(N <sub>2</sub> ) | 150<br>(N <sub>3</sub> ) | 180<br>(N <sub>4</sub> ) | Average<br>Prosek |
|                   |                    | 180  | I/WUE                           | 0.054                   | 0.080                    | 0.101                    | 0.046                    | 0.070             |
| 2004              | 569                |  | ETm/WUE                         | 0.162                   | 0.176                    | 0.199                    | 0.185                    | 0.180             |
|                   | 417                |  | ETa/WUE                         | 0.206                   | 0.210                    | 0.228                    | 0.232                    | 0.219             |
|                   |                    | 135  | I/WUE                           | 0.011                   | 0.024                    | 0.061                    | 0.001                    | 0.024             |
| 2005              | 534                |  | ETm/WUE                         | 0.147                   | 0.165                    | 0.177                    | 0.171                    | 0.126             |
|                   | 534                |  | ETa/WUE                         | 0.144                   | 0.159                    | 0.161                    | 0.169                    | 0.158             |
|                   |                    | 180  | I/WUE                           | 0.062                   | 0.050                    | 0.041                    | 0.052                    | 0.051             |
| 2006              | 602                |  | ETm/WUE                         | 0.112                   | 0.156                    | 0.162                    | 0.155                    | 0.146             |
|                   | 440                |  | ETa/WUE                         | 0.127                   | 0.193                    | 0.205                    | 0.191                    | 0.179             |
|                   |                    | 165  | I/WUE                           | 0.042b                  | 0.051ab                  | 0.068a                   | 0.033b                   | 0.048             |
| Average<br>Prosek | 568                |  | ETm/WUE                         | 0.140b                  | 0.166ab                  | 0.179a                   | 0.170a                   | 0.164             |
| TTOSEK            | 464                |  | ETa/WUE                         | 0.159b                  | 0.187a                   | 0.198a                   | 0.197a                   | 0.185             |

Values with no common superscript are significantly different ( $P \le 0.05$ ) Vrednosti koje nemaju isto slovo u superskriptu su statistički značajno različite ( $P \le 0.05$ )

Various authors provided different values of this coefficient, but noting that they depended on the method of irrigation, soil and climatic conditions, time of sowing, cultivars, plant density, control of disease and weeds (Bos, 1985). Draycott (2006) reported the average value of I/WUE for sugar beet of 0.050 t ha<sup>-1</sup>. Kenan and Cafer (2004) gave the value of 0.047 t ha<sup>-1</sup> for the conditions of Turkey, Kavazza (1976) the value of 0.055 t ha<sup>-1</sup> for the conditions of Italy. Based on the comparison of the I/WUE values obtained in the study period with those from the literature, it can be concluded with certainty that a cost-efficient irrigation schedule was designed which took into account the water requirement of sugar beet plants and water-physical soil properties.

The average value of the coefficient ETm/WUE for all fertilization variants was  $0.164 \text{ t} \text{ ha}^{-1}/\text{mm}$ , the actual values ranging in the interval from  $0.140 \text{ t} \text{ ha}^{-1}/\text{mm}$  in the variant with 90 kg N ha<sup>-1</sup> to  $0.179 \text{ t} \text{ ha}^{-1}/\text{mm}$  in the variant with 120 kg N ha<sup>-1</sup> (Table 2). The

average value of the coefficient ETa/WUE for all fertilization variants was 0.185 t ha<sup>-1</sup>/mm, with the actual values ranging from 0.159 t ha<sup>-1</sup>/mm in the fertilization variant with 90 kg N ha<sup>-1</sup> to 0.198 t ha<sup>-1</sup> / mm in the fertilization variant with 150 kg N ha<sup>-1</sup> (Table 2).

The higher productivity of applied water at  $T_0$  (ETa/WUE 0.185 t ha<sup>-1</sup>/mm) in relation to the values established in the  $T_1$  variant (ETm/WUE 0.164 t ha<sup>-1</sup>/mm) indicated the supplementary character of irrigation in the Vojvodina Province. In the variable climate of Vojvodina, where precipitation cannot be predicted for long term, negative effect of irrigation may occur if it is done before a heavy rain, because in that case the soil may become overwatered, and the excess water may percolate into deep soil layers taking the nutrients with it. This was the case in the rainy year of 2005. Takac et al. (2008) also emphasized the supplementary character of irrigation for conditions of Slovakia, and higher values ETa/WUE (0.115 t ha<sup>-1</sup>/mm) of sugar beet than ETm/WUE (0.098 t ha<sup>-1</sup>/mm).

#### CONCLUSION

Based on the analysis of sugar beet yield achieved under conditions of irrigation and with different doses of nitrogen fertilizer, it can be concluded that the irrigation did not significantly affect yield performance, although the root yield in irrigation was higher than that in dry farming by 9.4% or 8.01 t ha<sup>-1</sup>.

As there were no statistically significant differences in sugar beet root yield either among the variants with high nitrogen doses or among the values of I/WUE, ETm/WUE and ETa/WUE, a conclusion was drawn that the variant of nitrogen fertilization with 120 kg ha<sup>-1</sup> is adequate for both irrigation and rainfed conditions.

The higher values of ETa/WUE in relation to ETm/WUE indicated that sugar beet water use was more productive under rainfed conditions than under irrigation conditions.

The low values of I/WUE observed in some years and the higher values of ETa/ WUE than ETm/WUE in the study period indicated that irrigation in the Vojvodina Province is supplementary in character.

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# UTICAJ NAVODNJAVANJA I DJUBRENJA AZOTOM NA PRINOS I PRODUKTIVNOST UTROŠENE VODE ŠEĆERNE REPE

BORIVOJ PEJIĆ, LIVIJA MAKSIMOVIĆ, STANKO MILIĆ, MILORAD RAJIĆ

#### Izvod

Eksperimentalna istraživanja su obavljena na oglednom polju Instituta za ratarstvo i povrtarstvo na Rimskim Šančevima, na zemljištu tipa karbonatni černozem lesne terase u periodu od 2004-2006. godine. U ogledu su bile zastupljene varijanta sa navodnjavanjem (T<sub>i</sub>) i kontrolna, nenavodnjavana varijanta (T<sub>o</sub>) i četiri varijante djubrenja azotom N<sub>1</sub> 90, N<sub>2</sub> 120, N<sub>3</sub> 150 i N<sub>4</sub> 180 kg ha<sup>-1</sup>. Koeficijenti iskorišćenosti vode dodate navodnjavanjem (I/WUE, t ha-1/mm) i vode utrošene na evapotranspiraciju (ETm/WUE and Eta/WUE, t ha<sup>-1</sup>/mm) su korišćeni za ocenu efekta realizovanog zalivnog režima na prinos korena šećerne repe, odnosno za ocenu produktivnosti utrošene vode kako u navodnjavanju tako i u uslovima prirodne obezbedjenosti biljaka vodom, na raličitim varijantama djubrenja azotom. Navodnjavanje nije signifikantno uticalo na visinu prinosa korena šećerne repe ali je prinos u uslovima navodnjavanja bio veći za 9,4%, odnosno 8,01 t ha-1. Kako nisu utvrdjene statistički značajne razlike u postignutim prinosima korena šećerne repe i koeficijenata I/WUE, ETm/WUE, ETa/WUE izmedju varijanti sa visokim dozama djubrenja azotom, upućuje na zaključak da je varijanta djubrenja azotom 120 kg ha<sup>-1</sup> prihvatljiva kako za uslove navodnjavanja tako i za uslove prirodne obezbedjenosti biljaka vodom. Veće vrednosti ETa/WUE u odnosu na ETm/WUE ukazuju na produktivniju potrošnju vode u uslovima prirodne obezbedjenosti biljaka vodom u odnosu na proizvodnju šećerne repe u uslovima navodnjavanja. Niske vrednosti I/WUE u pojedinim godinama kao i veće vrednosti ETa/WUE u odnosu na ETm/WUE u periodu istraživanja ukazuju na dopunski karakter navodnjavanja u klimatskim uslovima Vojvodine.

Ključne reči: šećerna repa, navodnjavanje, doze azota, prinos, produktivnost utrošene vode.

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# INFLUENCE OF COW NUTRITION COSTS ON THE EFFICIENCY OF MILK PRODUCTION

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SUMMARY: Productivity in milk production is often low and depends on the diet of diary cows. By changing the nutrition the volume of milk produced can be increased. It is possible to achieve the same volume of milk with different rations, which, depending on their composition, have different costs. Simulating different rations with a fixed price of raw milk and applying the same calculation model on the basis of direct nutrition costs, we calculate different values of contribution margins.

*Key words: dairy production, cow nutrition, nutrition costs, contribution margins.* 

#### **INTRODUCTION**

The productivity of dairy cows (dairy production) in the RS/BiH is still low, 1.560 to 2.400 kg per animal per year (depending on the source). Besides genetics, the influence of diets on such a low dairy production is significant as well. The rations the cows receive are quite monolithic and do not contain all necessary nutrients that would be inevitable to exploit the production capabilities of the existing cattle.

Roughages represent the basic feed in cattle nutrition. For cows with a high genetic potential, not all needs can be met with roughages, but it is necessary to add concentrated feed and vitamin and mineral supplements (Ševković et al., 1983).

Among roughages, the most significant ones when it comes to cow nutrition are the following: green forage, hay and silage.

Pasture is the most economic way of using the green supply. The animals graze and in doing so, there are no expenditures relating to picking and transporting green supply, whereas the losses of nutritive matters are kept at a minimum. However, when stored, significant nutritive matters are lost, 27-35% in unfavourable conditions (Čobić i Antov, 1996; Cilev et al., 2007). Silage is very important in dairy cow nutrition and

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represents a special way of food conservation with the aid of lactic acid bacteria. In cow nutrition, maize silage is most significant. As for concentrated feed in cow nutrition, maize plays the most important role, other wheat is less significant, followed by rapeseed, by-products of the milling industry etc.

As for the season and availability of specific roughages, we distinguish between winter and summer rations. Green supply is the basis of the summer feed, and the goal of nutrition is to let the cattle graze als long as possible, i.e. to eat green forage, which at the same time is the cheapest solution. In winter hay and silage represent the basic nutrition, depending on the area and tradition of conserving roughages or a combination of both.

The production period of a cow can conditionally be divided into several phases: beginning, middle phase and end of lactation as well as dry period. In ideal conditions this cycle should last exactly one year. However, in practice this always takes a bit longer.

The breed structure of diary cows in our country is distributed quite unevenly. The Simmental is most common (Čobić and Antov, 1996). This is a breed with double properties, both for milk and meat production, and significant characteristics are a rapid growth of young animals, harmonious build, a good adaptibility, good use of roughages etc. The average milk production of this breed according to Čobić (Čobić and Antov, 1996) hovers around 5,000 kg.

Data on a controlled dairy cow herd of the Simmental breed from Mrkonjic Grad area served as basic material for this paper (Važić et al., 2005).

Since there is still no system for following production results of dairy cows on small farms, a model has been created that deals with the impact of different diets on the amount of milk in a hypothetical manner, so as to make sure that the simulated results reflect the real situation as realistically as possible. This has been made possible thanks to the knowledge of relations of cause and effect between various diets (balancing of feeds) and milk production of the diary cows, as well as the knowledge of production and market prices of various kinds of cattle feed.

#### MATERIAL AND METHODS

The starting point for the analysis was the regular feed ration dominating cow nutrition, called the traditional feed ration. A ration of this kind is based on pasture in summer and predominant proportion of hay during winter time, with a minimum of added concentrated feeds (maize). Such a diet leeds to a production result (milk production) of 3,167 kg, which has been established by the experiment conducted by Važić (Važić et al., 2005) and indicates that the herd's genetic potential has not been sufficiently exploited.

This feed implies three other rations that are based on several varying feed components in proportions that can offer an annual milk production of 5,000 kg for 305 days of lactation for the original genetic potential. All three recommended feed rations securing a better use of the animals' genetic potential possess the same nutrititive value achieved by a combination of 5-7 different components of feed rations. The original assumption, that a better diet secures a higher milk yield, has also been confirmed by some earlier researchers (Paleševski et al., 2007), whereas some researchers focussed on the monitoring of changes in milk quality depending on the diet (Đorđević et al., 2007). All feed rations are divided into summer and winter rations, and the consumption of single components has been projected for two periods – lactation period (305 days) and dry period (60 days). The lactation period is divided into two sub-periods – the first 100 days and the following 205 days.

The balance of the feed rations is such that they completely meet the daily needs of the cows in relation to consumption of dry matter, energy, proteins, mineral matters and vitamins. The daily needs of the cows have been calculated on the basis of standards for ruminants (Obračević, 1990 i NRC 1989). The nutritive value of specific feed used to balance the rations have been taken over from tables of cattle feeds' nutritive values (Obračević, 1990). Since in case of each single animal the phases of dry periods and periods of lactation can occur during different seasons, an ideal distribution was assumed, meaning that  $\frac{1}{2}$  of the feed ration consists of feeds during winter, and the second  $\frac{1}{2}$  of feed rations during summer time.

In order to transform the amounts of feed into cost, the projected amounts of necessary feed have been multiplied with the prices of single components. As for the prices, current market or production prices were combined, depending on the character of specific feedstuffs, or rather the possibility of its production on the farm itself or its purchase.

The project-related consumption of single feed components of dairy cows and the prices of these components led to the total feed costs, or rather costs for the diet of dairy cows. As for their character, the diet costs are direct and variable. Proceeding from this fact, the costing of milk production has been made by applying calculations on the basis of incomplete (direct) costs (Andrić, 1998; Berberović and Jovičić, 2007).

The production value has been established based on the production volumes of milk and its selling price. The price comprises the purchase price paid by the processor (dairy factory) and the premium paid by the ministry. The average milk price that dominated at the moment of calculation has been used, i.e. 0.54 KM/kg (purchase price) +0.20 KM/kg (premium of the ministry). The fixed exchange rate for the KM is 1 KM = 1.955 EUR.

By deducting variable feed expenditures from the value of milk producers, we get the remainder, called "contribution margin" in economic terminology. This is actually the remaining value of milk production, supposed to cover all other, mainly indirect, fixed costs. In this case we are talking about all costs except feed costs for dairy cows.

In general, small dairy farmers do not calculate with fixed costs (depreciation of cow/basic herd, stable, mechanisation) and their labor costs, so that the method of calculation based on direct costs is a convenient method to (halfway) calculate their efficiency in milk production. Practically, the (interim) result established this way represents the remainder of milk production value that the producer combines (associates) with the remaining revenues from other productions, and from this money supply defrays common farm and other costs of his houshold.

#### **RESULTS AND DISCUSSION**

The model analysed examines the influence of 4 different cow feed rations through feed expenditures on milk production efficiency. The so-called direct costing method was applied, i.e. the calculation of milk production costs on the basis of direct (variable) costs. Each feed ration has cost of its own, depending on its structure and the price

of single components. The shaping and valorisation of single input parameters of the model led to the results illustrated in the table below.

| Milk production line      |       | Feed<br>1 | Feed<br>2 | Feed<br>3 | Feed<br>4 |
|---------------------------|-------|-----------|-----------|-----------|-----------|
| Milk production volume    | kg    | 3.167     | 5.000     | 5.000     | 5.000     |
| Selling price of the milk | KM/kg | 0,74      | 0,74      | 0,74      | 0,74      |
| Milk market value         | KM    | 2.344     | 3.700     | 3.700     | 3.700     |
| Variable feed costs       | KM    | 1.084     | 1.133     | 1.243     | 1.046     |
| Contribution mannin       | KM    | 1.260     | 2.567     | 2.457     | 2.654     |
| Contribution margin       | KM/kg | 0,40      | 0,51      | 0,49      | 0,53      |

Table 1. Contribution margin in the application of four different cow feed rations

The highest contribution margin, i.e. the remainder after the deduction of feed costs, is represented by the fourth, and the lowest by the first feed. A reason for this is not only feed expenditures, but also the milk yield, which in case of the first feed is lower due to the poor nutrition value of that feed ration.

This simulation was meant to show that the logic still behind the methods of most of the milk producers, i.e. that the 1<sup>st</sup> feed – minimizing feed expenditures and maximum reliance on cattle feed produced on their own farms (pasture and hay), with minimized purchase of feedstuffs, leed to the lowest efficiency of their milk production in economic and financial terms.

A further calculation reveiled that the annual fixed costs (depreciation of the basic herd and stable, electricity and water costs, artificial insemination, veterinary services and animal insurance) would amount to 8,575 KM for a farm of 10 dairy cows. This amount does not include any expenditures for labor force, since the farm owner and his family members usually perform additional tasks and receive other revenues. The compensation for their work is actually part of the total profit achieved. A further analysis for a farm of 10 dairy cows offers the following financial result.

| Milk production line         |            | Feed<br>1 | Feed<br>2 | Feed<br>3 | Feed<br>4 |
|------------------------------|------------|-----------|-----------|-----------|-----------|
| Cartailation manain          | КМ         | 1.260     | 2.567     | 2.457     | 2.654     |
| Contribution margin          | KM/kg      | 0,40      | 0,51      | 0,49      | 0,53      |
| Capacity unit (herd)         | animals    | 10        | 10        | 10        | 10        |
| Contribution margin per herd | КМ         | 12.600    | 25.670    | 24.570    | 26.540    |
| Total fixed costs            | КМ         | 8.573     | 8.573     | 8.573     | 8.573     |
| Profit                       | Per farm   | 4.027     | 17.097    | 15.997    | 17.967    |
| Profit                       | Per animal | 403       | 1.710     | 1.600     | 1.797     |

Table 2. Profit from milk production for a farm of 10 dairy cows

All producers make a gain, but those who have a lower milk production and apply the traditional diet, generate the lowest profit. Other producers, who thanks to a better optimization of feed rations generate an annual milk production of 5,000 kg per animal, generate revenues of 1,600 to 1,797 KM per animal. Since the labor costs are not included in the fixed expenditures, the compensation for the work of one or more members of the household who take care of the herd is contained in the realized remainder

of the gain (336-1,497 KM per month). Also other researchers (Popović and Jovanović, 2007) came to a similar conclusion; they established the marginal profitability of milk production based on full expenditures by varying the number of cattle in the basic herd and arrived to the conclusion that in case of a milk production of 4,500 litres per cow a profit can exclusively be generated with a herd of more than 30 cows.

The next step of the analysis makes assumptions as to what would happen if we suspended the payment of premium for milk, while the purchase price paid by milk processors would remain unchanged, meaning 0.20 KM less? In that case the above calculation would change to a certain degree.

In case of identical feed expenditures, the contribution margin would decrease in all four groups of milk producers, due to the lower selling (purchase) price. However, those who thanks to a better diet generate an annual milk production of 5,000 kg per animal, have a reserve to withstand this price cut, so that they still have sufficient revenues left for covering fixed costs of the farm and keeping a profit per animal of 600 to 797 KM per year. The only group who suffered a loss is the first group of producers (-231 KM per animal per year), or rather, they cannot cover the total fixed charges from contribution margins.

This analysis is indicative when it comes to considerations whether it is necessary that ministries still earmark significant means in order to pay premiums for milk production (e.g. in the Republika Srpska in 2008 ¼ of the agricultural budget was spent only for this purpose), and how high the premium should be? The answer is that the addition of a premium to the purchase price of milk is necessary as long as the number of milk producers is being dominated by those who in this analysis are represented by the traditional feed ration. The other answer lies in one of the goals of RS Agriculture Development Strategy, being a radical change of quality of primarily roughages and in animal nutrition in general. When the first group of producers "move" with their approach to diary cow nutrition to the second, third or fourth, the necessity of subsidizing their insufficient milk production will cease as well.

#### CONCLUSION

In the model created, the total amount of milk has been valorized as gain, supposing that all the milk produced would be sold. The feed costs have been treated as direct, variable costs, and all other costs as fixed costs. The influence of three different feed rations was examined, with the same nutritive value. These recommended feed rations replace the traditional feed, which still dominates in most of the rural estates. 5-7 feed components varied in the rations in proportions that can secure an annual milk production of 5,000 kg in 305 days of lactation for the original genetic potential of the Simmental breed.

Calculating production costs on the basis of incomplete costs (direct costing method) led to the results that all 4 feed rations ensure a positive contribution margin ranging between 1,260 and 2,654 KM per animal per year, or rather 0.40 - 0.53 KM/kg of milk. If we extend the analysis to a farm model of 10 dairy cows with fixed annual expenditures of 8,573 KM, the profit per farm ranges between 4,027 KM and 17,967 KM (403 - 1,797 KM/animal per year).

The above profit assumes a purchase price of milk of 0.74 KM/kg, comprising also the premium of the ministry of 0,20 KM/kg. Without this premium (purchase price

of 0.54 KM/kg) the efficiency of milk production (profit) decreases. The first group of producers who apply the traditional feed and achieve an average milk production of 3,167 kg per animal per year do not generate a margin sufficient to cover all fixed expenditures (a loss of 2,313 KM per year). Other producers who achieve a milk production of 5,000 kg per animal due to a better diet, generate a profit between 5,997 and 7,967 KM per year per farm of 10 diary cows. This analysis shows that in current conditions of low productivity (milk production) and with the current subotpimal diet of diary cows, it is necessary to continue paying premiums for cow milk production, because only in doing so, the majority of small producers generate profits in their production, or rather manage to realize sufficient profits to cover both variable and fixed costs, whereas they keep a minimum difference as a compensation for their whole-year work.

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# UTICAJ CENE KOŠTANJA ISHRANE KRAVA NA EFIKASNOST PROIZVODNJE MLEKA

# ŽELJKO VAŠKO, MILANKA DRINIĆ

#### Izvod

Produktivnost u proizvodnji mlijeka je često niska i zavisi od načina ishrane muznih grla. Izmjenom načina ishrane krave može se povećati proizvedena količina mlijeka. Istu količinu mlijeka mogu obazbijediti različiti hranidbeni obroci koji, zavisno od njihovog sastava, imaju različite troškove. Simuliranjem različitih hrandibenih obroka uz fiksnu cijenu sirovog mlijeka i korišćenje modela kalkulacije na bazi direktnih troškova ishrane izračunavaju se različite vrijednosti marže porkića preostalih troškova.

Ključne riječi: mliječnost, ishrana krava, troškovi ishrane, marža pokrića.

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# ANALYSIS OF ECONOMIC EFFECTS OF THE BASIC FIELD CROPS PRODUCTION ON FAMILY FARMS\*

VELJKO VUKOJE, TODOR MARKOVIC<sup>1</sup>

SUMMARY: This paper analyzes the most significant parameters of cost effectiveness of wheat, corn, soybean and sunflower production on privately owned farms in the Autonomous Province of Vojvodina. The soybean production makes the highest gross margins per unit of the capacity (55.615 RSD/ha), as well as the best relation between the production value and the overall investment (profitability coefficient of 2.31). Soybean is followed by wheat (49.420 RSD/ha; coefficient of 1.93), sunflower (46.554 RSD/ha; 1.83) and corn (27.235 RSD/ha; 1.31) Sensitive analysis shows that the observed productions can sustain a significant decrease of yield and/or market prices (corn for about 46%).

*Key words:* Field crop production, analysis, private farms, economic results.

#### INTRODUCTION

The Republic of Serbia possesses very significant natural, human and technical and technological resources for agricultural production. With around 0.56 ha of arable land per capita, our county is above the average of the EU countries. A long tradition, great experience as well as remarkable knowledge obtained within the domain of selection and agricultural machinery represent important prerequisites for advancing this branch of economy to a much higher level. The Province of Vojvodina is an area with extremely favourable natural conditions for development of field crops as well as the total agricultural production.

According to the basic capacities it possesses (over 85%) and the production value it realizes (over 80%), the section of privately owned agricultural farms is extremely dominant compared to agricultural firms and associations. This sector was neglected

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in domestic agro-economic researches for a long time, compared to large agricultural firms. As our country has approached the final phases of property transformation process and has gradually been approaching EU membership, the family farms have been positioned in the centre of interest, thus taking the place where they really belong to.

#### MATERIAL AND METHODS

This paper provides a comparative analysis of the most significant parameters of cost effectiveness of wheat, corn, soybean and sunflower production. This analysis has been conducted according to analytic calculations on family farms for the year 2008. Within their regular activities, extension service consultants made calculations of all important field production, which were subsequently submitted to the Department of Agricultural Economics, at the Faculty of Agriculture, Novi Sad. At the Department agricultural and economic analyses were made for the needs of the Extension Service of AP Vojvodina. The sample includes 26 family farms from the area of Vojvodina. Only representative producers dealing with intensive production were chosen.

The original calculations by the extension service consultants were based on the calculation of direct variable costs, realized production values and gross margin, as the basic result. With the purpose of gaining a complete insight into profitability, the additional indicators of success have been determined (total costs, profit, overall price of cost, efficiency coefficient and the profit rate).

By using the method of sensitive analysis, the movement of the achieved results compared to the yields and/or market prices changes for products +/-20% has been analyzed.

#### **RESULTS AND DISCUSSION**

The problems of providing reliable data have represented great limitations for implementation of comprehensive agricultural and economic analyses on individual farms. In our country there is no regulation which would enforce bookkeeping to the family farms owners, except for those included in VAT system, which makes a small number at the moment. Also, there is no system of regular collection of economic data such as FADN system (Farm Accounting Data Network) in EU countries (Vukoje and Maletic, 2007). Besides, the agricultural producers are traditionally suspicious and not ready to share the data related to the business results of their farms, especially those related to economic data.

However, certain measures have been conducted in order to establish the system of surveillance of economic indicators of family farms. As a result of the cooperation between the Faculty of Agriculture of Novi Sad and the Extension Service of AP Vojvodina, a methodology and software for collection of basic "production and economical indicators on the farms" have been created (Vukoje and Koci, 2004). This model has been operating for 3 years in agricultural stations which are the basic supporters of extension work on the territory of Vojvodina.

The calculations on which analyses in this paper are based are the results of this model. After generating original calculations by consultants, the calculations were checked, and subsequently, average calculations of wheat, corn, sunflower and soybean

Table 1. Average calculations: Wheat, Corn, Soybean and Sunflower, Area 1 ha:Year 2008.

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Tabela 1: Prosečne kalkulacije: Pšenice, Kukuruza, Soje i Suncokreta, Površina 1 ha;2008. godina

|            |  |  | Weat / <i>Pšenica</i>     | šenica | Corn / Kukuruz            | ukuruz | Soybean / Soja            | 1 / Soja | Sunflower/<br>Suncokret   | wer/<br>kret |
|------------|--|--|---------------------------|--------|---------------------------|--------|---------------------------|----------|---------------------------|--------------|
|            | COSTS  | COSTS / TR O ŠK O V I                              | Amount/<br>Iznos<br>(RSD) | (%)    | Amount/<br>Iznos<br>(RSD) | (%)    | Amount/<br>Iznos<br>(RSD) | (%)      | Amount/<br>Iznos<br>(RSD) | (%)          |
| 1.         | 1. Seed / Seme   |  | 7543                      | 20.8   | 8399                      | 18.5   | 4420                      | 14.5     | 4314                      | 11.5         |
| 2.         | 2. Manure / <i>Đubrivo</i>                                 |  | 12330                     | 34.1   | 15255                     | 33.6   | 6154                      | 20.3     | 8677                      | 23.2         |
| 3.         | 3. Plant protection chemicals / Zaštitna sredstva          | edstva   | 1509                      | 4.2    | 4278                      | 9.4    | 6743                      | 22.2     | 5125                      | 13.7         |
| 4          | 4 Sources of energy / Energenti                            |  | 5703                      | 15.8   | 7165                      | 15.8   | 6467                      | 21.3     | 6294                      | 16.8         |
| 5.         | 5. Other materials / <i>Ostali materijal</i>               |  | 550                       | 1.5    | 0                         | 0      | 0                         | 0        | 0                         | 0            |
| I)         | I) Material costs / <i>Troškovi materijala</i>             |  | 27636                     | 76.4   | 35096                     | 77.3   | 23784                     | 78.3     | 24411                     | 65.1         |
| 6.         | 6. Direct services / Direktne usluge                       |  | 6584                      | 18.2   | 7665                      | 16.9   | 4297                      | 14.1     | 11038                     | 29.4         |
| 7.         | 7. Labour cost / <i>Troškovi rada</i>                      |  | 1972                      | 5.4    | 2656                      | 5.8    | 2301                      | 7.6      | 2033                      | 5.4          |
| <b>A</b>   | A) Variable costs / Varijabilni troškovi ( $\Sigma$ 1 to7) | 1 to7)   | 36192                     | 100    | 45418                     | 100    | 30383                     | 100      | 37482                     | 100          |
| Achieve    | Achieved results / Ostvareni rezultati                     |  |                           |        |                           |        |                           |          |                           |              |
| 8.         | 8. Yield / Prinos (t / ha) Vari                            | Variable costs / Varijabilna cena koštanja (RSD/t) | 5.53                      | 6550   | 7.92                      | 5737   | 3.10                      | 9790     | 3.13                      | 11962        |
| 9.         | 9. Production value / Vrednost proizvodnje                 | 0)   | 77612                     |        | 64653                     |        | 77997                     |          | 76036                     |              |
| 10.        | 10. Income from subventions / Prihodi od subvencija        | ubvencija  | 8000                      |        | 8000                      |        | 8000                      |          | 8000                      |              |
| <b>B</b> ) | B) PRODUCTION VALUE / VREDNOST                             | VREDNOST PROIZVODNJE (8 -10)                       | 85612                     |        | 72653                     |        | 85997                     |          | 84036                     |              |
| Û          | C) GROSS MARGIN / BRUTO MARŽA (B - A)                      | (P - 8   | 49420                     |        | 27235                     |        | 55615                     |          | 46554                     |              |
| Addition   | Additional indicators / Dodatni indikatori:                |  |                           |        |                           |        |                           |          |                           |              |
| 11.        | 11. Interest on Working Assets / Kamata na c               | ' Kamata na obrtna sredstva                        | 1629                      |        | 2044                      |        | 1367                      |          | 1687                      |              |
| 12.        | 12. Fixed costs / Fiksni troškovi                          |  | 6515                      |        | 8175                      |        | 5469                      |          | 6747                      |              |
| D          | D) Total costs / Ukupni troškovi (A+11+12)                 |  | 44335                     |        | 55636                     |        | 37219                     |          | 45915                     |              |
| E)         | E) PROFIT / DOBIT ( B - D)                                 | Full costs / Puna cena kostanja (RSD/t)            | 41277                     | 8024   | 17016                     | 7028   | 48778                     | 11993    | 38120                     | 14654        |

production were made (Table 1).

Gross margin (the margin pay, contribution margin, margin results, etc.) represents the difference between the production value and variable costs. It can be calculated on several levels, i.e. with different level of inclusion of variable costs. In calculations, almost all direct variable costs were included, including the costs of machinery maintenance, external production services, insurance, storage, etc (presented within the "direct services").

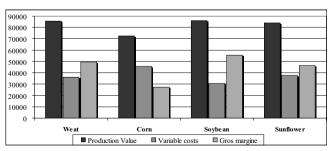
Fixed costs (depreciation, fixed costs of maintenance, interest and insurance of permanent property, appertaining general costs on a farm – electricity, water, taxes, heating, phone, travelling costs, intellectual services, etc), are covered from the overall gross margin of the farm.

Fixed costs of the farm are mostly permanent within a short period of time, i.e. they cannot be avoided considerably. This is why the gross margin is a more reliable indicator of success than profit, because the arbitrariness of the fixed costs distribution on certain productions can be thus evaded by using more or less imprecise keys.

Interest and fixed costs were not given in original calculations, but they are subsequently determined by the regular methodological procedure (Tab. 1, row 11). The interest is calculated only for loaned assets used for acquisition of working assets. Interest calculated for the loans for fixed assets represents the fixed costs of exploitation of these assets. It has been assumed that a farm finances a half of working assets from the loaned sources, and that the rate of interest amounts 9% (for wheat: 36.192; 2x 9%= 1.629 RSD). Fixed costs are approximately 18% of the amount of variable costs. Their precise calculation cannot be done without the bookkeeping records of the farm.

The comparative analysis of **the variable costs structure** shows the prevailing share of the costs of manure for wheat (34.1%) and corn (33.6%). In the soybean production, the most significant costs are the costs for plant protection chemicals (22.2%) while in the sunflower production those are the direct production services – primarily harvest, transportation, sowing, etc (29.4%). The position of the "direct service" includes a wide spectrum of costs and it represents a significant item in all productions (over 14%). The fuel costs share is unsurprisingly high, and it amounts between 15.8% for wheat to 21.3% for soybean. Since these are field crops, it is logical that labour expenses are not significantly high (5.4-7.6%).

The highest investment of variable factors of production (Chart 1) has been shown in corn production (45.418 RSD /ha) which is 21.2% higher than for sunflower (37.482 RSD/ha) and 49.5% higher than for the soybean (30.383 RSD/ha).



Graph. 1. The production value, variable costs and gross margin (RSD/ha) *Graf. 1. Vrednost proizvodnje, varijabilni troškovi i bruto marža (din/ha)* 

Apart from the yield market value, **production value** also includes all the subventions, i.e. it represents the overall achieved production profit. The model presumes that the products used for own consumption (semi-products) are calculated per their cost price. In these specific cases of the selected farms, the accessory products of the examined productions have not been used, so they do not contribute to the production value. The highest production value per capacity unit (1 ha) has been achieved in the soybean production (85.997 RSD/ha), while the lowest one has been realized with the production of corn (72.653 RSD/ha). As these are different productions, the comparison of the yields per 1 ha is not possible, and neither is it possible to compare the cost prices per unit of the product (shown as variable and full).

Soybean has also the highest gross margin per capacity unit (55.615 RSD/ha) which is about 2 times higher than with corn, while wheat and sunflower achieve approximate values of this result (46.762 RSD/ha, and 46.554 RSD/ha, respectively). Taking into the account the above stated indicators, it is logical that the soybean production gives the highest profit (48.778 RSD/ha).

The last two indicators (efficiency and profitability of products) make the so-called **relative indicators.** They are expressed in the form of coefficients, i.e. percents, and enable the efficient comparison of different productions. The efficiency coefficient shows that each dinar (RSD) of the overall costs in soybean production realizes 2.31 RSD of the production value (1.93 for wheat, 1.83 for sunflower, and 1.31 for corn). These can be regarded as very good indicator values, especially for the first three crops.

The profit rate, i.e. the profitability yield rate indicates that each 100 dinars of soybean production value provides 56.72 dinars of profit, which is also a very good indicator (wheat 48.21 dinars, sunflower 45.36 dinars and corn 23.42 dinars).

**Sensitive analysis** shows the movement of gross margin related to the change of product yield and sale price for +/-20%. It measures the sensibility of the results to variation of the key physical and financial parameters, and it is of special importance for the process of planning. Table 2 shows the movement of margin in relation to the cumulative variation of yield and /or market prices.

|                       |       | change and/or market price<br>omena prinosa i/ili tržišnih |       |  |  |
|-----------------------|-------|--|-------|--|--|
|                       | -20%  | Realized /Ostvareno  | +20%  |  |  |
| Wheat / Pšenica       | 33898 | 49420  | 64943 |  |  |
| Corn / Kukuruz        | 14305 | 14305 <b>27235</b> 40166                                   |       |  |  |
| Soybean / Soja        | 40015 | 55615  | 71214 |  |  |
| Sunflower / Suncokret | 31347 | 46554  | 61761 |  |  |

Table 2: Movement of gross margin in relation to the yield and market prices changes (RSD/ha) *Tabela 2. Kretanje bruto marže sa promenom prinosa i tržišnih cena (RSD/ha)* 

It is evident that the margin pay is not questionable with the examined productions. For the least profitable production, i.e. corn production, the margin becomes negative only with the overall decrease of yield or/and market price for about 46%.

#### CONCLUSION

The results of the research show that there are significant differences among the examined productions, related to the height of the gross and net success indicators. According to all the indicators, soybean is clearly distinguishably the most profitable in production. It accomplishes the highest gross margin of 56.615 RSD/ha per the capacity unit (wheat 49.420 RSD/ha, sunflower 46.554 RSD/ha and corn 27.235 RSD/ha). The level of necessary current investments can often be a limiting factor for the intenseness of farm production. Soybean requires the lowest investment per 1 ha (37.219 RSD/ha), and at the same time it provides the highest profit per unit of investment amounting 131 RSD per 1 RSD of the overall costs (wheat 93 RSD, sunflower 83 RSD and corn 31 RSD).

Despite the relatively good yield in the observed year, corn production has realized the worst but still sufficient level of economic results (7.92 t/ha). In our conditions it is very difficult to give general evaluations or long-term predictions of profitability of certain productions due to their dependence on climate, and especially market conditions which fluctuate very much and are unpredictable. Decreasing these risks and gradual increasing of subventions, as well as providing cheap sources of financing will contribute to accelerated development of the whole agricultural sector.

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# ANALIZA EKONOMSKIH EFEKATA PROIZVODNJE VODEĆIH RA-TARSKIH USEVA NA PORODIČNIM GAZDINSTVIMA

## VELJKO VUKOJE, TODOR MARKOVIĆ

#### Izvod

U radu se analiziraju najvažniji parametri isplativosti proizvodnje pšenice, kukuruza, soje i suncokreta, na privatnim gazdinstvima AP Vojvodine. U proizvodnji soje se ostvaruje najveća bruto maržu po jedinici kapaciteta (55.615 din/ha), kao i najbolji odnos vrednosti proizvodnje i ukupnih ulaganja (keoficijent ekonomičnosti 2,31). Potom slede pšenica (49.420 din/ha; koef. 1,93), suncokret (46.554 din/ha; 1,83) i kukuruz (27.235 din/ha; 1,31). Senzitivna analiza pokazuje da posmatrane proizvodnje mogu podneti značajno smanjenje prinosa i/ili tržišnih cena (kukuruz za oko 46%).

Ključne reči: ratarska proizvodnja, privatne farme, analiza, ekonomski rezultati.

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# ORGANIZATION OF THE NATIONAL WATER MANAGEMENT SYSTEM

### MARKO BAJČETIĆ<sup>1</sup>

SUMMARY: Water Management in the state is a complex issue because it reflects a number of factors and influences. These factors and influences entail various proprietary and ownership grounds and relations, technicaltechnological characteristics of water, facilities and other resources and economic specifics of goods, income and assets. The complexity of the elements and the system requires the construction of an autonomous and coherent system which functions in order to satisfy the interests of the population, economy and other users. Satisfying the users' needs implies the provision of clean and sufficient water, and especially the protection from water. In different segments of water management the interests and needs of users are met by different hierarchically set organizations. Each organization must establish a system that is developing and realizing positive results. The systems are in the form of directorates and agencies for water, public water enterprises, scientific research institutions, insurance entities and public and private legal entities and individuals.

*Key words*: water management, directorate, agency, company, unique and decentralized system.

#### INTRODUCTION

The organization of water management is complex because it reflects parts of many systems in society and elements of the economy that are related to different factors and methods of water management. Various entities are involved in water management, from individual users to government institutions which require the adaptation of the water activity and institutions to natural conditions and socio-economic relations (Vlada RS, 2002.). The management of water and the water activity is also a complex system because it responds to a number of different influences, pressures and demands from the dynamic environment. This requires an arrangement of units in the manage-

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ment of water and the water activity. Furthermore, the environment, water institutions and organizations are at the same time recipients and suppliers of various inputs and outputs.

#### METHODOLOGY

The research in the paper is set so that the analysis of areas and segments of water management contributes to the improvement of the organizational system of water management and the water activity. This required defining the use of water, water services and other activities in the protection against the harmful effects of water, the protection of water, use of water and protection of the environment and surroundings. The importance of the research required the application of the historical method to compare different concepts of attitudes towards water and the water activity up to the modern ones, developed from the EU Framework Directive on Water (Ministarstvo, 2005).

Establishing a rational organization is not possible without the application of the methods of induction and deduction or the determination and establishment of systems with individual and generic elements. Individual elements display characteristics that the system is composed of, not by removing but by applying the general attitudes and opinions.

The research material and results are also the consequences, or theoretical-practical effects, of the years of work of the author on managerial business affairs in the water activity of Vojvodina and Serbia. The methods applied in the research are goal determined for reexamining the fundaments established from transitional and integration processes.

#### WATER MANAGEMENT AND ORGANIZATION

The organization system starts from the functioning of the water activity in certain areas, or the characteristics of the area on different levels of jurisdiction. The levels of jurisdiction in water management start from the interests of the state, the interests of joint and collective users and the population (Wilcox i sar., 2007), over to the holders of management of the water system. Public (state) and market interests (Stiglitz, 2004), are achieved at different levels in the river basin and water area under the influences and requirements for the improvement of water management and elimination of risks. Each holder of water management has an obligation in:

- The determination and implementation of policies and strategies in the management of water and the water activity that evolve into plans and actions for support in decision-making from elements of a developed water and business information system;
- Focusing human resource management towards a planned improvement of the role of employees in accomplishing objectives by developing quality relationships between the employees in organizations;
- 3) Planning and monitoring water resources in space and time for specifically targeted quantities and quality of water and changes for manageable and implementation of specific measures for unmanageable water regimes. Water resources require different procedures, technology, technical systems and other, with results

that can be compared to the planned;

- Planning, monitoring and effectiveness of the use of resources contained in income, financial assets, fixed and current assets, input and output (Potkonjak, 1991), property, etc.;
- Identifying, reexamining and improving the process of providing and performing services, particularly through demonstrated initiative and creativity of employees of different specialties (Bajčetić, 2008);
- 6) Fulfillment of user satisfaction, which is expressed by a positive assessment of the achieved quality and quantity of water, especially the safety from water and the water service provided;
- 7) Acceptance of the assessment on the impact of the water activity on the development of the local and regional community;
- 8) The realization of public and private interests in measurable individual and overall results of the water activity (the capacity of the water facilities and systems and the scope of services);
- 9) Providing conditions for employees to achieve social, water, entrepreneurial and personal goals.

The system approach (Djordjevic, 1990) to the establishment of an organizational form allows for the assessment of individual elements and segments that can be linked into a harmonious and complete whole. Each of the wholes has goals that are based on water principles (integrality, vigilance, flexibility, efficiency, etc.) and norms (technical, economic, etc.), and in institutional and organizational relationships for achieving public and private interests.

Holders of the allocation and use of water, provision of water services and other activities in the policy for protection against the harmful effects of water, protection of water, use of water and preservation of the quality of the environment, must go through specific and planned organizational changes. The main directions of change are conditioned by the status and role of the ratepayers and users from which the organization (Janicijevic, 2007) of the water activity requires:

- Firstly, the process of deconcentration of resources and decentralization of management of water services in a unique system of water management on the river basin.
- Secondly, the inclusion of the elements of competition for water services in conditions of relations between public and private corporations in the water activity.
- Thirdly, institutions and organizations in water management should not be exclusively based on a bureaucratic (administrative) basis, but should be guided by a mission and directed vision towards real effects (outputs), or in other words be less focused on entry documentation that is currently dominant.
- Fourthly, the transition from a system of command to a system of cooperation, less administration and greater application of new methods in coordinating and strengthening the public sector and managerial functions. For too long has administration in water management been unknown and hidden. It gives off an impression of functional discontinuity without mutual relations and influences of individual units on the total result.
- Fifthly, the deregulation of the system which is realized through the adoption of strategies, defining a mission and clear vision on the principle of new public and business management, according to a code of conduct, ethical principles and

standards that are transformed into norms of laws, decrees, orders, conventions, etc.

- Sixthly, the changes in the relationship of holders of management of water and water services towards the ratepayer and user that was manifested in bureaucratic behavior. Bureaucratic behavior holds slowness, arbitrariness and formalism which create discontent in the ratepayer and user. In new conditions, the relationship should be towards a valued and desired ratepayer and user, with a tendency to expand the range of users through the quality of services.
- Seventhly, the changes in the water activity in the future, for which it is necessary to predict events and determine methods for preventive action.
- Eighthly, the creation and development of entrepreneurial orientation (Penezić, 2005) into a winning individual and collective interest of public and water companies (enterprises).
- Ninthly, the application of developed information technologies in adequate and complete information systems. This application should create the conditions and basis for the improvement of management, establishment of functions, realization of business objectives, implementation of strategies and tactics for mass phenomena and events, static and dynamic elements and processes, unique decision-making and interaction in a hierarchical structure of government, etc.
- Tenthly, the constant review of priorities, scope and targeted results in organizations by areas and segments of water management connected into a unified system.

# ORGANIZATION IN WATER MANAGEMENT

The organizational model in water management can be different and depends on the set goals in the socio-economic system, excess or shortage, purity or pollution of water, size and structure of the water infrastructure network, the user layout, etc. The model of organization must take into account:

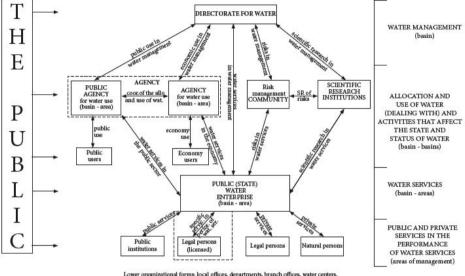
- 1) The configuration of the water management system of the state where each of the segments and each of the areas are specific;
- Interconnection of the elements of the allocation and use of water, water services and other activities for determining the beginning of the procedures and ways of linking and merging into a single system of water management (Ministarstvo, 2005);
- 3) Quality standards (Vulanović i sar., 2003), which enable compatibility of various elements in the system for achieving strategic objectives in the balance of water, especially in the use of potential or built-in capacities for the provision of water services. Standards are partially and functionally determined by the requirements (needs) of the users.

In the organizational forms of water management it is important to separate the functions of management from ownership and property rights. The functions of management are related to the processes of planning, management, control, analysis and information (Duričin, Janošević, 2007) which each of the segments contains in the case of action. Ownership and property rights refer to the rights of management, allocation and use that are different for water, the facilities and systems.

The institutionally most important entities in the organization of the water activ-

ity are the Directorate for Water, the agency (s) for water and a public enterprise. Apart from them, also important are the scientific-technical institutions, and those whose subjects of operations are risks, water companies and other legal and natural persons.

A possible model of organization of water management in the state is given in the following illustration:



Lower organizational forms: local offices, departments, branch offices, water centers, internal directorates for certain functions, business units, sectors, services, divisions

# CHARACTERISTICS OF THE ORGANIZATIONAL MODEL OF WATER MANAGEMENT

The given organizational structure is based on the stated facts and structure of the subjects in the water activity with activities in the measures and instruments that are required by transition and integration processes. The transition processes determine the relations contained in the public and private ownership, management, and economic characteristics of goods for specifically targeted states and statuses of water, sources of funds, revenues, expenses and profits. The integration processes are in accordance with those contained in the EU directives on water for a unique water management in river basins.

- A possibility of application on the water area of the state because it includes all areas and segments of water management, river basins, water areas and water systems;
- Takes into account the uniqueness of the system that reflects the hierarchical relations in water management from general to individual activities;
- 3) The model can be established and applied in a relatively short period of time with the engagement of the existing human resources of the staff;
- 4) There is the possibility of rapid adaptation to transitional changes that occur in the public and real sector;
- 5) Orientation towards integrated management on the river basin (EU, 2004);

- 6) Identifies the priorities of water management, especially the relationship between the leading organizational units;
- Reflects the complexity of the elements (Stojanović, 2008) and the system for various processes in water management;
- 8) It is conditioned by the strengthening of the integration of water entities and particularly with the environment;
- 9) Focus of the organization towards specific goals of water management in river basins, which are balanced with the goals of the state by eliminating duplication and inaccuracies in the jurisdiction and authority.

A special issue in the organization of water management refers to the hierarchy of a specific degree and tasks for achieving optimal relations in the allocation and use of water, and especially in water services that are a function of time and space. A hierarchical relationship implies that the organization forms collective goals at different levels, whose realization is based on the division of labor in a unique water management. An organization in hierarchical coordination implies relations of the subjects from the water activity and the state. The relations are directed towards the activities of allocation and use of water through arrangement by types of processes, functions and elements. The state determines and encourages institutions to cooperate and improve management, take risks and engage in the setting and achieving of collective goals. State (administrative) coordination cannot create a substitute for unperformed actions or water services (market coordination) but through planned relations between subjects reaches adequate solutions. Therefore, it is important that water management is a process of planned and organized management of changes in the state and status of water or changes in the allocation of water through the performance of water services. They are divided into a vertical hierarchy, but also in the vertical integration of water functions.

Through planned organization of the water activity we reduce the complexity of the problem of water management and create conditions for new values of the individuals in the regime (customers and employees), water stakeholders and the society. Complete operation in water management is in the formation of public and collective (common) goals in the allocation and use of available water and water services. This especially applies in the construction, reconstruction and revitalization of water facilities, and the application of scientific research solutions in risk management of the water activity on the state and interstate level.

#### CONCLUSION

Rational organization of water management in the country means accurate and complete determination of the areas and segments of water management. They are basically contained in the systems for protection against the harmful effects of water, the protection of water and the use of water and for segments in the allocation and use of water, water services and other activities. Each of the areas and segments contains a number of legal, technical-technological and economic elements. Organization requires the establishment of mutual elements in the basic systems that create a complete system of water management. It is realized on different levels and not through formal but functionally established relationships between the hierarchical levels. An established system of functions that are always active gives specific results in water management and special benefits to the water activity. The benefits are also significant in satisfying the interests of the ratepayers and users in the prevention of damages and gain of profit.

The results of the paper can serve as a basis for the establishment of a new organizational structure of a coexistent system in conditions of new bases and principles of water management. All this is aimed at the realization of historically unchanging objectives: security, stability and usability of water for life of the population and economy in a good and natural environment.

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# ORGANIZOVANJE SISTEMA NACIONALNOG GAZDOVANJA VODAMA

## MARKO BAJČETIĆ

### Izvod

Gazdovanje vodama u državi je kompleksno pitanje jer odražava veliki broj faktora i uticaja. Oni su u različitim vlasničkim i svojinskim osnovama i odnosima, tehničko-tehnološkim karakteristikama voda, objekata i drugih resursa i ekonomskih specifičnosti dobara, prihoda i sredstava. Složenost elemenata i sistema zahtevaju izgradnju autonomnog i koherentnog sistema koji funkcionisanjem efektuira u zadovoljavanju interesa stanovništva, privrede i ostalih korisnika. Zadovoljavanje korisnika je u obezbeđivanju čiste i dovoljne vode, a posebno je obezbeđenje sigurnosti od vode. U različitim segmentima upravljanja vodama interese i potrebe korisnika ispunjavaju hijerarhijski uspostavnjene različite organizacije. Svaka od organizacija mora uspostaviti sistem koji se razvija i ostvaruje pozitivne rezultate. Sistemi su u formi direkcija i agencija za vode, javnom i vodnom preduzeću, naučno-istraživačkim institucijama, osiguravajućim subjektima i u javnim i privatnim pravnim i fizičkim licima.

Ključne reči: upravljanje vodama, direkcija, agencija, preduzeće, jedinstveni i decentralizovani sistem.

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# THE VALUES OF ORGANIC AND INORGANIC BLOOD PARAM-ETERS IN DAIRY COWS DURING THE PERIPARTAL PERIOD

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SUMMARY: The objective of this study was to evaluate organic and inorganic blood parameters, i.e. indicators of metabolic condition in dairy cows (n=30) during the peripartal period. The cows were divided into two groups. The first group (n=15) comprised clinically healthy prepartal cows, and the second one included clinically health postpartal cows (n=15). Blood samples were taken from all the examined dairy cows. Blood glucose levels were statistically significantly lower (P < 0.05) in the puerperal cows than in the late pregnant ones, suggesting an increased glucose uptake by the mammary gland and decreased gluconeogenesis in the liver. Significantly lower(P<0.05) blood levels of triglyceride, total protein, albumin and urea were found in the puerperal cows, which suggested the decreased synthetic capacity of liver cells. Blood bilirubin levels were significantly higher (P < 0.01) in the puerperal cows than in the late pregnant cows clearly indicated the decreased excretory capacity of the liver. The analysis of the blood parameters as indicators of the functional capacity of liver cells suggested that puerperal cows revealed different degrees of fatty liver, as opposed to the late pregnant cows, in which the morphological and functional capacity of hepatocytes was preserved. Blood calcium, phosphorus and magnesium levels in the postpartum cows were lower (P>0.05), which suggesting a reduced supply from alimentary sources and/or increased utilization by the mammary gland.

Key words: cows, fatty liver, protein, lipids, bilirubin, macroelements.

## INTRODUCTION

During the peripartal period (transition period), from day 15 before to day 15 after parturition, the organism in high-yielding dairy cows is pushed to its physiological lim-

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its, reaching maximum until day 120 of lactation, resulting in a substantial load on the organism, specifically on the digestive organs, liver, udder and the reproductive organs (Grummer, 1995, Overton and Waldron, 2004).

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 physiological integrity and morphology of the liver (Veenhuizen et al., 1991; Reist et al., 2002; Bobe, 2004; Doković et al., 1998, 2007, 2008, 2009). Mild fatty infiltration of liver cells in dairy cows during peripatral period is considered to be almost physiological. The fat content of liver can range from 10-60%, as dependent on the degree of pathology (Gaal, 1993). Increased metabolic load on the dairy cows' organism and fat accumulation in liver cells induce disturbances in the morphological and functional integrity of hepatocytes, resulting in decreased blood levels of individual liver-synthetized indicators of liver function (glucose, total protein, albumin, globulin, cholesterol, triglycerides, urea). Furthermore, the excretory function of hepatocytes is reduced and, accordingly, the levels of certain metabolic products in the blood (bilirubin, ammonia, bile acids) are generally increased (Herdt et al., 1983; Holtenius, 1989; Veenhuizen et al., 1991; Vazguez-Anon et al., 1994; Reynolds et al., 2003; Sevinc et al., 2003; Lubojacka et al., 2005; Đoković et al., 1998, 2007, 2008, 2009). The blood levels of calcium, inorganic phosphorus and magnesium in cows during the peripartal period reflect the intake of these macroelements through diet and their utilization by the mammary gland. Any reduction in these blood parameters as compared to the physiological values in cows at the beginning of lactation, or their deficiency, as well as the abnormal relationship between them most commonly lead to subclinical and clinical manifestations which adversely affect cow health and fertility (Ivanov et al., 1993; Sevinc et al., 1997; Kupczynski et al., 2002).

Considering the increased metabolic loads of the dairy cows during peripartal period, the objective of the study was to determine blood parameters, i.e. indicators of the functional condition of the liver, being the following: glucose, triglyceride, total cholesterol, total protein, albumin, urea, bilirubin and blood inorganic parameters: calcium, magnesia and inorganic phoshorus and show theirs importance in functional activity of liver.

### MATERIAL AND METHOD

Late pregnant cows and puerperal cows (n=30) were randomly selected from the Simmental herd (Farm-Farmad-Vrdila-Kraljevo) for examination. The cows were divided into two groups. The first group (group A) included clinically healthy cows from day 15 to day 1 before parturition (n=15). The second group (group B) comprised clinically healthy cows from day 1 to day 15 after parturition (n=15). The examined cows were four to six years old. Their average weight was 650kg during the prepartum period and 600kg after parturition. There were three lactations on average with an average milk yield of 6,825 l of milk over the period of 305 days of lactation. The examinations were conducted during the same season, in mid July. The cows were kept in free stalls in a closed barn. The diet and the housing facilities were adapted to research purposes. The diet suited the energy needs of the cows in late pregnancy and lactation. Blood was sampled from all examined cows by punction of vena jugularis, from 10:00 to 12:00 a.m, i.e. four to six hours after milking and feeding. Two test tubes of blood (approxi-

mately 20 ml) were taken per punction. Serum separation after spontaneous coagulation at room temperature was performed by centrifugation at 3000 rotations/min. The serum samples were kept refrigerated at -18 °C until analysis. An enzymatic spectrophotometric assay was used to determine the levels of glucose (cat. No. 11803), triglyceride (cat. No. 11828), total cholesterol (cat. No. 11828), urea (cat. No. 11536), total protein (ct. No. 11500), albumin (cat. No. 11547) and bilirubin (cat. No. 11515) in the blood serum. All biochemical blood parameters were assayed using a Cobas Mira device at the biochemical laboratory Medicus in Kraljevo. Blood serum levels of calcium and magnesium were determined by flame atomic absorption spectrophotometry (AAS), and those of inorganic phosphorus by spectrophotometry at the Specialist Veterinary Institute in Kraljevo.

The statistical analysis of the obtained data was carried out by ANOVA-procedure. The analysis of variance and LSD test were used to evalute the probability of the significance of the statistical differences of mean blood parameter values between the groups of cows used in the experiment at P<0.05 and P<0.01 (Microsoft STATISTICA ver.5.0 Stat.Soft.Inc.1995).

### RESULTS

Table 1 shows the research results on the blood levels of glucose, triglyceride, total cholesterol, total protein, albumin, urea and bilirubin as well as blod levels calcium, magnesium and inorganic phosphorus in dairy cows during peripartal period.

Table 1. Blood levels of glucose, triglyceride, total cholesterol, total protein, albumin, urea, bilirubin, calcium, magnesium and inorganic phosphorus in the late pregnant cows (group A) and in the puerperal cows (group B)

Tabela 1. Nivo glukoze, triglicerida, ukupnog holesterola, ukupnog proteina, albumina, uree, bilirubina, Ca, Mg i neorganskog fosfora u krvi visoko steonih i puerperalnih krava

| Group                         | А               | В               | P<0.05 | P<0.01 |
|-------------------------------|-----------------|-----------------|--------|--------|
| n                             | 15              | 15              |        |        |
| Glucose (mmol/l)              | 2.73±0.70       | 2.21±0.48       | A:B    |        |
| Triglycerides (mmol/l)        | 0.29±0.14       | 0.18±0.08       | A:B    |        |
| Total cholesterol (mmol/l)    | 2.66±0.58       | 2.26±0.59       |        |        |
| Total protein (g/l)           | 70.26±10.54     | 63.51±7.70      | A:B    |        |
| Albumin (g/l)                 | 33.98±4.63      | 29.54±3.89      | A:B    |        |
| Urea (mmol/l)                 | 4.47±1.60       | $3.85 \pm 0.98$ | A:B    |        |
| Bilirubin (µmol/l)            | 2.92±0.83       | 4.44±1.11       |        | A:B    |
| Calcium (mmol/l)              | $2.35 \pm 0.24$ | 2.17±0.22       |        |        |
| Magnesium (mmol/l)            | 1.02±0.26       | 1.01±0.32       |        |        |
| Inorganic phosphorus (mmol/l) | 2.09±0.34       | 1.97±0.39       |        |        |

Table 1 shows that blood levels of glucose, total protein, albumin and urea were statistically significantly lower in the late pregnant cows than in the puerperal ones (P<0.05). Statistically significantly lower blood levels of triglyceride (P<0.05) were found in the puerperal cows than in the late pregnant cows. Total cholesterol values were lower in the puerperal cows than in the other group of cows, but no statistical significance was recorded (P>0.05). Blood bilirubin values was statistically significantly higher (P<0.01) in the puerperal cows than in the cows before parturition. Blood levels of calcium, magnesium and inorganic phosphorus were lower, but no statistical significance (P>0.05) in puerperal cows than in late pregnant cows.

### DISCUSSION

Glucose is a blood parameter defining the energy metabolism in late pregnancy and lactating cows. Blood glucose levels in all examined groups of cows was within the physiological limits (2.2-4.0 mmol; Jovanović, 1984). The puerperal cows showed statistically significantly lower (P<0.05) blood glucose values as compared to the late pregnant cows. The above results are in agreement with the literature data (Veenhuizen et al., 1991; Grummer, 1995; Reist et al., 2002; Doković et al., 1998 2007, 2008, 2009) indicating that physiological glycemia in early lactation cows is at the lower physiological limit due to the sudden activity of the mammary gland and increased lactose synthesis. Furthermore, the negative energy balance, lipomobilization and increased fat accumulation in hepatocytes induce a considerable reduction in glucose synthesis by gluconeogenesis in the liver. Lipid metabolism parameters include the blood levels of triglyceride and total cholesterol. Significantly lower (P<0.05) blood triglyceride levels were determined in the puerperal cows, the total cholesterol values being lower but statistically insignificant (P>0.05) as compared to those in the other group of cows. The results suggested an increased accumulation of triglyceride and total cholesterol in liver cells in the puerperal cows. The data are in agreement with the results obtained by other authors (Pechova et al., 1997; Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Sevinc et al., 2003; Đoković et al., 1998; 2007, 2008, 2009). Nitrogen metabolism parameters include determination of the blood levels of liver-synthesized total protein, albumin and urea, the values there of decreasing in cases of liver cell damage (Jovanović et al., 1993;, Lubojacka et al., 2005). Albumin is an indicator of the synthetic capacity of the liver, its decrease in the blood to values as low as 20% being induced by chronic liver diseases (Sevinc et al. 2003). The values of the above blood parameters were within the physiological limits (total protein 60-80 g/l; albumin 30-40 g/l; urea 1.66-6.66 mmol/l) in all examined groups of cows (Jovanović, 1984). They were statistically significantly lower (P < 0.05) in the puerperal cows than in the other group of cows, suggesting the reduced synthetic capacity of the liver cells in the early lactation cows. The reduced synthesis of total protein, albumin and urea at the puerperal cows is induced by the development of fatty infiltration and degeneration of liver cells (Pechova et al., 1997; Sevinc et al., 2003; Overtron and Waltron, 2004; Lubojacka et al., 2005; Đoković et al., 2009).

The total bilirubin value is a sensitive indicator of liver damage. A significant correlation between the amount of lipids in the liver and total bilirubin concentrations has been reported by West (1990). In the present study, higher concentrations of total bilirubin in the blood serum (P<0.01) were found in the puerperel group and it was within phisiological range of 0.17-5.13  $\mu$ mol/l (Jovanović, 1984). Significantly higher values of total bilirubin in the puerperal cows suggesting the disturbance in the excretory capacity of the liver cells due to fat accumulation in the hepatocytes. Similar results were obtained by other authors (Herdt et al., 1983; Holtenius, 1989).

The obtained results on the blood levels of macroelements showed that the calcium and inorganic phosphorus values (Ca: 2.0-3.0 mmol/l; P:1.6-2.3 mmol/l; Jovanović, 1984) in the blood serum were within the physiological range in both groups of cows. These values in the cows during the initial period of lactation were at the lower physiological limit, which resulted from the sudden activity of the mammary gland and its utilization of these macroelements. The obtained results suggested the possible development of subclinical puerperal paresis. Similar results were obtained by other authors (Ivanov et al., 1993; Sevinc et al., 1997).

The blood level of magnesium was lower, but no statistically significant differences were observed in the cows at the beginning of lactation as compared to the cows before parturition. Magnesium homeostasis depends on an optimum supply from alimentary sources and, hence, magnesium levels depend on ruminal resorption. Magnesium resorption is insufficient in diets rich in potassium and proteins, but lacking cellulose. Magnesium values in the cows after parturition were low within the physiological range (0.7-1.2 mmol/l; Jovanović,1984) and conformed with the results of other authors (Ivanov et al., 1993; Sevinc et al., 1997; Kupczynski et al., 2002), suggesting a reduced supply from alimentary sources and/or increased utilization by the mammary gland at the beginning of lactation.

### CONCLUSION

Blood glucose levels were statistically significantly lower (P<0.05) in the puerperal cows than in the late pregnant ones, suggesting an increased glucose uptake by the mammary gland and decreased gluconeogenesis in the liver at the beginning of lactation.

Significantly lower (P<0.05) blood triglyceride values and low values of total cholesterol (P>0.05) in the puerperal cows suggest lower activity of the liver cells.

Significantly lower (P < 0.05) blood levels of total protein, albumin and urea were found in the puerperal cows, which suggested the decreased synthetic capacity of liver cells.

Blood bilirubin levels were significantly higher (P<0.01) in the puerperal cows than in the late pregnant cows clearly indicated the decreased excretory capacity of the liver.

The analysis of the blood parameters as indicators of the functional capacity of liver cells suggested that puerperal cows revealed different degrees of fatty, as opposed to the late pregnant cows, in which the morphological and functional capacity of hepatocytes was preserved.

Blood calcium, phosphorus and magnesium levels in the postpartum cows were lower, but they were not statistically significant (P>0.05), which suggesting a reduced supply from alimentary sources and/or increased utilization by the mammary gland.

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## VREDNOSTI ORGANSKIH I NEORGANSKIH PARAMETARA KRVI KOD MLEČNIH KRAVA U PERIPARTALNOM PERIODU

## RADOJICA ĐOKOVIĆ, ZORAN ILIĆ, VLADIMIR KURĆUBIĆ, VLADIMIR DOSKOVIĆ

#### Izvod

Cilj ovog rada je bio da se odrede organski i neorganski parametri krvi, indikatori stanja metabolizma kod mlečnih krava (n=30) u peripartalnom periodu. Krave su podeljene u dve grupe. U prvoj grupi (n=15) nalazile su se klinički zdrave visoko gravidne krave, a u drugoj grupi klinički zdrave krave (n=15) u puerperiumu. Od svih ispitivanih mlečnih krava uzeti su uzorci krvi. Koncentracije glukoze u krvi su bile statistički značajno manje (P<0.05) kod grupe krava na početku laktacije u odnosu na vrednosti u krvi kod grupe krava pre telenja, što ukazuje na povećanu potrošnju glukoze od strane mlečne žlezde i smanjenu sintezu putem glukoneogeneze u jetri. Kod grupe krava posle telenja utvrđene su značajno manje (P<0.05) vrednosti triglicerida, ukupnih proteina, albumina i ureje u krvi, što ukazuje na smanjenu sintetsku sposobnost ćelija jetre. Koncentracije bilirubina u krvi kod krava na početku laktacije su bile značajno veće (P<0.01) u odnosu na vrednosti kod krava pre telenja, što jasno ukazuje na smanjenu ekskrecionu sposobnost jetre. Na osnovu ispitivanja organskih parametara krvi indikatora funkcionalne sposobnosi ćelija jetre može se pretpostaviti da kod krava na početku laktacije je prisutna masna jetra različitog stepena, za razliku od grupe krava pre teljenja, kod kojih je očuvana morfološka i funkcionalna sposobnost hepatocita. Vrednosti kalcijuma, neorganskog fosfora i magnezijuma u krvi su bile niže, ali bez statističke značajnosti (P>0.05) kod grupe krava na početku laktacije, što jasno ukazuje na smanjeno snabdevanje ovim makroelementima iz alimentarnih izvora i/ili povećano korišćenje od strane mlečne žlezde.

Ključne reči: krave, masna jetra, proteini, lipidi, bilirubin, makroelementi.

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## SEASONAL DIFFERENCES IN SOW REPRODUCTIVE PERFORMANCES

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SUMMARY: The effects of the warm and cold period of the year on the parameters of sow reproductive performances were examined on a big pig farm in Vojvodina. The farm has the capacity of 5,000 sows. It was determined that the values of all the examined parameters (weaning-to-estrus interval, farrowing rate and litter size) are statistically significantly lower (P>0.01) in the warm period compared to the cold period of the year. Higher temperature and extended photoperiod during summer months are the main factors of this phenomenon. Physiological mechanisms of this phenomenon are not entirely clarified. Therefore, it is not possible to determine a single practical technology which would provide solution for it. However, the results of scientific research conducted so far, as well as practical experience, indicate that by optimization of nutrition, ambient temperature and sow health status, it is possible to significantly reduce the negative effects of summer infertility syndrome on sow reproductive performance.

Key words: reproduction, performance, summer infertility, sow.

### INTRODUCTION

The reproduction efficiency of breeding herd in industrial pig production is assessed by the number of weaned pigs per sow per year. However, this value can vary widely, as it is subject to the effects of a number of genetic and paragenetic factors. Since genetic heritability of all reproductive traits is low, and the level of heritability for the average number of weaned pigs per litter is among the lowest amounting around 10% (See, 2002), the phenotypic value of this parameter is significantly more affected by paragenetical factors. Regarding these factors, the basic fertility parameters are: weaning-to-estrus interval, successful conception rates (i.e. the percentage of farrowing), the level of prenatal embryonic or fetal survival rate, the number of live-born pigs and the level of pig survival rate during lactation period (*Nielsen, 1981a; Tomes i sar. 1982;* 

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*Stančić, 1994).* The value of the above stated parameters of sow fertility can be influenced by a number of infectious and non-infectious etiologic factors (*Vanroose i sar. 2000*). The infectious agents which are highly significant are the causes of intrauterine infections, caused by porcine parvovirus (PPV), porcine **reproductive** and **respiratory syndrome virus (PRRS)**, and Aujeszky's disease virus (ADV) (*Straub, 1994; Gagrčin, 2003*), as well as bacterial infections, such as brucellosis, leptospirosis, actinobacillosis, infections caused by E. colli, and so on (*Yeske, 2007*).

Non-infectious causes of reduced fertility are numerous and in production conditions their influences, most frequently, interact with each other. Therefore, it is very difficult to isolate these effects and define them precisely. However, some of the basic causes are nutrition, housing, insemination technologies, stamina, and general health status of breeding sows (*Stančić, 2005*). On the other hand, under modern production conditions, the phenomenon known as "seasonal infertility in pigs" represents the most significant factor of reduced parameters of sow fertility. Namely, for as long as 40 years, considerably lower values of sow fertility parameters have been evident in the warmer part of the year (*Almond, 1992*). Thus, *Aumaitre i sar. (1976*) 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 (June – September). This phenomenon is related to the negative effect of increased ambient temperature on sow reproductive functions (*Gordon, 1997*).

Summer infertility syndrome inflicts substantial economic, zoo-technological and veterinary-medicine damage on sow production. Therefore, examination of this problem is always important. Accordingly, the objective of this paper is to examine the effects of the warm and cold period of the year on the basic parameters of sow reproductive performance in domestic conditions of intensive pig production.

### MATERIAL AND METHOD

The research was conducted on a big Vojvodinian farm for the year. The capacity of the farm is approximately 5,000 breeding sows. The sows are kept in enclosed facilities, except for the weaned sows until the moment of successful insemination. Pregnant sows are kept around 30 days in separate boxes, and later, up to 7 days before farrowing, in group boxes with pens. During lactation, which lasts around 30 days, sows are kept in individual boxes. The nutrition of sows is adequate, with meals prepared for certain categories of breeding sows. Estrus testing is performed in the presence of boars, once within 24h. Artificial insemination is performed with liquid diluted sperm, with the dozes of 100ml, consisting around 5x10° spermatozoids with progressive motility. Insemination is performed two times: firstly, a couple of hours after estrus is detected, and secondly, 24h later. Rebreeding control is conducted in facilities for inseminated (pregnant) sows, staring around 14 hours after insemination. The data for this research were acquired from the reproductive data taken from farm records for 2535 sows in total (455 gilts and 2080 second and higher parity sows). The following data were recorded: (a) Estrus emergence within  $\leq$  7, 8 up to 14, and  $\geq$  15 days after litter weaning, (b) duration of weaning-to-estrus interval (days), (c) the farrowing rate (%) after insemination in the first post-lactation estrus, (d) the reasons for farrowing failure, and (e) the number of litter size per farrowing.

The values of all the above stated parameters of reproductive performance were

analyzed in relation to the parity of farrowing and a season of the year. The year is divided in the *warm* and *cold* season. The warm season refers to the period from May to September, while the cold period lasts from October until April.

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

## RESULTS

By distribution analysis of occurrence of estrus after weaning, it was concluded that, in the cold period of the year, in a substantially higher number of sows (83.4%) of all parities of farrowing, estrus was manifested within the first 7 days after weaning compared to the warm period of the year (63.9%). In the warm period of the year, the number of sows with extended weaning-estrus interval (WEI) is significantly increased. Namely, 8 to 14 days after weaning, estrus was manifested in 9.1% of sows in the cold, and 13.5% in the warm period of the year. The occurrence of estrus 15 and more days after weaning was determined in 22.6% of sows in the warm, and in only 7.5% of sows in the cold period of the year. These differences were statistically significant (P>0.01) (Table 1).

|           |                         |       | Weaning-to-estrus interval (days) / Interval zalučenje – estrus (dani) |        |       |       |       |              |      |                 |
|-----------|-------------------------|-------|--|--------|-------|-------|-------|--------------|------|-----------------|
| Parity Pa | <i>Paritet</i> $\leq 7$ |       | 7  | 8 - 14 |       | ≥ 15  |       | Total Ukupno |      | Total           |
|           |                         | Н     | Т  | Н      | Т     | Н     | Т     | Н            | Т    | Ukupno<br>(H+T) |
| 1.        | n                       | 175   | 95   | 32     | 30    | 57    | 66    | 264          | 191  | 455             |
| 1.        | %                       | 66.3a | 49.7b  | 12.1a  | 15.7a | 21.6a | 34.5b | 204          | 191  | 455             |
| ~ 2       | n                       | 1056  | 582  | 102    | 113   | 53    | 174   | 1211         | 860  | 2080            |
| $\geq 2.$ | %                       | 87.2a | 67.0b  | 8.4a   | 13.0b | 4.4a  | 20.0b | 1211         | 869  | 2080            |
| Total     | n                       | 1231  | 677  | 134    | 143   | 110   | 240   | 1475         | 1060 | 2535            |
| Ukupno    | %                       | 83.4a | 63.9b  | 9.1a   | 13.5b | 7.5a  | 22.6b | 14/5         | 1060 | 2555            |

Table 1. Distribution of total estrus reaction in sows after weaning Tabela 1. Distribucija ukupnog estrusnog reagovanja krmača posle zalučenja

 $\rm H-Cold\ period\ /\ H-Hladni\ period$ 

 $T-Topli \ period \ / \ T-Warm \ period$ 

<sup>a, b</sup> The values with different superscripts are statistically significantly different (P>0.01).

<sup>a, b</sup> Vrednosti sa različitim superskriptima se statistički značajno razlikuju (P>0,01).

Estrus reaction in the first 7 days after weaning is significantly lower in gilts, compared with the sows with a higher number of parities. It is important to stress that the number of sows for which estrus occurred during the first 7 days after weaning in the warm period is reduced by 19.5% compared with the cold period. The number of sows with extended WEI is increased in the warm period compared with the cold period, from 4.4% to 15% (Table 1).

The average duration of the weaning-to-estrus interval in all the sows in both of the seasons was 7.2 days. It was observed that this interval was extended for over 2.5 days, lasting 8.7 days on average, compared with the cold period, when the average interval lasted for 6.1 days (Table 2).

|                   | Weanir        | ng-to-estrus in | strus interval (days) / Interval zalučenje – estrus (dani) |                |              |                |  |
|-------------------|---------------|-----------------|--|----------------|--------------|----------------|--|
| Denites / Dunited | P             | eriod of year   | Total / Ukupno   |                |              |                |  |
| Parity / Paritet  | Cold / Hladni |                 |  |                | Warm / Topli |                |  |
|                   | n             | $\overline{x}$  | n  | $\overline{x}$ | Ν            | $\overline{x}$ |  |
| 1.                | 264           | <b>8.</b> 6a    | 191  | 10.7b          | 455          | 9.5            |  |
| ≥ 2.              | 1211          | 5.6ab           | 869  | 8.3bc          | 2080         | 6.7            |  |
| Total / Ukupno    | 1475          | 6.1ab           | 1060   | 8.7bc          | 2535         | 7.2            |  |

Table 2. Average duration of weaning-to-estrus interval (days) Tabela 2. Prosečno trajanje intervala zalučenje – estrus (dani)

<sup>a, b, c</sup> Values with different superscripts are statistically significantly different (P>0.01).

a, b, c Vrednosti sa različitim superskriptima se statistički značajno razlikuju (P>0,01).

In gilts, during the whole year, WEI was longer for 2.2 days (9.5 days on average) compared with sows, in which this interval lasted for 6.7 days on average. In both gilts and sows, the average WEI was longer in the warm than in the cold period (8.6 and 10.7 for gilts, and 5.6 and 8.3 days older sows). The differences between all the established values were statistically significant (P>0.01).

Significant difference (P>0.01) was established also regarding the achieved farrowing rate, after insemination in the first post-lactation estrus, between the cold (79.7%) and warm (69.6%) period of the year (Table 3).

| Table 3. Farrowing rate after insemination in first estrus after weaning        |
|---|
| Tabela 3. Vrednost prašenja posle osemenjavanja u prvom estrusu posle zalučenja |

| Parity / Paritet |                                |             | Period of year | Period of year Period godine |              |  |
|------------------|--------------------------------|-------------|----------------|------------------------------|--------------|--|
|                  | Tunity / Tuniti                |             | Cold / Hladni  | Warm / Topli                 | Total Ukupno |  |
|                  | Inseminated (n) semi           | enjeno (n)  | 264            | 191                          | 455          |  |
| 1.               | n n                            |             | 182            | 107                          | 289          |  |
|                  | Farrowed Oprašeno              | %           | 68.9a          | 56.0b                        | 63.5         |  |
|                  | Inseminated (n) Ose            | menjeno (n) | 1211           | 869                          | 2080         |  |
| ≥ 2.             | Ormiterre                      | n           | 993            | 631                          | 1624         |  |
|                  | Oprašeno                       | %           | 82.0b          | 72.6a                        | 78.1         |  |
|                  | Inseminated (n) Osemenjeno (n) |             | 1475           | 1060                         | 2535         |  |
| Total Ukupno     | Farrowed                       | n           | 1175           | 738                          | 1913         |  |
|                  | Oprašeno                       | %           | 79.7b          | 69.6a                        | 75.5         |  |

<sup>a, b</sup> Values with different superscripts are statistically significantly different (P>0.01).

<sup>a, b</sup> Vrednosti sa različitim superskriptima se statistički značajno razlikuju (P>0,01).

Gilts achieved a lower farrowing rate (63.5%), compared with sows (78.1%). Furthermore, both gilts and sows had considerably lower farrowing rate in the warm (56%) and cold period (68.9%), compared with the same rates in sows (82% and 72.6%) (Table 3).

During the observed period, only 24.5% of the inseminated sows had farrowing failure. The main reasons why the inseminated sows had farrowing failure were the

following: rebreeding (91.5%), abortion (2.2%), paragravidity (1.5%), and other reasons (1.3%). The obtained results indicate that the reproductive disorders (rebreeding, abortion and paragravidity) are the reason for farrowing failure for 95.5% (588/622) of sows (Table 4).

|                                    | Peri          | od of year | Total / Ukupno |       |              |      |
|------------------------------------|---------------|------------|----------------|-------|--------------|------|
| Reasons / Razlozi                  | Cold / Hladni |            |                |       | Warm / Topli |      |
|                                    | n             | %          | n              | %     | n            | %    |
| Sows inseminated Osemenjeno krmača | 1475          | -          | 1060           | -     | 2535         | -    |
| Rebreeding Povađanje               | 240           | 16.3a      | 253            | 23.9b | 493          | 19.5 |
| Abortion / Abortus                 | 19            | 1.3a       | 37             | 3.5b  | 56           | 2.2  |
| Paragravidity1 Paragravidnost1     | 18            | 1.2a       | 21             | 2.0a  | 39           | 1.5  |
| Other / Ostalo                     | 23            | 1.5a       | 11             | 1.0a  | 34           | 1.3  |
| Total / Ukupno                     | 300           | 20.3a      | 322            | 30.4b | 622          | 24.5 |

Table 4. Reasons for farrowing failure, from number of sows inseminated *Tabela 4. Razlozi izostanka prašenja, od broja osemenjenih krmača* 

<sup>1</sup> Not-in-pig / Krmača je osemenjena, ne povađa, a nije se oprasila (nije suprasna).

<sup>2</sup> Dead/uginuće, old age/starost, locomotor and other disorders/lokomotorna i druga obolenja.

<sup>a, b</sup> Values with different superscripts are statistically significantly different (P>0.01). <sup>a, b</sup> Vrednosti sa različitim superskriptima se statistički značajno razlikuju (P>0,01).

Table 5. Average litter size at farrowing Tabela 5. Prosečan broj prasadi u leglu kod prašenja

|   | Per    | iod of year /  | Totol / Ultumus |                |                |                |
|---|--------|----------------|-----------------|----------------|----------------|----------------|
|   | Cold / | Hladni         | Warm / Topli    |                | Total / Ukupno |                |
|   | n      | $\overline{x}$ | n               | $\overline{x}$ | n              | $\overline{x}$ |
| Total number of litters <i>Ukupan</i> broj legala | 1175   | -              | 738             | -              | 1913           | -              |
| Live-born / Živih                                 | 12314  | 10,48a         | 7616            | 10,32a         | 19930          | 10,42          |
| Dead / Mrtvih                                     | 493    | 0,42a          | 472             | 0,64b          | 965            | 0,50           |
| Avital / Avitalnih                                | 258    | 0,22a          | 125             | 0,17a          | 383            | 0,20           |
| Abnormal Abormalnih                               | 106    | 0,09a          | 44              | 0,06a          | 150            | 0,08           |
| Mummification Mumifikovanih                       | 47     | 0,04a          | 66              | 0,09b          | 113            | 0,06           |
| Total / Ukupno                                    | 13218  | 11,25a         | 8323            | 11,28a         | 21541          | 11,26          |

<sup>a, b</sup> Values with different superscripts are statistically significantly different (P>0.01).and <sup>a, b</sup> *Vrednosti sa različitim superskriptima se statistički značajno razlikuju (P>0,01).* 

The average litter size was almost identical in the cold (11.25) and warm (11.28) period of the year. Likewise, the differences between the average number of live-born, avital and abnormal pigs in the cold and warm period were not statistically significant, either (P<0.05). However, the average number of dead and mummified pigs was statistically much bigger (P>0.01) during the warm, compared with the cold period (Table 5).

#### DISCUSSION

Basically, infertility or reduced fertility in pigs can be of infectious and non- infectious etiology. Infectious factors, bacterial or viral etiology, most frequently affect uterus or conceptus (fetus and /or fetal membrane), which results in embryo or fetus mortality, and consequently, abortion, irregular rebreeding, dead-born, avital or mummified pigs (*Christianson, 1992; Hogg i Levis, 1989; Floss i Tubbs, 1993; Varoose i sar.,* 2000; Gagrčin, 2003).

Non-infectious factors which cause pig infertility are numerous and can be genetic or paragenetic. The most important paragenetic factors are nutrition, housing, parity structure of breeding herds, climatic conditions (ambient temperature and daily photoperiod duration), application of hormonal preparations and general health (*Tomes i sar. 1982; Stančić, 2005*). Unfavourable effects of these factors are reflected in significant reduction of sow fertility during the warm period of the year. This phenomenon is known as "summer or seasonal infertility syndrome" (*Love, 1978; Rozeboom i sar., 2000*).

Although the domestic pig breeds are reproductively active throughout the year (manifesting ovulatory estrus and being capable of insemination) (*Mauget, 1982*), there is the difference in values of all fertility parameters (duration of the weaning-to-estrus interval, successful conception rates, the rebreeding and abortion rates, as well as the average litter size), between the cold and warm period of the year (*Rozeboom i sar., 2000; Stančić i sar., 2002*). Namely, considerably lower values of the above stated fertility parameters, during the warm summer months, have been detected as a significant factor of economic lossess in intensive domestic pig breeding, since 1970s (*Aumaitre i sar., 1976*). Summer infertility is considerably more prominent in gilts, compared with sows (*Britt i sar., 1983*). Research conducted by a number of authors, as stated by *Gordon (1997*), point to the general conclusion that the parameters of sow fertility during the warm period are reduced by 15 to 20% compared with the cold period.

The results of the research in this paper showed that the number of sows which manifest estrus in the first 7 days after weaning is lower (63.9%) in the warm period, compared with the cold period (83.4%), as well as that the number of sows with extended weaning-to-estrus interval (WEI) is increased in the warm period of the year. Therefore, the average duration of WEI was longer in the warmer period (8.7 days) in comparison with the cold period of the year (6.1 days). Both the level of estrus reaction and duration of WEI had lower values in gilts than in sows. These differences were statistically significant (P>0.01). Very similar differences in duration of WEI between the cold and warm period of the year were identified also by certain other authors (Aumaitre i sar., 1976; Hurtgen i sar., 1980; Peltoniemi i sar., 1999; Stančić i sar., 2002; Almond i Bilkei, 2005). Thus, for instance, in Eastern European countries Almond i Bilkei (2005) ascertained that this interval lasts 5.9 days on average in the cold and 7.8 days in the warm period of the year. The extended duration of WEI reduces reproductive efficiency of breeding herds both directly and indirectly. Firstly, the reproductive efficiency is reduced directly, as sows with extended WEI achieve lower farrowing rates (%) after insemination in the first post-lactation estrus and have a significantly lower number of pigs per litter (Stančić, 1994; Kemp i Soede, 1996; Stančić, 1997a i 1997b; Borchardt Netto, G., 1998; Stančić, 2000; Stančić i sar., 2002; Timotijević i sar., 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 i sar., 1982; Tubbs, 1990; Stančić, 2005*). According to the research conducted by *Prunier i sar. (1996*), the extended WEI during the warm period of the year is a consequence of the decreased capability of hypothalamus to re-establish the normal pulsatile secretion of Gn-RH. This inhibits the release of hypophyseal gonadotropin (FSH and LH), which results in postponement of the first post-lactation ovulation and estrus manifestation.

The farrowing rate after insemination in the first post-lactation estrus is also statistically lower (69.6%) in the warm than in the cold period of the year (79.7%) (P>0.01). This value is lower in gilts (69% in the cold and 56% in the warm period of the year), compared with sows (82% in the cold and 72.6% in the warm period of the year). The results of other authors also indicate lower values of farrowing during the warm period compared with the cold period of the year. Thus, Almond i 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, reestablishment of estrus 25 to 35 days after insemination (so-called irregular rebreeding). Such early disruption of pregnancy is a consequence of embryo mortality (Xue i sar., 1994) or the regression of yellow body of pregnancy (Wrathall i sar., 1986). Namely, recent research indicate that high ambient temperature leads to the increased embryo mortality, and consequently, to disruption of pregnancy (Stančić i sar., 2004). Besides, it seems that the increased temperature inhibits prolactin release (LTH) from hypophysis, which is necessary for enhancement of secretory activity of yellow body of pregnancy after 16<sup>th</sup> day of gestation, which also causes disruption of pregnancy and irregular rebreeding (Tast i sar., 2002; Kirkwood, 2009). In this research, it was identified that the abortion rate during the warm period of the year (2%) is higher compared with the cold period (1.2%), but this difference was not statistically significant (P<0.05). 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 results of this research show that the average number of live-born and the total number of pigs per litter was very similar in the cold (10.48 and 11.25, respectively) and in the warm period of the year (10.32 and 11.28, respectively). However, it was ascertained that there is a substantially higher number of dead (0.64) and mummified (0.09)pigs per litter in the warm period compared with the cold period of the year (0.42 and 0.04). Similar results in Eastern Europe were obtained by Almond i Bilkei (2005). However, the views on the effects of a season on litter size are quite contradictory (Stančić *i sar.*, 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 weaningto-estrus interval, which is frequently in the summer period, have significantly lower number of pigs per litter (Borchardt Netto, 1998; Wettemann i Bazer, 1985; Stančić, 1997a i 1997b; Stančić i sar., 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 i sar., 1994; Stančić, 1995; Tast i sar., 2002; Stančić i sar. 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 i Marley, 2008).

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

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

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 (*Rozeboom i sar., 2000; Stančić i sar., 2002*).

## CONCLUSION

The results of this research clearly indicate that there are negative effects of the warm period of the year on the examined parameters of sow fertility. These effects are as follows:

- a) During the warm period of the year there are statistically significantly less sows which manifest the first post-lactation estrus in the warm period (63.9%) compared with the cold period of the year (83.4%).
- b) The farrowing rate, after insemination in the first post-lactation estrus, during the warm period is statistically lower to a considerable degree (79.7%) compared with the cold period (69.6%).
- c) The average numbers of live-born and the total number of pigs are very similar in the cold and warm part of the year (10.48 and 10.32, and 11.25 and 11.28). However, the numbers of dead-born and mummified pigs per litter were statistically significantly increased in the warm period (0.42 and 0.04) compared with the cold period of the year (0.64 and 0.09).
- d) 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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# SEZONSKE PROMENE REPRODUKTIVNE PERFORMANSE KRMAČA

# JELENA STANČIĆ, MLADEN GAGRČIN, SAŠA DRAGIN

## Izvod

Ispitivan je uticaj toplog i hladnog perioda godine na parametre reproduktivne performanse krmača, na jednoj velikoj farmi svinja u Vojvodini, kapaciteta oko 5.000 krmača. Pokazalo se da su vrednosti svih ispitivanih parametara (interval zalučenje – estrus, vrednost prašenja i veličina legla) statistički značajno (P>0,01) niži u toplom, u odnosu na hladniji period godine. Povišena temperatura i produžen fotoperiod, tokom letnjih meseci, su glavni faktori ovog fenomena. Fiziološki mehanizmi delovanja ovog fenomena nisu potpuno razjašnjeni. Zbog toga, nije moguće definisati jedinstvenu praktičnu tehnologiju njegovog rešavanja. Ipak, rezultati dosadašnjih naučnih istraživanja, kao i praktična iskustva, pokazuju da je optimalizacijom ishrane, ambijentalne temperature i zdravstvenog statusa krmača, moguće značajno umanjiti negativno delovanje fenomena letnjeg infertiliteta na reproduktivnu performansu krmača.

Ključne reči: reprodukcija, performansa, letnji infertilitet, krmača.

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# GENETIC ANALYSIS OF ANOTHER CULTURE RESPONSE IN WHEAT GENOTYPES\*

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SUMMARY: Androgenic responses of 10  $F_1$  wheat hybrids and 13 parental genotypes were studied. It was observed that the studied androgenic traits (androgenic capacity, callus yield, and frequency of green plants) were genotype dependent. On average for all genotypes, 10.6% of the anthers were responsive and 12.2 calluses were produced per 100 anthers. Line 9D-27-262 and the hybrid 9D-27-262/Posavka-2, had the highest frequencies of green plants, 11% and 12% respectively. It was found that 30.4% of the genotypes produced 2 or more green plants per 100 anthers, which can be considered as sufficient number for use in breeding. Heterotic effects as well as additive and dominant gene actions were found to control the inheritance of all characters.

Key words: androgenesis, inheritance, breeding, wheat

## **INTRODUCTION**

Production of doubled haploid lines (DHL) is commonly used by plant breeders in order to obtain pure lines in the shortest possible time. The anther culture method, in addition to wide hybridization, is a usual way to produce DH plants in cereals (Maluszynski et al. 2003; Ljevnaić and Kondić, 2008). Success in anther culture has been shown to be highly dependant on genotype of anther donor plants (Bruins and Snijders, 1995). Androgenic capacity in hexaploid wheat is controlled by independently inherited traits:

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callus induction, plant regeneration and the ratio of green plants. In determination of these parameters, nuclear gene effects were reported to be most important, including mainly additive effects besides dominance, epistasis and heterosis (Ekiz and Konzak, 1994; Chaudhary et al. 2003; Zamani et al. 2003).

The objective of this study was to investigate the androgenic response of  $F_1$  wheat crosses and their parents as well as the genetic control of the component traits of anther culture.

### MATERIAL AND METHODS

The plant material used in this experiment included 10 randomly selected single cross  $F_1$  hybrids of wheat (*Triticum aestivum* L.) and their 13 parental genotypes (Obrij, NSP1, Szegedi-746, Žitnica, 9D-27-262, Posavka-2, Balkan, Košuta, Pobeda, Rodna, Sremica, NS-0-694 and Fundulea-4). The  $F_1$  generation and parental lines were grown at the experimental fields of the Institute of Field and Vegetable Crops, Small Grains Department during the season 2005/2006. The sampled spikes were cold pretreated at 4-6°C for 5-10 days.

After the surface sterilization of spikes in 1.3% NaOCl, anthers were plated onto a modified Potato 2 (Chuang et al. 1978) inductive medium. Four to six weeks after isolation, the developing embryogenic calli were transferred to the 190-2 (Zhuang and Jia, 1980) regeneration medium. The number of responding anthers, calluses/100 anthers, and green plants/100 anthers were recorded.

The experimental design was completely randomized, with four replications. Statistical analyses were carried out in order to determine the effect of genotype on the traits studied. In order to normalize the distributions, all data were transformed before statistical analysis. LSD was used for comparing means for the analyzed traits. Inheritance of androgenic component traits was estimated by using t-test (Kraljević-Balalić et al., 1991).

### **RESULTS AND DISCUSSION**

Data obtained with respect to androgenic capacity, calli and green plantlet development were recorded on per cent basis and the performance of parents and  $F_1$  hybrids is presented in Table 1. The average androgenic capacities in parental genotypes and  $F_1$ s were 8.2% and 13.8%, respectively. Callus yield ranged between 0.6% (NS-0-694) and 43.3% (NSP 11) in homozygous genotypes, and between 3.9% (NS-0-694/Košuta) and 40.0% (Szegedi-746/Zitnica) in heterozygous  $F_1$  hybrids. Line 9D-27-262 and the hybrid which involved this line, 9D-27-262/Posavka-2, had the highest frequencies of green regenerants, 11% and 12%, respectively.

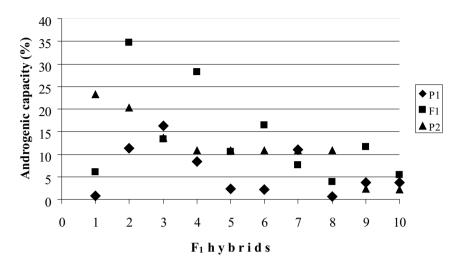
It was found that 30.4% of the genotypes produced 2 or more green plants per 100 anthers, which can be considered as a sufficient number for use in breeding. Three homozygous genotypes (Obrij, Szegedi-746 and NS-0-694) and one heterozygote (NS-0-694/Košuta) had no regenerated green plants.

Out of the 10  $F_1$  combinations, three combinations (Obrij/NSP11, Szegedi-746/ Žitnica and NS-0-694/Košuta) had the androgenic capacity close to the mid-parental values, and two combinations (9D-27-262/Posavka-2 and Pobeda/Košuta) had the capacity at the level of the parent value. Four combinations (Balkan/Košuta, Rodna/ Košuta, Fundulea 4/Pobeda and Fundulea 4/Rodna) had a higher number of responding anthers than the better parent, while only one combination (Sremica/Košuta) had the value lower then both parents (Graph. 1).

The  $F_1$  combinations Obrij/NSP11, NS-0-694/Košuta and Fundulea 4/Rodna had callus yield close but significantly different from the parent value, indicating partially dominant inheritance and additive gene action. Callus yield values of the  $F_1$  hybrids 9D-27-262/Posavka-2 and Pobeda/Košuta were not significantly different from the parent value, indicating dominant inheritance. All other  $F_1$  combinations had higher callus yield than the better parent, except Sremica/Košuta, which had a lower value than the parent with low callus yield (Graph. 2).

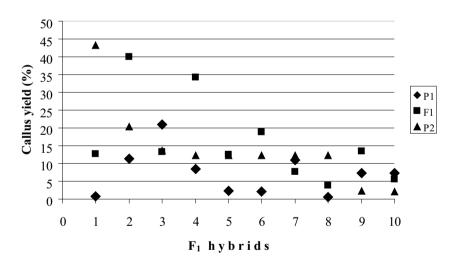
|                        | Androgenic capacity | Callus yield  | Green plants  |
|------------------------|---------------------|---------------|---------------|
| Genotype/cross         | Androgeni kapacitet | Prinos kalusa | Zelene biljke |
| Genotip/Ukrštanje      | (%)                 | (%)           | (%)           |
| Parents/Roditelji:     |                     |               |               |
| 1. Obrij               | 0.7                 | 0.7           | 0.0           |
| 2. NSP11               | 23.3                | 43.3          | 0.7           |
| 3. Szegedi-746         | 11.3                | 11.3          | 0.0           |
| 4. Žitnica             | 20.3                | 20.3          | 4.7           |
| 5. 9D-27-262           | 16.3                | 21.0          | 11.0          |
| 6. Posavka-2           | 13.7                | 13.7          | 4.3           |
| 7. Balkan              | 8.4                 | 8.4           | 0.6           |
| 8. Košuta              | 10.8                | 12.3          | 1.0           |
| 9. Pobeda              | 2.3                 | 2.3           | 0.4           |
| 10. Rodna              | 2.1                 | 2.1           | 0.6           |
| 11. Sremica            | 11.0                | 11.0          | 1.8           |
| 12. NS-0-694           | 0.6                 | 0.6           | 0.0           |
| 13. Fundulea-4         | 3.7                 | 7.3           | 0.4           |
| Mean/Prosek (parents): | 9.6                 | 11.9          | 2.0           |
| F1 hybrids/F1 hibridi: |                     |               |               |
| 1. Obrij/NSP11         | 6.0                 | 12.7          | 0.7           |
| 2. Szegedi-746/Žitnica | 34.7                | 40.0          | 3.0           |
| 3. 9D-27-262/Posavka-2 | 13.3                | 13.3          | 12.0          |
| 4. Balkan/Košuta       | 28.2                | 34.3          | 5.1           |
| 5. Pobeda/Košuta       | 10.5                | 12.5          | 1.6           |
| 6. Rodna/Košuta        | 16.5                | 18.8          | 3.1           |
| 7. Sremica/Košuta      | 7.6                 | 7.6           | 0.6           |
| 8. NS-0-694/Košuta     | 3.9                 | 3.9           | 0.0           |
| 9. Fundulea 4/Pobeda   | 11.6                | 13.5          | 0.6           |
| 10. Fundulea 4/Rodna   | 5.5                 | 5.5           | 0.2           |
| Mean/Prosek (F1s):     | 13.8                | 16.2          | 2.7           |
| LSD/NZR 0.05           | 0.49                | 0.52          | 0.36          |
| 0.01                   | 0.65                | 0.70          | 0.48          |

Table 1. Anther culture response of  $F_1$  wheat hybrids and their parents *Tabela 1. Reakcija u kulturi antera*  $F_1$  *hibrida pšenice i njihovih roditelja* 



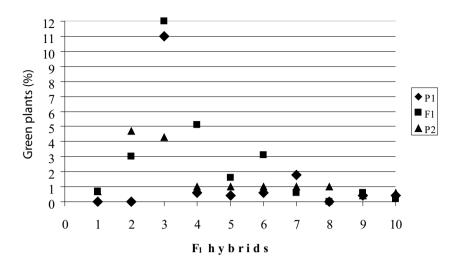
Graph. 1. Relative performance of F<sub>1</sub> wheat hybrids and their parents with respect to androgenic capacity Graf. 1. Relativni odnos F<sub>1</sub> hibrida pšenice i njihovih roditelja u pogledu androgenog kapac-

iteta



Graph. 2. Relative performance of  $F_1$  wheat hybrids and their parents with respect to callus yield Graf. 2. Relativni odnos  $F_1$  hibrida pšenice i njihovih roditelja u pogledu prinosa kalusa

In terms of green plant frequency, one cross (Szegedi-746/Žitnica) showed partially dominant inheritance, two combinations (Obrij/NSP11 and NS-0-694/Košuta) showed dominance, five combinations (9D-27-262/Posavka-2, Balkan/Košuta, Pobeda/ Košuta, Rodna/Košuta and Fundulea 4/Pobeda) exhibited positive and two (Sremica/ Košuta and Fundulea 4/Rodna) negative heterosis in green plant yield (Graph. 3).



Graph. 3. Relative performance of  $F_1$  wheat hybrids and their parents with respect to per cent green plant formation

Graf. 3. Relativni odnos  $F_1$  hibrida pšenice i njihovih roditelja u pogledu formiranih zelenih biljaka

Considerable variation was obtained for all component traits of androgenesis, indicating genotypic specificity of wheat androgenic response. It is in agreement with our earlier results (Ljevnaić et al. 2007; Kondić-Špika et al. 2007), as well as with the results of other authors (Tuvesson et al. 2000; Tersi et al. 2006). Genotypes with high per cent responding anthers and calli induction mostly did not respond favorably to green plants regeneration. This suggests that three component traits of androgenesis are governed by different genes leading to independent inheritance of the parameters. The results are in agreement with earlier reports of Chaudhary et al. (2003), Zamani et al. (2003), Vukosavljev (2009).

Significant differences between the average values of the  $F_1$  hybrids and their parents were found for all characters. The potential heterotic cross combinations identified in this study are Balkan/Košuta and Rodna/Košuta for androgenic and 9D-27-262/Posavka-2 for green plantlet regeneration ability. In the earlier studies (Ekiz and Konzak, 1994; Kondić and Šesek, 1999; Chaudhary et al. 2003) similar results were proposed suggesting that heterozygosity is important for androgenic response in wheat.

### CONCLUSSION

As one parent with a high androgenic response lead to the production of sufficient number of green plants from the  $F_1$  hybrids, it was concluded that screening inbred lines for anther culture response could increase the efficiency of this method if used in combination with well-responsive parent lines.

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# GENETIČKA ANALIZA REAKCIJE GENOTIPOVA PŠENICE U KULTURI ANTERA

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#### Izvod

U radu je ispitana androgena sposobnost kod 10  $F_1$  hibrida pšenice (*Triticum aes-tivum* L.), kao i 13 roditeljskih genotipova.

Utvrđeno je da su ispitivana androgena svojstva (androgeni kapacitet, prinos kalusa i frekvencija zelenih biljaka) u velikoj meri zavisila od genotipa. U proseku za sve ispitivane kombinacije ukrštanja androgeni kapacitet je iznosio 10.6%, sa prosečno formirana 12.2 kalusa na 100 izolovanih antera. Linija 9D-27-262, kao i hibrid koji uključuje ovu liniju (9D-27-262/Posavka-2) imali su najviše frekvencije zelenih biljaka (11% i 12%). Rezultati su pokazali da je kod 30.4% genotipova dobijeno 2 ili više zelenih biljaka na 100 izolovanih antera, što se smatra dovoljnim brojem za korišćenje u oplemenjivanju.

Heterotični efekat, kao i aditivno i dominantno delovanje gena utvrđeno je u kontroli ispitivanih androgenih svojstava.

Ključne reči: androgeneza, nasleđivanje, oplemenjivanje, pšenica

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# PROTEIN AND TRYPTOPHAN CONTENT IN KERNELS OF MAIZE HYBRIDS\*

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SUMMARY: In this work, proteins and tryptophan content were analyzed in kernels of semi-flint, semi-dent, dent and specialty ZP maize hybrids. Protein content, as well as protein fraction, differed significantly among the analyzed maize genotypes. Globulin was the lowest fraction in kernels of all maize hybrids (6.69-11.03% of total protein). The content of albumin was the highest in sweet hybrids ZP 441su and ZP 461su i.e. 20.27% and 19.76% of total protein, respectively.  $\alpha$ -Zein and G-3 glutelin were dominant protein fractions in all maize genotypes. The highest content of  $\alpha$ -zein and G-3 glutelin was in the kernels of popping maize hybrid ZP 611k, i.e. 29.25 and 25.71% of total protein, respectively. All hybrids could be classified according to the tryptophan content in three groups – over 0.08% (sweet and waxy hybrids), from 0.06 to 0.07% (three standard dent hybrids, one semi-flint and two semident hybrid) and from 0.05 to 0.06% (two semi-flint, one semi-dent hybrids and popping).

Key words: maize hybrids, protein fractions, tryptophan

## **INTRODUCTION**

The diets consumed in developed countries usually contain various sources of dietary protein (cereals, legume, meat, etc.). However, in some less developed countries in which a single cereal may account for a major part of the total protein intake, the nutritional quality of the protein as well as the amount may be important.

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Cereals are a major source of dietary protein for humans. Cereal grains have a low protein content and the protein quality is limited by deficiencies in some essential amino acids, mainly lysine and tryptophan. The contents of amino acids in whole cereal grains are largely determined by the starchy endosperms which typically comprise about 80% of the grain dry weight and consist of starch and prolamin rich proteins. The aleurone and embryo tissues of grains contain higher contents of essential amino acids but these are often not available for human nutrition as they are removed by milling (wheat), polishing (rice), pearling (barley) and decorticating (sorghum) (Jensen and Martens, 1983).

The protein quality of maize is similar to other cereals except rice and oats, in which the major storage proteins are related to the 11S globulins ("legumins") of legumes with the prolamins (zein) being only minor components. Protein solubility is an important functional property that affects the utilization and nutritional value of cereal grain. According to the solubility in different solvents, proteins from maize grain can be separated on albumins - water soluble, globulins - salt soluble, zeins - alcohol soluble and glutelins - alkali soluble. The albumins and globulins represent about 6% of total protein, while glutelins account for approximately 30-45% (Konzak, 1977). Zeins and glutelins constitute storage proteins of maize kernels. Zein fraction accounts for about 50 % of the total endosperm protein. Zeins can be separated into four distinct subfractions:  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ .  $\alpha$ -Zein are by far the most abundant, making up to approximately 70 % of the total. It is located in large central portion of the protein body with  $\beta$ - and  $\gamma$ -zeins surrounding it on periphery, which can be the reason for higher thermo stability of zein fraction (Lending and Larkins, 1989). Also, zein has some of the properties of wheat gluten but is not able to form viscoelastic fibrils at room temperature, though it can be made functional in this way at higher temperatures (Bugusu et al., 2001; Schober et al., 2008). Three glutelin subgroups, denoted G1, G2 and G3, constitute alkali soluble maize storage protein. Interpolypeptide disulfide bonds make glutelin poorly soluble (Landry and Moureaux, 1981), except for G3-glutelins, which have an amino acid composition similar to that of salt-soluble proteins.

Zein is characterized by high contents of glutamine, leucine, proline, and partially is devoid of two essential amino acids - lysine and tryptophan, which determine corn protein as nutritionally inadequate. In contrast, albumins and globulins are high in lysine and arginine while glutelins are intermediate.

To improve the nutritional value of maize varieties, both for animal feed and for human consumption, the content and quality of amino acids of grain storage proteins have to be changed. The first biofortified crop was *opaque-2* (*o2*) maize, bred for its improved protein quality. At the time, this discovery was considered a potentially major breakthrough to help reduce protein deficiency, which was then considered the world's most pressing nutrition problem. Subsequent conventional breeding led to *o2* maize varieties with improved agronomic characteristics, now known as QPM - quality protein maize (Vasal, 2000).

This research is focused on analyses of protein and tryptophan content in kernels of standard, sweet, popping and waxy ZP maize hybrids. A more detailed knowledge of chemical properties of ZP maize genotypes will be beneficial in the future selection of maize hybrids, as well as production of maize food with improved nutritional quality.

## MATERIALS AND METHODS

The kernels of 13 maize (*Zea mays* L.) hybrids developed at the Maize Research Institute, Zemun Polje, (MRIZP) Belgrade, Serbia, were used for this study. Kernels were collected at full maturity stage from plants grown in a field-trial at the MRIZP in 2008 growing season. The maize genotypes were chosen on the basis of their differences in agronomic traits and physical characteristics of the kernels: three semi-flint hybrids (ZP 209, ZP 745, ZP Prado), three semi-dent hybrids (ZP 505, ZP 643, ZP 717), three dent hybrids (ZP 434, ZP 544, ZP 688) and four specialty maize hybrids (popping ZP 611k; waxy ZP 704xw and two sweet ZP 441su, ZP 461su). The wholemeal flour (particle size<500 µm), obtained by grounding maize on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden) was used in the analyses.

Different protein fractions were obtained by succesive extractions of defatted maize flour with a series of solvents (in a ratio 1:10 w/v) according to the Landry and Moureaux (1970) method, with some modifications. Distilled water, 0.5 M NaCl, 70% ethanol and 0.0125 M borate buffer, pH 10, containing 5% sodium dodecyl sulfate (SDS), were used to extract albumin, globulin,  $\alpha$ -zein and glutelin (G3-glutelin) fractions, respectively. Extraction of each protein fraction was done by repeated stirring three times for 30 min at 4°C, followed by centrifugation at 20 000 g for 15 min. Protein content was calculated, in each fraction, from the nitrogen content determined by micro Kjeldahl method, using 6.25 as the conversion factor. The results are given as percentage of total protein (protein solubility index).

Tryptophan content was determined according to Nurit et al. (2009). Shortly, flour hydrolysate (obtained by overnight digestion with papain solution at 65°C) was added to 3 ml reagent containing Fe<sup>+3</sup> (1 g FeCl<sub>3</sub> dissolved in 50 ml 7 N H<sub>2</sub>SO<sub>4</sub>), 30 N H<sub>2</sub>SO<sub>4</sub> and 0.1 M glyoxilic acid. After incubation at 65°C for 30 min, absorption was read at 560 nm. Tryptophan content was calculated using a standard (calibration) curve, developed with known amounts of tryptophan, ranging from 0 to 30  $\mu$ g/ml. Besides, tryptophan content quality index (QI), defined as tryptophan to protein ratio in the sample, was also calculated.

All chemical analyses were performed in three replicates and the results were statistically analysed. Significant statistical differences of observed chemical maize parameter means were determined by the Fisher's least significant differences (LSD) test, after the analysis of variance (ANOVA) for trials set up according to the RCB design. Correlations between parameters were examined using the Peterson correlation.

### **RESULTS AND DISCUSSION**

Data in Table 1 indicate that protein content, as well as soluble protein fractions among analyzed maize genotypes, differs significantly (P < 0.05). The sweet and popping hybrids ZP 441su and ZP 611k had higher content of total protein (12.73 and 12.41% of d.m., respectively) than any of the other eleven maize genotypes, which protein content varied from 9.11% (ZP 688) to 11.69% (ZP 461su).

Protein fractions were isolated according to their solubility in different solutions. In our study globulin was the lowest fraction in kernels of all maize hybrids (6.69-11.03% of total protein). The content of albumin was 20.27% and 19.76% of total protein in ZP 441su and ZP 461su hybrids, respectively. In the kernels of the other hybrids,

the albumin content was lower by 33% to 54%, ranging from 9.43% of total protein in kernels of popping maize hybrid ZP 611k to 15.48% of total protein in typical dent genotypes ZP 688. Also, typical dent genotypes ZP 434 and ZP 544 had higher content of albumin (14.08 and 14.32% of total protein, respectively) than other standard semi-flint and semi-dent hybrids. Content of soluble proteins is affected by the kernel structure. Bulk of the proteins in a mature maize kernel is present in the endosperm and germ, but the germ protein is superior in both quantity and quality. The albumins and globulins are present mainly in the germ (Shukla and Cheryan, 2001). The kernel of sweet hybrid ZP 441su with the highest content of albumin fraction had the highest portion of germ, i.e. 20.0% of the grain weight. Also, kernels of popping maize hybrid ZP 611k with the lowest portion of germ (9.7%) had the lowest content of albumin fraction (Table 2).

α-Zein and G-3 glutelin were the dominant protein fractions in all maize genotypes. The highest content of  $\alpha$ -zein and G-3 glutelin was in the kernel of popping maize hybrid ZP 611k i.e. 29.25 and 25.71% of total protein, respectively. Almost all the zein is present in the maize endosperm, whereas glutelin is distributed between the endosperm and the germ (Shukla and Chervan, 2001). In normal maize, proportions of various endosperm storage protein fractions, on an average, are: albumins (3%), globulins (3%), zeins (60%) and glutelins (34%) (Prasanna et al., 2001). Zeins have never been detected in any part of the plant other than the kernel. In our study the popping maize hybrid ZP 611k had the highest portion of hard endosperm per the grain weight (80.0%) (Table 2). Semi-dent and dent maize hybrids (ZP 505 and ZP 434) had higher content of  $\alpha$ -zein (25.5 and 24.26% of total protein, respectively) than other semi-flint, semi-dent and dent hybrids. The lowest content of  $\alpha$ -zein was in kernels of sweet maize ZP 441su (17.05% of total protein) and in the kernels of dent maize hybrids ZP 688 (18.44% of total protein). Malumba et al. (2008) reported that zein was the most abundant protein group extracted from the whole maize kernel, followed by glutelin-G1 and glutelin-G3. Content of zein was 33.5%, while G-3 glutelin was 18.1%. Differences observed between the present study and previous study is possibly due to the maize genotypes used. A by-product (so-called "gluten-meal") of corn-starch manufacturing can be relied on for the supply of zein sources, but it is not effectively used for food materials. The main reason is that the maize protein is, not only poor in its nutritive value (low in tryptophan and lysine), but also cumbersome for food processing because of its gummy cohesion in a hydrated state. Deamidation, or partial cleavage of the peptide bond, leads to a pronounced change in the functional properties of zein. As the fragmentation reaction proceeds, however, a favorable feature, represented by antioxidation, vanishes. Additionally, the possibility cannot be excluded that a crude zein preparation contains physiologically active substances which benefit rather than damage health (Chiue et al., 1997). The highest content of G-3 glutelin was in kernels of semi-dent maize hybrids ZP 643 and ZP Prado i.e. 23.44 and 24.06% of total protein, respectively. However, the kernel of sweet hybrid ZP 461su had the lowest content of G3-glutelin i.e. 16.25% of total protein. Our results are in accordance with results obtained by Fageer and El Tinay (2004). These authors reported that the content of true glutelin (G3-glutelins) varied from 10.8% to 21.9% in grains of twelve maize genotypes, as well as albumins and globulins from 16.8% to 22.7%.

Table 1. Content of soluble protein fractions in the kernel of ZP maize hybrids. Results are presented as % of total protein

| Hybrids  | Protein | Albumin | Globulin | α-Zein | G3-Glutelin |
|----------|---------|---------|----------|--------|-------------|
| ZP 209   | 10.15g  | 12.71h  | 9.36d    | 22.48d | 21.38ef     |
| ZP 434   | 9.52j   | 14.08e  | 9.98bc   | 24.26c | 19.96h      |
| ZP 441su | 12.73a  | 20.27a  | 7.62f    | 17.05i | 20.42gh     |
| ZP 461su | 11.69c  | 19.76b  | 7.10g    | 20.79g | 16.25i      |
| ZP 505   | 9.70i   | 12.26i  | 11.03a   | 25.05b | 20.31gh     |
| ZP 544   | 9.50jk  | 14.32d  | 9.68c    | 22.84e | 21.26f      |
| ZP 611k  | 12.41b  | 9.431   | 6.69h    | 29.25a | 25.71a      |
| ZP 643   | 9.43k   | 12.94f  | 8.59e    | 22.80e | 23.44c      |
| ZP 688   | 9.111   | 15.48c  | 10.76a   | 18.44h | 20.64g      |
| ZP 704wx | 11.34d  | 13.76e  | 10.32b   | 22.75e | 20.37gh     |
| ZP 717   | 9.98h   | 11.72j  | 8.52e    | 22.24f | 22.34d      |
| ZP 745   | 10.41f  | 13.06g  | 8.84e    | 22.00f | 21.71e      |
| ZP Prado | 10.85e  | 10.78k  | 9.86c    | 20.92g | 24.06b      |
| LSD0.05  | 0.099   | 0.281   | 0.351    | 0.291  | 0.477       |

Tabela 1. Sadržaj rastvorljivih proteina u zrnu ZP hibrida kukuruza. Rezultati su iskazani kao % od ukupnih proteina

<sup>a-l</sup> Means followed by the same letter within the same columns are not significantly different (P<0.05)

<sup>a-l</sup> Srednje vrednosti označene istim slovima unutar iste kolone nisu značajno različite (P<0.05)

Table 2. The portion of pericarp, germ and endosperm per kernel weight of ZP maize hybrids (%)

Tabela 2. Udeo perikarpa, klice i endosperma u zrnu ZP hibrida kukuruza (%)

| Hybrids  | Pericarp | Germ   | Whole<br>endosperm | Hard endosperm | Soft<br>endosperm |
|----------|----------|--------|--------------------|----------------|-------------------|
| ZP 209   | 6.0e     | 11.9de | 82.1a              | 71.5d          | 28.5f             |
| ZP 434   | 7.3c     | 13.3c  | 79.4d              | 62.5g          | 37.5c             |
| ZP 441su | 6.2e     | 20.0a  | 73.7e              | 61.0h          | 38.9b             |
| ZP 461su | 9.0a     | 19.1b  | 71.9f              | 58.5i          | 41.5a             |
| ZP 505   | 6.2e     | 12.2d  | 81.7b              | 71.1de         | 28.9f             |
| ZP 544   | 6.4d     | 13.0c  | 80.6               | 61.5h          | 38.5b             |
| ZP 611k  | 8.6b     | 9.7f   | 81.7b              | 80.0a          | 20.0i             |
| ZP 643   | 7.8c     | 12.6cd | 79.6d              | 69.1f          | 30.9e             |
| ZP 688   | 6.7d     | 11.8de | 81.5b              | 61.3h          | 38.7b             |
| ZP 704wx | 5.8ef    | 13.4c  | 80.8c              | 65.7g          | 34.3d             |
| ZP 717   | 6.1e     | 12.6cd | 81.4b              | 72.2c          | 27.8g             |
| ZP 745   | 6.3de    | 11.4e  | 82.3a              | 73.1b          | 26.9h             |
| ZP Prado | 6.1e     | 12.9cd | 81.1bc             | 71.8d          | 28.2fg            |
| LSD0.05  | 0.325    | 0.480  | 0.534              | 0.582          | 0.560             |

 $^{\rm a-i}$  Means followed by the same letter within the same columns are not significantly different (P<0.05)

 $a^{-i}$  Srednje vrednosti označene istim slovima unutar iste kolone nisu značajno različite (P<0.05) In our study, the albumin content was negatively correlated to the content of glob-

ulin,  $\alpha$ -zein and G3-glutelin concentrations (r = 0.20, r = 0.70 and r = 0.80 respectively, P < 0.05). However,  $\alpha$ -zein content was positively correlated to the content of G3-glutelin (r = 0.44, P < 0.05).

The quality of maize proteins is poor because they are deficient in the essential amino acids, lysine and tryptophan (Shewry, 2007). Since these two amino acids are highly correlated, tryptophan is usually used as a single parameter for evaluating the nutritional quality of the grain protein (Hernandez and Bates, 1969a). Results of tryptophan analysis in kernels of maize genotypes determined by Nurit et al. (2009) colorimetric method and levels of statistical significance obtained from analysis of variance, are summarized in Table 3

The data indicate that the levels of tryptophan in kernels of semi-flint hybrids ZP 209 and ZP 745, semi-dent hybrid ZP 505 and popping maize ZP 611k were similar and lower than the other maize genotypes (in average 0.055% of d.m.). According to our results, the highest tryptophan content was detected in kernels of sweet hybrid ZP 441su i.e. 0.093% of d.m. Also, this hybrid had the highest portion of germ, i.e. 20.0% of the grain weight (Table 2), as well as the highest content of albumin. However, this hybrid had the highest content of total protein and this could be the reason for QI somewhat below the QPM treshold level, i.e. 0.72. (Table 3).

Table 3. Tryptophan content and quality index in in the kernel of ZP maize hybrids *Tabela 3. Indeks kvaliteta proteina i sadržaj triptofana u zrnu ZP hibrida kukuruza* 

| Hybrids  | Tryptophan (%) | QI     |
|----------|----------------|--------|
| ZP 209   | 0.056ef        | 0.56g  |
| ZP 434   | 0.066bcd       | 0.70cd |
| ZP 441su | 0.093a         | 0.72bc |
| ZP 461su | 0.083a         | 0.72bc |
| ZP 505   | 0.058def       | 0.60f  |
| ZP 544   | 0.070bc        | 0.73ab |
| ZP 611k  | 0.055ef        | 0.443i |
| ZP 643   | 0.071b         | 0.75a  |
| ZP 688   | 0.061cde       | 0.67d  |
| ZP 704wx | 0.086a         | 0.75a  |
| ZP 717   | 0.063bcde      | 0.64e  |
| ZP 745   | 0.051f         | 0.49h  |
| ZP Prado | 0.062bcde      | 0.57f  |
| LSD0.05  | 0.015          | 0.022  |

 $^{a-g}$  Means followed by the same letter within the same columns are not significantly different (P<0.05)

 $^{ag}$  Srednje vrednosti označene istim slovima unutar iste kolone nisu značajno različite (P<0.05)

Waxy and sweet maize (ZP 704wx and ZP 461su) also had high tryptophan content (0.086 and 0.083% of d.m., respectively) and QI (0.75 and 0.72, respectively) (Table 3). Content of tryptophan in kernels of typical dent hybrids (ZP 434, ZP 544 and ZP 688) ranged from 0.061 to 0.070% of d.m. It was interesting that semi-dent hybrid ZP 643 had higher content of tryptophan (0.071% of d.m.) and QI (0.75) than typical dent hybrids (Table 3). The maize genotypes analyzed by Vyu and Yollenaar (1998) contained on average 0.072% of tryptophan. The values obtained in our study were close to this average. In the study of Ignjatović-Micić, et al., (2009) content of triptophan in kernels of normal and *opaque*-2 maize inbred lines ranged from 0.071 to 0.136% of d.m.

Three out of 13 ZP hybrids had tryptophan over the QPM threshold limit, but five hybrids had QI close to the threshold value (ZP 441su, ZP 461su, ZP 544, ZP 643

and ZP 704wx) (Table 3). The QPM threshold values for tryptophan content are 0.07% (endosperm) and 0.075% (whole grain), while for QI they are 0.7 and 0.8%, respectively (Vasal, et al., 1996). The nutritional benefits of QPM for people, who depend on maize for their energy and protein intake, and for other nutrients, are indeed quite significant. QPM protein contains, in general, 55% more tryptophan, 30% more lysine and 38% less leucine than that of normal maize (Prasanna et al., 2001).

Our results are in accordance with the data presented by Segal et al. (2003) and Huang et al. (2006) that the decrease in zein resulted in the increased grain lysine and tryptophan content. Tryptophan content was negatively correlated to contents of globulin,  $\alpha$ -zein and G3-glutelin (r = 0.27, r = 0.53 and r = 0.51 respectively, P < 0.05) and positively correlated to the albumin content (r = 0.78, P < 0.05).

Considering that a significant number of metabolic disorders and diseases are caused by malnutrition, and the fact that the majority of the world population consumes maize as the main bread grain, one of the future important breeding objectives in the Maize Research Institute will be development of maize genotypes with the improved nutritive value.

### CONCLUSION

Currently, limited information is available on the biochemical and genetic mechanisms that regulate high-proteins. Essentially two avenues of improving maize protein have to be utilized. The first is through traditional breeding using high-protein germplasm and the second is the use of biotechnology.

Our results showed differences in the concentration of soluble proteins and tryptophan among studied ZP maize genotypes. The highest content of albumin and tryptophan was in kernels of sweet maize hybrids. Popping maize hybrid had the highest content of  $\alpha$ -zein and the lowest content of tryptophan. Standard dent hybrids had higher content of albumin than other standard maize hybrids. Among the standard ZP hybrids, semi-dent and dent hybrids (ZP 643, ZP 544) had the highest content of tryptophan.

In conclusion, it appears that among ZP maize genotypes there are genotypes with different amount of protein fractions and tryptophan that should be bred in the future for a desired level and quality of the protein components.

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# SADRŽAJ PROTEINA I TRIPTOFANA U ZRNU HIBRIDA KUKURUZA

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#### Izvod

U ovom radu ispitivan je sadržaj rastvorljivih proteina i triptofana u zrnu polutvrdunaca, poluzubana, zubana i specifičnih ZP hibrida kukuruza. Sadržaj ukupnih proteina, kao i sadržaj rastvorljivih proteinskih frakcija se statistički razlikovao između analiziranih genotipova kukuruza. Globulin je bio najmanjie zastupljena frakcija u kukuruznom zrnu (6.69-11.03% od ukupnih proteina). Sadržaj albuminske frakcije u zrnu hibrida šećeraca ZP 441su i ZP 461su iznosio je 20.27% i 19.76% od ukupnih proteina, dok je u zrnu ostalih hibrida bio značajno niži.  $\alpha$ -zein i G3-glutelin bile su dominantne frakcije u zrnu svih ispitivanih genotipova kukuruza. Najviši sadržaj  $\alpha$ -zeina i G3-glutelina bio je u zrnu kukuruza kokičara ZP 611k i iznosio je 29.25% i 25.71% od ukupnih proteina. Prema sadržaju triptofana svi analizirani hibridi mogu se podeliti u tri grupe: preko 0.08% (šećerci i voksi hibridi – ZP 441su, ZP 461su i ZP 704wx), od 0.06 do 0.07% (tri standardna zubana - ZP 434, ZP 544, ZP 688, poluzubani - ZP 643, ZP 717 i polutvrdunac ZP Prado), od 0.05 do 0.06% (dva polutvrdunca – ZP 209, ZP 745, jedan poluzuban i kokičar – ZP 505, ZP 611k).

Ključne reči: hibridi kukuruza, rastvorljive proteinske frakcije, triptofan.

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# NUTRITIVE VALUE OF RAPESEED MEAL IN NUTRITION OF BROILER CHICKENS

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SUMMARY: In this paper, results relating to nutritive value of rapeseed meal on production performances of fattening chickens are presented. Investigation was carried out on Ross 308 chickens in duration of 42 days. Control group of chickens (group 1) was fed standard mixture (starter, grower and finisher) based on corn, sovbean meal and full fat sovbean extruded as basic feeds, and share of rapeseed meal in diets for trial chickens was 10% (group 2) and 15% (group 3). Results showed that control group of chickens had realized final body mass of 2.250 kg, chickens of group 2 body mass of 2.243 kg, and group 3 had realized the lowest value - 2.181 kg. Feed conversion was equal in all three groups of chickens (1-1.864; 2-1.880; 3-1.861). Mortality was 3.16% in control group, 3.13% in group 2 and 7.16% in group 3. Production index had value of 270 in group 1, 268 in group 2 and 251 in group 3. Differences in all investigated parameters, with the exception of feed conversion, between group 3 and other groups were statistically significant (P<0.05). Presence of 15% of rapeseed meal in diets had exhibited depressive effect on production parameters of chickens and inclusion of this feed in higher percentage can have negative effect on success of broiler fattening.

Key words: rapeseed meal, broilers, production traits

## INTRODUCTION

Rape seed is important plant culture for production of oils, plant proteins and renewable energy. There are several species within the group of *Brassica sp.* But two are the most popular *B. napus L.* and *B. rapa L.*, and many cultivars and hybrids grown within said species (Sovero, 1993, Straková et al., 2008). Two type most present are rapeseed (Sweden) and canola, which are very similar plant species.

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Significance of rapeseed as oil crop is increasing since rapeseed grain contains over 40% of oil and 18-23% of protein (Munoz-Valenzuela et al., 2002, Milošević et al., 2007). Reason for growing interest in this plant is also the fact that rapeseed oil is used as source of renewable energy (bio-fuel) and raw material for motor oils and hydraulic lubricants.

By-products in processing of rapeseed grain are cake and meal, rich in biologically valuable proteins used in nutrition of most of livestock species and categories. Until recently,

rapeseed meal was not used intensively in poultry nutrition, but it was established that it can be included into diets even for fattening broiler chickens (Stanaćev et al., 2006, Stanaćev et al., 2007, Milošević et al., 2007, Milošević et al., 2008, Lukić et al., 2009). Rapeseed meal is standardized feed which is sold on the market with protein content of 35-38%, carbon hydrate content of 15-16%, content of crude fibre of 11-12%, moisture 8-10%, ashes 6-7%, oil 3-4% (Newkrik and Classen,1999, Slominski et.al., 1999, Canola Council of Canada, 2005, Milošević et al., 2008). This feed also contains significant amounts of mineral matters and vitamins, especially phosphorus, of good availability even though phytine acid is present (Zeb, et al., 2002, Milošević et al., 2007). Disadvantage is increased content of anti-nutritive matters. It containes higher amounts of glucosinolate, some cultivars up to 100 µmol/g and 4-5% of erucic acid (Donald and Basin, 1990, Canola Council of Canada, 2005). Disadvantage of this feed is also low content of metabolic energy of approx. 2000 kcal/kg (Jokić, et al., 2004, Canola Council of Canada, 2005). New rapeseed cultivars contain significantly less anti-nutritive matters (glucosinolate and erucic acid). There are numerous examples where rapeseed meal exhibited significant production impact in broiler nutrition, and considering its very acceptable price it is used more and more in nutrition of this poultry category.

In great number of studies and researches it was confirmed that chickens fed diets containing rapeseed meal had realized production performance equal to those fed diets based on soybean meal or slightly behind (Tadelle, et al., 2003, Kralik, et al., 2003, Stanaćev, et al., 2006, Milošević, et al., 2005, Milošević, et al., 2008).

Rapeseed meal is interesting for use in livestock production because of the price, since use of this meal significantly substituted use of soybean meal which is three times more expensive. According to the recommendations of the Canola Council of Canada, (2005) heat treated rapeseed meal or rapeseed meal deriving from cultivars with low content of anti-nutritive matters can be included in diets for pigs and poultry up to 20%. Stanaćev, et al., (2006) and Milošević, et al., (2005) recommend that rapeseed meal in diets for fattening chickens can be used in the amount of up to 8 % without detrimental effect on production results and health condition of chickens.

Objective of this study was to investigate potential of significant inclusion of rapeseed meal in diets for fattening chickens.

## MATERIAL AND METHODS

Investigation of the influence of rapeseed meal on production performances of fattening chickens was carried out on three groups of chickens in 4 repetitions on total of 300 chickens in each treatment. Trial lasted 42 days. In this trial Ross 308 chickens were used. Chickens were weighed individually and weekly. During the trial health con-

dition of chickens was regularly controlled as well as dead and rejected birds recorded. Chickens were reared according to standard technology for Ross 308 strain. Feed and water were ad libitum.

Mixtures used in this study were chemically analyzed in the Laboratory for examination of the quality of livestock feed of the Faculty of Agriculture, Novi Sad. Three mixtures were used in nutrition of chickens: initial or starter mixture, grower and final mixture or finisher. During the last week of the trial chickens were fed mixture without coccidiostats. Composition of used mixtures is presented in table 1. EPEF was represented aggregate number and calculated as EPEF = average body mass (g) x % surviving chickens x 100 / feed conversion x duration of fattening, days.

| No. | Termediant Innetice                                   | Star  | ter - Sta | arter | Grower - Grover |       |       | Finisher - Finišer |       |       |
|-----|---|-------|-----------|-------|-----------------|-------|-------|--------------------|-------|-------|
| Br. | Ingredient - hraniva                                  | 1     | 2         | 3     | 1               | 2     | 3     | 1                  | 2     | 3     |
| 1   | Maize - <i>kukuruzna prekrupa</i>                     | 49.00 | 45.00     | 43.00 | 52.80           | 49.40 | 47.80 | 60.00              | 56.00 | 55.00 |
| 2   | Soybean meal - <i>sojina sačma</i>                    | 28.00 | 21.00     | 16.80 | 23.00           | 15.00 | 12.20 | 18.00              | 11.80 | 8.00  |
| 3   | Full fat soybean extruded - sojin griz                | 13.80 | 14.10     | 15.00 | 14.20           | 15.00 | 14.00 | 12.00              | 11.00 | 10.90 |
| 4   | Rapeseed meal - sačma uljane repice                   | -     | 10.00     | 15.00 | -               | 10.00 | 15.00 | -                  | 10.00 | 15.00 |
| 5   | Yeast torula - kvasac torula, (sušen)                 | 3.00  | 3.00      | 3.00  | 3.00            | 3.00  | 3.00  | 3.00               | 3.00  | 3.00  |
| 5   | Oil - <i>ulje</i>                                     | 1.80  | 2.70      | 3.00  | 2.60            | 3.40  | 4.00  | 2.60               | 4.00  | 4.20  |
| 6   | MCP (monocalcium phosphate)-MKF                       | 1.55  | 1.35      | 1.25  | 1.55            | 1.35  | 1.25  | 1.60               | 1.50  | 1.20  |
| 7   | Limestone - stočna kreda                              | 1.40  | 1.40      | 1.50  | 1.40            | 1.40  | 1.30  | 1.40               | 1.30  | 1.30  |
| 8   | Salt - so   | 0.30  | 0.30      | 0.30  | 0.30            | 0.30  | 0.30  | 0.30               | 0.30  | 0.30  |
| 9   | Methionine - metionin                                 | 0.15  | 0.15      | 0.15  | 0.15            | 0.15  | 0.15  | 0.10               | 0.10  | 0.10  |
| 10  | Premix - premiks                                      | 1.00  | 1.00      | 1.00  | 1.00            | 1.00  | 1.00  | 1.00               | 1.00  | 1.00  |
|     | Total - <i>ukupno</i>                                 | 100.0 | 100.0     | 100.0 | 100.0           | 100.0 | 100.0 | 100.0              | 100.0 | 100.0 |
|     | Chemical composition - hemijski sastav                | , %   |           |       |                 |       |       |                    |       |       |
| 1   | ME MJ/kg- (calculated - <i>izračunato</i> )           | 12.83 | 12.82     | 12.83 | 13.26           | 13.29 | 13.28 | 13.44              | 13.48 | 13.46 |
| 3   | Crude protein - sirovi protein                        | 22.61 | 22.84     | 22.95 | 20.98           | 21.04 | 21.14 | 18.63              | 18.70 | 18.69 |
| 6   | Calcium - <i>kalcijum</i>                             | 0.90  | 0.92      | 0.97  | 0.89            | 0.91  | 0.88  | 0.88               | 0.88  | 0.86  |
| 7   | Phosphorus - <i>fosfor</i> – (total - <i>ukupni</i> ) | 0.76  | 0.78      | 0.80  | 0.74            | 0.76  | 0.77  | 0.73               | 0.77  | 0.74  |
| 8   | Methionine - metionin                                 | 0.51  | 0.53      | 0.54  | 0.49            | 0.50  | 0.51  | 0.41               | 0.42  | 0.43  |
| 9   | Lysine - <i>lizin</i>                                 | 1.26  | 1.30      | 1.32  | 1.14            | 1.16  | 1.17  | 0.95               | 0.99  | 0.99  |

Table 1. Composition of starter, grower and finisher diets, % Tabela 1. Sastav starter, grover i finišer smeša, %

## Group 1 (Grupa 1): Control - Kontrola

Group 2 (*Grupa 2*): Rapeseed meal - content in diet 10% - *Sačma uljane repice - nivo u smeši 10%* Group 3 (*Grupa 3*): Rapeseed meal - content in diet 15% - *Sačma uljane repice - nivo u smeši 15%* 

At the end of the 6<sup>th</sup> week of fattening chickens were selected (10 males and 10 females per treatment) of average body mass, slaughtered manually and carcasses were processed for the purpose of investigation of slaughter parameters and share of abdominal fat in carcasses. Quantity of abdominal fat was placed into relation to body mass prior to slaughtering, and in this way relative share of abdominal fat in carcasses was calculated. Computer program Statistica 8, (2009) was used to establish mean values and variability measures. In the same way the variance analysis was done, and in regard to demonstration of statistical significance in variance analysis LSD test at the level of probability of 0.05% was used.

#### **RESULTS AND DISCUSSION**

Obtained results presented in table 2 show that final body masses of chickens fed diets with 15% of rapeseed meal at  $42^{nd}$  day of fattening were significantly lower compared to chickens of control group which were fed diets based on soybean and corn, whereas chickens fed diets containing 10% of rapeseed meal had final body masses similar to those of the control group. Depression of the chicken growth when rapeseed meal was used in percentage above 10% was established also by other authors (Zeb, et al., 2002, Stanaćev, et al., 2007, Milošević, et al., 2008). Adequate to final body masses also values for daily gains according to treatments varied. Average daily gains were realized at the level of slightly over 50g. Mortality of chickens was within technological norms in control group and group with 10% of rapeseed meal in diet, but significantly higher in group of chickens fed diet containing 15% of rapeseed meal. Difference was statistically significant (P<0.05) in relation to K and T2 group of chickens. Increased mortality in T2 group can not exclusively be attributed to increased amount of rapeseed meal, but also to random effect, since only few authors have obtained similar results (Karunajeewa, et al., 1999, Tadelle, 2003).

|                      | -   |               | -                                    |                                  |                          |                     |
|----------------------|---|---------------|--------------------------------------|----------------------------------|--------------------------|---------------------|
| Treatment<br>Tretman | Start- <i>Početna</i> ,<br>B.W <i>T.M.</i> <b>1</b> g | Final-Zavisna | Growth/day, g<br>Dnev.<br>prirast, g | Mortality,%<br><i>Uginuće,</i> % | Conversion<br>Konverzija | EPEF - <i>P.I.2</i> |
| C (K)                | 41.2  | 2250 <b>b</b> | 52.29b                               | 3.16b                            | 1.863                    | 270                 |
| T1                   | 41.1  | 2243 <b>b</b> | 52.43b                               | 3.13b                            | 1.880                    | 268                 |
| T2                   | 40.5  | 2181 <b>a</b> | 50.96a                               | 7.15a                            | 1.860                    | 251                 |

Table 2. Parameters of chicken meat production (42. day of age) Tabela 2. Proizvodni parametri brojlerskih pilića (42. dana starosti)

<sup>a-b</sup> Means within a column with different superscripts differ significantly ( $P \le 0.05$ )

<sup>*a-b*</sup> Vrednosti unutar kolone sa različitim slovima značajno se razlikuju ( $P \le 0.05$ )

<sup>1</sup>T.M. - Body weight of chicken - Telesna masa pilića

<sup>2</sup> EPEF - P.I. - Proizvodni indeks

Feed conversion was satisfactory in all groups, with minimal diffrences between treatments, which were not statistically significant. According to EPEF chickens of the control group had the best values, followed by the group fed diets with 10% of rapeseed meal and the lowest value, but these differences were not statistically significant. Obtained results for feed conversion and production index are in concordance with most of literature references (Tadelle, 2003, Stanaćev, et al., 2006, Milošević, et al., 2005).

| Treatment | Sex B.W.       | B.W. g | Processing percentage - Randmani |       |        |        |        |       | Ab. fat-Ab. mast |      |
|-----------|----------------|--------|----------------------------------|-------|--------|--------|--------|-------|------------------|------|
| Tretman   | pol            | Ũ      | KO, g                            | KO, % | SZP, g | SZP, % | SZR, g | SZR,% | g                | %    |
|           | М              | 2228   | 1848                             | 82.96 | 1728   | 77.55  | 1571   | 70.53 | 18.80            | 0.84 |
| C(K)      | $F(\check{Z})$ | 1908   | 1591                             | 83.39 | 1493   | 78.22  | 1356   | 71.07 | 17.00            | 0.89 |
|           | M+F            | 2138   | 1778                             | 83.18 | 1665   | 77.89  | 1514   | 70.80 | 17.90            | 0.84 |
|           | М              | 2184   | 1832                             | 83.87 | 1706   | 78.12  | 1549   | 70.91 | 15.20            | 0.66 |
| T1        | $F(\check{Z})$ | 1860   | 1548                             | 83.25 | 1455   | 78.24  | 1314   | 70.62 | 19.80            | 1.06 |
|           | M+F            | 2046   | 1710                             | 83.56 | 1600   | 78.18  | 1448   | 70.77 | 16.00            | 0.78 |
|           | М              | 2176   | 1832                             | 84.18 | 1707   | 78.44  | 1543   | 70.90 | 16.60            | 0.76 |
| T2        | $F(\check{Z})$ | 1792   | 1478                             | 82.45 | 1381   | 77.06  | 1236   | 68.96 | 17.80            | 0.99 |
|           | M+F            | 1984   | 1653                             | 83.32 | 1543   | 77.75  | 1387   | 69.93 | 17.20            | 0.87 |

 Table 3. Slaughter parameters according to treatments

 Tabela 3. Klanični parametri po tretmanima

KO - Processing percentage traditional - Klasična obrada trupa

SZP - Processing percentage ready to roast - Trup spreman za pečenje

SZR - Processing percentage ready to broil - Trup spreman za roštilj

Rapeseed meal in diets had no effect on quality of carcasses. Differences in content of abdominal fat were also minimal except in group T1 where in male chickens lower content of fat in carcasses was established compared to other groups of chickens.

## CONCLUSION

Based on these studies, it can be concluded that rapeseed meal is feed of high quality which can be used with certain limitations in broiler nutrition. It can be used as substitute for plant protein feeds in different combinations. It can be used in the amount of up to 10% in mixtures without any potential threat of serious depression in regard to production traits of broiler chickens, or negative consequences on health condition of chickens. Recommendation to farmers is to use this feed in nutrition of broiler chickens, because by introduction of this feed into diets other more expensive feeds can be substituted, primarily soybean meal, and this will reduce the cost of complete mixtures and increase the profitability of the poultry meat production.

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# NUTRITIVNA VREDNOST SAČME ULJANE REPICE U ISHRANI BROJLERSKIH PILIĆA

# NIKO MILOŠEVIĆ, VIDICA STANAĆEV, LUKIĆ MILOŠ, SINIŠA BJEDOV

#### Izvod

U radu su prikazani rezultati nutritivne vrednosti sačme uljane repice na proizvodne parametre tovnih pilića. Ispitivanje je izvedeno na pilićima Ross 308 hibrida u trajanju od 42 dana. Kontrolna grupa pilića (gupa 1) hranjena je standardnom smešom (starter, grover i finišer) na bazi kukuruza, sojine sačme i sojinog griza kao osnovnih hraniva. Udeo sačme uljane repice u obrocima oglednih pilića bio je 10% (grupa 2) i 15% (grupa 3). Rezultati su pokazali da je kontrolna grupa pilića ostvarila završnu telesnu masu 2.250 kg, dok je grupa 2 imala telesnu masu 2.243 kg, a grupa 3, je bila najlošija sa 2.181 kg. Konverzija hrane je bila ujednačena kod sve tri grupe pilića (1-1.864; 2-1.880; 3-1.861). Mortalitet je bio 3.16% kod kontrolne grupe, 3.13% kod grupe 2 i 7.16% kod grupe 3. Proizvodni indeks je imao vrednost 270 kod grupe 1, 268 kod grupe 2 i 251 kod grupe 3. Razlike po svim ispitivanim parametrima, osim po vrednosti konverzije hrane između grupe 3 i ostalih grupa su bile statistički značajne (P<0.05). Pokazalo se da učešće sačme uljane repice od 15% u obrocima depresivno deluje na proizvodne parametre pilića i da njeno uključivanje u većem procentu može imati negativnog uticaja na uspeh tova brojlerskih pilića.

Ključne reči: sačma uljane repice, brojleri, proizvodne osobine.

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# SURVIVAL, REPRODUCTION AND DEVELOPMENT OF INDIAN MEAL MOTH (*PLODIA INTERPUNCTELLA* HBN.) ON DRIED FRUITS\*

# RADMILA ALMAŠI, DANIJELA POSLONČEC<sup>1</sup>

SUMMARY: The present paper discusses the survival, reproduction, development, harmfulness, and F, reproduction potential of the Indian meal moth (Plodia interpunctella Hbn.) on dried fruits. The Indian meal moth (IMM) is a species that can develop on the surface of dried fruit. The IMM was grown on three different kinds of store-bought dried fruit. On average, the females laid a relatively high number of eggs per pair: 45.36 in raisins, 41.52 in dried apricots, 13.94 in prunes. However, the mortality of the eggs and caterpillars was high, ranging from 94.4% in dried apricots to 98.7% in raisins and prunes. The highest number of offspring was obtained on dried apricots (1.5/pair), followed by raisins (0.7/pair), and prunes (0.2/pair). The F, population size was small, the moths had a short lifespan, the males outnumbered the females, and the eclosion of females did not coincide with that of males. As a result, the number of offspring in the F, generation was negligible. The results of the present study notwithstanding, the fact remains that the IMM adapts very easily to different media and can be a major pest on a wide variety of stored products, including dried fruits. The IMM causes the most damage by altering the appearance of dried fruits, as the presence of caterpillars, exuviae, bite marks, feces, and silken threads reduces the market value of the product.

*Key words*: *dried fruits, fecundity, progeny, survival, Indian meal moth, Plodia interpunctella Hbn.* 

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## INTRODUCTION

Efforts to increase dried fruit production in Serbia focus on the development of the best fruit drying methods, but not enough attention is paid to pests that attack this kind of

product. There are several insect species that feed on dried fruits. The most important among them are the Indian meal moth (*Plodia interpunctella* Hbn), the dried fruit beetle (*Carpophilus hemipterus* L.), the saw-tooth grain beetle (*Oryzaephilus surinamensis*), and, in high moisture conditions, the hairy fungus beetle (*Typhaea stercorea* L). All these pest species affect the storage life of dried fruit products, but the extent of damage they cause is highly variable and depends on many factors. The initial size of the insect population, the amount of food available, temperature, and moisture content of the dried fruit are just some of the factors influencing the quality and usability of a dried fruit product infested by the pests. A large initial population will increase competition for space and food amongst the individuals, as a result of which the life cycle of the insect is prolonged and the egg production reduced.

In the case of the Indian meal moth (IMM), the most polyphagous of the dried fruit storage pests and a species that has a high reproductive potential and rate of development and produces a large number of generations per year, special attention has to be paid to packaged dried fruits. Such fruits are kept in warehouses as well as at various retail outlets, where the pest transfers easily from one product to another. First generation larvae can enter the pack through an opening as small as 0.13 mm in size (Lindegran et al., 1992). The presence of caterpillars on dried fruits becomes noticeable only when the mature larvae begin to seek shelter and migrate across the surface of the product, leaving behind them a trail of excrement and silken threads resembling a spider web. Prunes, dried apricots, raisins and other kinds of dried fruit are all rich in sugar, protein, and vitamins and thus represent a good source of food for the caterpillars of the IMM. Feeding on dried fruits has a positive effect on IMM egg production, oviposition, development rate, and adult moth lifespan.

Many authors have studied how IMM develops on different products, including maize (Mbata, 1990), peanuts (Arthur, 1995), dried vegetables (Na, Ryoo, 2000), and dried fruits and walnuts (Johnson et al., 1995, Almaši i Veljković, 2006, Almaši 2007). Small IMM populations are often ignored, which may lead to sudden outbreaks of this pest that can cause great damage to stored products. Since over 90% of pest damage on dried fruits are caused by the IMM (Vail, 2008), the objective of the present paper was to determine the effects of the initial IMM population on the extent of damage caused by the insect to different kinds of dried fruit products in laboratory conditions. The experiment involved five different initial densities of IMM imagoes, and the goal was to examine the reproduction of the  $F_1$  and  $F_2$  generations and determine the damage levels caused by the caterpillars.

## MATERIAL AND METHODS

Serbian government regulations prescribe that all fruit products must be free of live and dead insects and insect fragments (Government Manual on Fruit Quality Standards, Anonymous, 2005). In actual practice, however, fruit products will often become infested with insects when stored for longer periods of time.

To study the development of the IMM on dried fruits, a laboratory experiment was set up with this species under controlled temperature and moisture conditions.

Dried fruits for the experiment were purchased in a health food store and had been imported. The moisture content was 35% in prunes, 5.7% in raisins, and 19.9% in dried apricots. The IMM individuals used in the experiment had been grown for generations in a thermostat-controlled environment at a temperature of  $27 \pm 1$  °C and a relative humidity of 40-60%.

The experiment was carried out in the entomological laboratory of the Faculty of Agriculture in Novi Sad during the summer months. Glass jars 700 ml in volume were filled with 100 g of prunes, raisins, or dried apricots. Pairs of 24-hour old IMM moths in the process of mating were also placed in the jars.

In order to determine the effects of initial IMM population size under optimal, controlled conditions, the dried fruits were exposed to different initial population densities of the IMM (one, two, three, five and 10 pairs of moths) and the results were compared to IMM development on wheat grain. The jars were closed shut with canvas. Each of the initial population densities had 12 replicates, with the goal being to obtain as much of the  $F_1$  progeny as possible.

Of particular interest in the present study was the development of the  $F_2$  generation. The  $F_1$  individuals obtained on a particular dried fruit medium were transferred to a new medium composed of the same kind of fruit in order to produce the  $F_2$  generation.

The fecundity of the females was determined by counting the number of newly hatched larvae on the different media under a binocular microscope, while the abundance of pupae and imagines was established by counting the number of individuals present on the fruit and in the open space above it.

After counting up the number of insects in the different stages of development (egg, larva, pupa, imago), measurements were made of the weight of the dried fruits and losses due to shrinkage and feeding by insects were calculated. The results were represented graphically.

## **RESULTS AND DISCUSSION**

During the experiment, the average daily temperature in the laboratory was around  $25^{\circ}$ C, while the relative humidity was in the 40-60% range. The females laid their eggs mostly on the surface of the dried fruit. After hatching, all the caterpillars moved about the surface of the fruits, as they fed themselves and spun silken webs resembling those produced by spiders. Full-grown caterpillars metamorphosed into pupae on the surface of the dried fruits and in their folds. The results on the effects of initial IMM density on the number of eggs, caterpillars, pupae and adult moths in the F<sub>1</sub> and F<sub>2</sub> generations are shown in Figures 1-5.

On average, the IMM females in the present study laid the most eggs on raisins (45.36/pair), followed by dried apricots (41.52/pair), and prunes (13.94/pair) (Fig. 1). Embryogenesis was fairly even on all three media and was not affected by the type of fruit the insects fed on. The fecundity of the females on dried fruits is 117-303 eggs per female according to Na and Ryoo (2000) and 170 eggs per female according to Simmons and Nelson (1975). In the present study, the females laid more eggs when the initial population size was smaller (Fig. 1). These findings are in agreement with those of

Nenses et al. (2006), according to which the females lay fewer eggs and produce fewer caterpillars and offspring when space is limited. Studies like this should be carried out in larger warehouses and on larger quantities of packaged dried fruits.

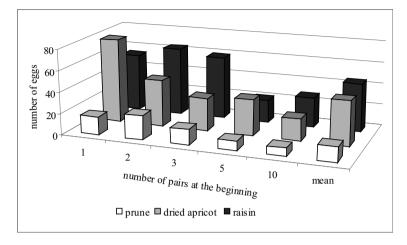


Figure 1. Fecundity of IMM females on different kinds of dried fruit Grafik 1. Komparativni prikaz nosivosti ženki bakrenastog moljca na različitim vrstama sušenog voća

Feeding on dried fruits had a great effect on IMM postembryonic development and the abundance of the adult offspring of the species. Population size was extremely small on all three media, especially compared to IMM populations found on wheat. Populations of the insect produced in the present experiment were 9.1-32 times lower than those obtained on wheat in a previous study (Almaši, 1984).

The highest number of offspring was found on dried apricots (1.5/pair), followed by raisins (0.7/pair), and prunes (0.2/pair) (Fig. 2).

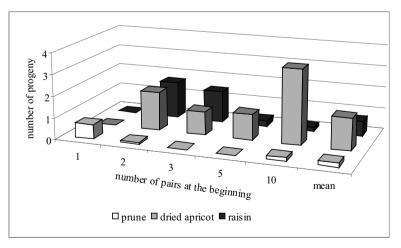


Figure 2. Effect of initial population size on the number of IMM offspring *Grafik 2. Uticaj početnog broja parova bakrenastog moljca na broj potomaka* 

The reasons for the low population size lay in a high mortality rate during embryonic development. Mortality rate during embryonic development (Tab. 1) is expressed as the percentage of unhatched larvae relative to the number of eggs laid. In the present study, this rate was the lowest in apricot (20.98 %) and the highest in raisins (75.86%). Johnson et al. (1992) reported embryonic mortality rates of 88-96% for pistachio and almond.

| Dried fruit                    | Iı    | Average |       |       |       |        |
|--------------------------------|-------|---------|-------|-------|-------|--------|
| Sušeno voće                    | 1     | 2       | 3     | 5     | 10    | Prosek |
| Prunes<br>suve šljive          | 62.05 | 67.41   | 59.72 | 77.01 | 84.21 | 70.08  |
| Dried apricots<br>suve kajsije | 35.41 | 43.02   | 11.41 | 1     | 15.05 | 20.98  |
| Raisins<br>suvo grožđe         | 89.13 | 76.06   | 82.51 | 73.61 | 58.01 | 75.86  |

 Table 1. Effect of food type on embryonic mortality rate in IMM

 Tabela 1. Uticaj vrste hrane na smrtnost bakrenastog moljca u toku embrionalnog razvića

Postembryonal mortality rate is defined as the percentage of individuals that suffer death during postembryonal development relative to the initial number of newly hatched larvae. Since IMM caterpillars develop on the surface of dried fruit, their mortality rate is affected not only by what they feed on but by other factors as well. In the present study, mortality rate during postembryonal development was the lowest in apricot (93.04%) and the highest in prunes (94.99%) (Tab. 2).

Table 2. Effect of food type on postembryonal mortality rate in IMM Tabela 2. Uticaj vrste hrane na smrtnost bakrenastog moljca u toku postembrionalnog razvića

| Dried fruit                    | Ini   | Average |       |       |       |        |
|--------------------------------|-------|---------|-------|-------|-------|--------|
| Sušeno voće                    | 1     | 2       | 3     | 5     | 10    | Prosek |
| Prunes<br>suve šljive          | 89.37 | 98.91   | 100   | 100   | 86.67 | 94.99  |
| Dried apricots<br>suve kajsije | 100   | 92.78   | 95.96 | 96.45 | 80    | 93.04  |
| Raisins<br>suvo grožđe         | 100   | 88.99   | 85.73 | 95.09 | 98.56 | 93.67  |

Overall mortality rate is a measure of the number of individuals that suffer death relative to the initial number of eggs laid. In this experiment, an overall mortality rate of 100% (meaning no larvae survived to reach the imago stage) was recorded in prunes at initial densities of two, three, and five pairs and in apricots and raisins at an initial population of one pair of moths (Fig. 3). Overall, the highest total mortality rate was found in prunes (98.7%), followed by raisins (98.6%), and apricots (94.4%). There are several possible reasons for such a low number of surviving offspring in the  $F_1$  generation, including a loss of moisture in the product, which was especially pronounced in prunes (although an effort was made to keep product moisture at an adequate level).

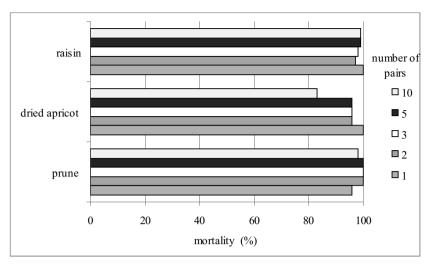


Figure 3. Effect of food type on overall mortality rate in IMM Grafik 3. Uticaj vrste hrane na ukupnu smrtnost bakrenastog moljca

As a rule, once a species has adapted to a particular type of food in the  $F_1$  generation, it will develop much faster and produce more offspring in the  $F_2$  generation.

 $F_1$  progeny from the present experiment was transferred to a new medium composed of the same kind of dried fruit. However, in spite of many attempts on the part of the experimenters, the insect failed to reproduce in the  $F_2$  generation. There are a number of reasons for this. The main ones are that the size of the  $F_1$  population was very small, the moths had a short lifespan, males were predominant, and the eclosion of the few females that were present did not coincide with that of males. Finally, when the very long period of development of a generation is added to the equation, it becomes apparent why the efforts to obtain an  $F_2$  generation were unsuccessful. When grown on the same kind of dried fruit obtained from a different supplier, the IMM behaved and developed differently.

Information on the chemicals used to protect dried fruit products from insects and microorganisms can be found in the literature. US regulations may be the most relevant in this regard, since the U.S. exports 1.2 million tons of dried fruit per annum and takes all the necessary steps to protect products of this kind. The best and most expensive such measure is to keep the dried fruit at low temperatures (below 7<sup>o</sup>C) and low humidity (55-70%) in the warehouse. Another measure, which is mandatory and also much more economical, is the fumigation of fruits prior to packaging. There are also various other protection measures that are in use, such as exposing the product to low oxygen levels (0.4%  $O_2$ ) or using special fumigants such as Eranol (ethyl formate) (Anonymous, 1998) or granuloviruses (Johnson, 2002).

The results of the present study suggest that the imported dried fruits bought in stores had been treated with coloring additives, protected from insect attacks and fungal diseases, or dried in various ways.

According to dried fruit sellers in Serbia, prunes are smoke-dried in smoking chambers, while raisins are sun-dried and potassium metabisulfite is added to the product in order to preserve its color. On a package of dried apricots imported from Turkey, it is declared that the product has been preserved by the addition of sulphur dioxide. Serbian regulations on the production of prunes prescribe that a total of 0.02% of sorbic acid, potassium sorbate or sodium benzoate can be used in the preservation of the product and that only surface treatments are allowed (Anonymus, 2005).

The Indian meal moth is a species that develops on the surface of the product, adapts very easily, and can survive on a variety of stored products, including dried fruits. The results of this experiment provide further confirmation that dried fruits must be protected from insects as part of the production process.

## CONCLUSION

In the present experiment, IMM females laid a relatively high number of eggs on all three kinds of dried fruit. The number of eggs laid was higher in treatments with lower initial population densities.

All three types of dried fruit had a large influence on IMM population size. The number of offspring in the  $F_1$  generation was small, and the  $F_2$  generation failed to develop.

The reasons for the low number of offspring were several, including the long time it took for one generation to develop, the preponderance of males over females, and the fact that the dried fruit had been treated for the purpose of protection from insects (the larvae died as soon as they tried to feed upon hatching). The IMM is a species that feeds on the surface of products, so if the product has been chemically treated, the caterpillars that try to survive on it will die. The overall IMM mortality rate in the present study was 98.7% for prunes, 98.6% for raisins, and 94.4% for dried apricots.

The IMM adapts very easily to different media and can thus be a major pest on a wide variety of stored products, including dried fruits (as evidenced by the photographs that were taken of the surface of dried fruits used in this experiment).

The quality of a dried fruit product can be maintained only if the product is protected from insects.

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# PREŽIVLJAVANJE, REPRODUKCIJA I RAZVIĆE BAKRENASTOG MOLJCA (*PLODIA INTERPUNCTELLA* HBN.) NA SUVOM VOĆU

# RADMILA ALMAŠI, DANIJELA POSLONČEC

## Izvod

Predmet istraživanja u ovom radu su preživljavanje, reprodukcija, razviće, štetnost i mogućnost razmnožavanja F, generacije bakrenastog moljca, Plodia interpunctella Hbn. u suvom voću. Bakrenasti moljac je vrsta koja se razvija na površini suvog voća. U sve tri vrste suvog voća, kupljenog u prodavnici, ženke bakrenastog moljca položile su relativno veliki prosečan broj jaja po jednom paru, najviše na suvo grožđe (45,36), skoro isto toliko na suve kajsije (41,52), a najmanje na suve šljive (13,94). Međutim, registrovana je visoka smrtnost jaja i gusenica, koja se kretala od 94,4 % na kajsijama do 98,7 % na grožđu i suvim šljivama. Najviše potomaka je dobijeno na suvim kajsijama (1,5/paru), zatim na suvom grožđu (0,7/paru), a najmanje na suvim šljivama (0,2/ paru). Potomstvo je bilo malobrojno, leptiri su kratko živeli, preovlađivali su mužjaci, pojedinačne ženke nisu eklodirale istovremeno sa mužjacima, zbog čega je brojnost F, generacije bila zanemarljivo mala. Bez obzira na dobijene rezultate, bakrenasti moljac se veoma lako adaptira na nove proizvode i može se održati na veoma različitim uskladištenim proizvodima pa i na suvom voću. Najveće štete nastaju u izgledu suvog voća, usled prisustva gusenica, egzuvija, izgrizina, izmeta i svilenkastih niti koje smanjuju tržišnu vrednost proizvoda.

Ključne reči: suvo voće, fekunditet, potomstvo, preživljavanje, bakrenasti moljac, *Plodia interpunctella* Hbn.

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UDC: 615.837:599.735.5

# **ULTRASOUND EXAMINIATION OF CATTLE ACROPODIUM\***

# BOJAN TOHOLJ, MILENKO STEVANČEVIĆ, VELIBOR KUJAČA IVANA DAVIDOV, OGNJEN STEVANČEVIĆ<sup>1</sup>

SUMMARY: The aim of this study was to assess the possibilities of application of diagnostic ultrasound examination of cattle acropodium. The research was conducted on 16 cows during the hoof trimming procedure correction. In order to evaluate the accuracy of the findings, ultrasound examination was done at the samples from abbatoir. Results obtained in this study indicate that ultrasound can be a useful diagnostic tool in view of acropodium tissues, in cases when other, diagnostic methods can not determine the exact nature of the pathological process:

Key words: cows, hoof, ultrasound examination.

## **INTRODUCTION**

Ultrasound uses energy in the form of sound waves that are a frequency higher than the normal range of hearing. These sound waves are transmitted into the body by an instrument known as a transducer, a device that converts one form of energy into another. Sound waves from the transducer are reflected off the internal organs and interpreted by the scanner to create an image on a video display The diagnosis of diseases of cows ultrasound diagnosis is already used for many years in the diagnosis of diseases of the reproductive system as well as for diagnosis of pregnancy (Ivančev, 1999). Application of ultrasonic diagnosis in other parts of the body of a cow encounters significant obstacles that hinder or prevent its implementation. First, due to body size of cattle use of ultrasound for transcutaneous examination of abdmen or thorax has no practice importance. The presence of thick hair cover prevents the penetration of ultrasonic waves. Ultrasound examination of acropodium also experiencing significant aggravating circumstances. First, the water content in hoof horn is significantly less than in the skin of cattle, which impedes the penetration of ultrasonic energy. Schmid 1995, found that the water content in hoof horn is 20% of the front hoofs and 25% at the rear hoofs, while

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this percentage is much higher for the skin and is 67-74%. In the preliminary study (Kofler, 1998) found that ultrasound examination is required before adequate preparations, which involves removing impurities and hoof trimming, with aim to make a flat surface. Also the same author observed that the transhorneal examination view required linear probe. For convex probe the limiting factor is the small area of contact with the surface, and poor visualization of structures that are in the surface layer immediately below the probe, which are the subject of research. Most diseases of acropodium can be diagnose by adspektion, especially when hoof trimming procedure was done. However, in some cases it's not possible to establish right nature of disease. Septic arthritis of distal interphalangeal joint (SADIJ), a disease that is usually manifested in the appearance of swelling in periople segment of hoof, which is more or less extended. However, a similar clinical picture has phlegmon, septic tenosinovitis of deep flexor tendon etc. Since the therapy of SADIJ involve amputation or arthrodesis, it is necessary to previously confirm the diagnosis.

## MATERIAL AND METHOD

Investigation was done on 16 Holstein-Freisian cows. Ultrasound examination was performed immediately after the hoof trimming at cows fixed in the box. Determination was done using the ultrasound device Falcovert (Esaote Pie Medical) and linear probe 6-8 Mhz of general purpose, using B mod ultrasonography. Linear probe has an advantage over the convex because it provides better visualization of tissue that is closer to probes. Investigated the possibility of review tabanskog region (Figure 2), distal interphalngeal joint (Figure 3) and deep flexor tendon (Figure 4).



Fig. 1. Ultrasound examination of acropodium. Slika 1. Ultrazvučni pregled akropodijuma

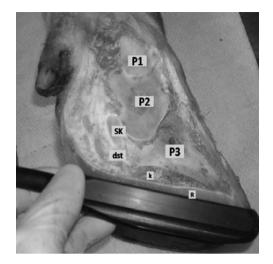


Fig. 2. Longitudinal section of samples from abbatoir.Placement of probe for examination of sole. Slika 2. Uzdužni presek akropodijuma. Položaj UZ sonde



Fig. 3. Placement of probe for examination of deep digital tendon *Slika 3. Položaj UZ sonde kod pregleda duboke sagibačke tetive* 

#### RESULTS

Transhorneal examination of acropodium in the solear area is possible only with adequate preparation of hooves. This means hoof trimming procedure in order to make a flat contact surface. In the cows in free system of housing, the front and rear hoofs were both available for review with ultrasound. However, at two cows in tie system of housing, front hoofs in a few cases it wasn't possible to see with the ultrasound. with ultrasound imaginig can be wieved next structures: solear surface of third phalanx, corium, deep digital flexor tendon, distal interphalangeal joint, thickness of horn (figure 4).

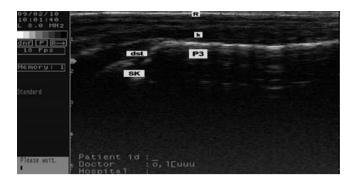


Fig. 4. Ultrasound image of solerar region . R- horn, P3-distal phalanx, DST- deep flexor tendon, SK - sesamoid bone.

Slika 4. Ultrazvučni nalaz tabanske regije. R-rožina, P3-papčana kost, DST-duboka savijačka tetiva, SK-sezamoidna kost

Placing a probe on the plantar or palmar side of hoof at place of skin-horn junction (figure 3) it's possible to wiev deep digital tendon and sesamoid bone (figure 5).



Fig. 5. Ultrasound image of acropodium structure, get by placing a probe at palmar or plantar side of the hooves at region of skin-horn junction. DST- deep flexor tendon, SK - sesamoid bone. *Slika 5. Ultrazvučni nalaz akropodijuma dobijen postavljanjem sonde na palmarnu ili plantarnu stranu akropodijuma u predelu spoja kože i rožine. DST-duboka sagibačka tetiva, SK-sezamoidna kost* 

## DISCUSSION

The first clinical use of ultrasound in cattle dating back to the 1971st when the ultrasound was first used for the diagnosis of pregnancy (Fraser, 1971). Basic guidelines in the use of ultrasound examination of muscle-skeletal system of cattle give prof. Kofler and colleagues during the nineties. The results of this study suggest that the use of ultrasound transducer frequency and linear 8Mhz can wiew some structure of acropodium. Therefore, ultrasound examination of cattle acropodium can be useful tool to diagnose certain diseases of acropodium such as septic arthritis of distal interphalangeal joint deep digital septic tendinitis (Heppelman, 2009).

#### CONCLUSION

Ultrasound diagnosis can be successfully applied in the examination of cattle acropodium. By this techniquesubsolear structures, deep flexor tendom and distal interphalangeal joint can be visualisied.

Further research are required to accurately define pathological alterations and made the appropriate criteria

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# ULTRAZVUČNI PREGLED AKROPODIJUMA KRAVA

# BOJAN TOHOLJ, MILENKO STEVANČEVIĆ, KUJAČA VELIBOR, IVANA DAVIDOV, OGNJEN STEVANČEVIĆ

## Izvod

Cilj ovog rada je bio da se procene mogućnosti primene ultrazvučne dijagnostike kod pregleda akropodijuma krava. Istraživanje je obavljeno na 16 krava prilikom korekcije papaka. U cilju evaluacije tačnosti ultrazvučnog nalaza ispitivanje je vršeno i na preparatima sa klanice. Rezultati dobijeni ovim istraživanjem ukazuju na to da ultrazvuk može biti korisna dijagnostička alatka u pregledu akropodijuma, pogotovo kod visokovrednih grla, u slučajevima kada se drugim, dijagnostičkim metodama ne može utvrditi tačana narav patološkog procesa:

Ključne reči: krave, oboljenja papaka, ultrazvučni pregled.

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# EFFECT OF DIFFERENT GARLIC LEVELS IN BROILER CHICKEN FOOD ON PRODUCTION PARAMETERS\*

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SUMMARY: The beneficial effects of garlic (Allium sativum L.) on human and animal organism, which result from its antimicrobial, antioxidative and antihypertensive properties, have been known from ancient times. These effects can be attributed to its bioactive components, the most important among which are sulphuric compounds alliin and allicin which make garlic an alternative to antibiotics that European Union prohibited 2006. The aim of study focused on examining the effects of garlic supplement in broiler feed on the production parameters and health status. At the start of the fattening period there were formed three groups of 37 one-day-old Ross 308 hybrid broilers of the same weight in five repetitions. Treatment groups were given: 1.5% commercial garlic in group II and 3% in group III. At the end of the experiment which lasted 42 days it was found that the addition of garlic led to a not significant increase of body mass in group II (2257,47g) in comparison to the control group (2250,26g), while it recorded depressive effect in group III (2229,39g) in comparison to the control group. A feed conversion ratio (FCR) was better in groups with addition of garlic, and the best FCR were in group II (1.83).

Key words: Garlic, broilers, production parameters.

## **INTRODUCTION**

In addition to nutritional substances necessary for growth and development of birds, the feed is regularly supplemented with pharmacological products, either for preventive purposes, as prevention against certain diseases (coccidiostats) or as growth stimulators (antibiotics), primarily in case of young chicks (Doyle, 2001., Šefer and

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Sinovec, 2003). These products have a positive effect on growth, feed conversion and meat quality, but they also have negative effects manifested through the emergence of pathogens resistant to the applied antibiotics in animals and people. For these reasons the European Union prohibited the use of such supplements in 2006.

The alternatives to antibiotics as growth stimulators are numerous, amounting to finding an adequate non-pharmacological products from the group prebiotics, probiotics, organic acids, essential and other oils, medicinal plants or parts of plants such as thyme, basil, oregano and others (Simon, 2005.; Ankri and Mirelman, 1999).

The beneficial effects of garlic (Allium sativum L.) on human and animal organism, which result from its antimicrobial, antioxidative and antihypertensive properties (Konjufca et al., 1997; Sivam, 2001; Prasad et al. 1981.; Prasad at al. 1995.), have been known from ancient times. Research has shown that these effects can be attributed to its bioactive components, the most important among which are sulphuric compounds, alliin, diallylsulphide, allyldisulphide and allicin (Ankri and Mirelman, 1999.; Kumar and Berwal, 1998; Amagase et al. 2001; Horton et al. 1991a). Garlic also manifests hypocholesterolemic effects on chickens through inhibition of the most important enzymes that participate in the synthesis of cholesterol and lipids (tri-hydroxy-tri-methyl-glutaril coenzym-A-reductase, cholesterol-7- $\alpha$ -hydroxylase and the synthesis of fatty acids. Cullen et al. (2005) examined the effect of garlic supplement in the amount of 1% in pig feed and recorded an increase in growth, conversion and meat quality in comparison to the control group. Horton et al. (1991b), Freits et al. (2001) and Bampidis et al. (2005) had similar results in their study of broilers, but they also concluded that lower concentrations, ranging between land 2%, were actually more effective. Stanaćev et al. (2008) carried out the parallel experiment with addition of 2% of garlic and 100 ppm of copper in broiler feed, both alone and in combination, on the production parameters and come to a conclusion that the group with the addition of 2% of garlic had the best results.

The study focused on examining the effects of different levels of commercial garlic supplement in broiler feed on the production parameters of chicks.

#### MATERIALS AND METHODS

Biological experiments were performed under production conditions in the experimental field "Pustara" of the Faculty of Agriculture in Novi Sad. At the start of the fattening period, three groups of 37 one-day-old Ross 308 hybrid broilers of the same weight were formed. The experiment was performed with five repetitions covering a total of 185 chickens per treatment, and was set according to the scheme given in Table 1.

Table 1. The plan of additives supllement

| Group    | Ι | II  | III |
|----------|---|-----|-----|
| Garlic,% | 0 | 1,5 | 3   |

There were three nutrient mixtures used. Starter during the first three weeks, then followed Finisher I in the next 14 days and at the and, last 7 days Finisher II, with 23%, 20% and 18% protein content respectively. The experiment lasted for 42 days. Composition and chemical analyses of feed mixtures are given in tables 2. and 3.

| Ingredients           | Starter | Finišer I | Finišer II |
|-----------------------|---------|-----------|------------|
| Soy oil               | 1.80    | 2.50      | 3.00       |
| Corn                  | 49.00   | 55.45     | 63.00      |
| DL Methionine         | 0.15    | 0.15      | 0.10       |
| Fullfat soya          | 13.80   | 12.00     | 8.00       |
| Soya bean meal        | 28.00   | 22.70     | 18.60      |
| Monocalcium phosphate | 1.55    | 1.50      | 1.60       |
| Premix                | 1.00    | 1.00      | 1.00       |
| Salt                  | 0.30    | 0.30      | 0.30       |
| Limestone             | 1.40    | 1.40      | 1.40       |
| Yeast                 | 3.00    | 3.00      | 3.00       |
| Total                 | 100     | 100       | 100        |

Table 2. Composition of feed for chicks, %

Table 3. Chemical analysis of feeds

| Mixture             | Starter | Finišer I | Finišer II |
|---------------------|---------|-----------|------------|
| Crude protein,%     | 22.91   | 20.57     | 17.97      |
| Fat,%               | 6.19    | 6.76      | 6.78       |
| Crude fiber,%       | 4.15    | 3.88      | 3.61       |
| Methionine, %       | 0.51    | 0.48      | 0.39       |
| ME:SP               | 133     | 153       | 178        |
| ME,MJ/kg            | 12.83   | 13.206    | 13.411     |
| Calcium,%           | 0.90    | 0.88      | 0.88       |
| Phosphorus total, % | 0.76    | 0.72      | 0.72       |

During the experimental period, chicks where fed *ad libitum*, while the microclimatic conditions were regularly monitored. Monitoring of body weight was carried out periodically every time with changing of food mixture. The research results are presented in table form, as average treatment values and the data processing statistical method applied was ANOVA and diferences between the groups are carried out by Dankan test.

## **RESULTS AND DISCUSSION**

Based on the obtained results it can be concluded that the introduction of commercial garlic in broiler feed in amount of 1.5% had effect on the intensity of the increase in body weight (Table 4), while the addition of 3% led to a slight depression in body weight.

|                          |          | Groups      |           |
|--------------------------|----------|-------------|-----------|
| De un un et e un         | I        | II          | III       |
| Parameters               | Control  | Garlic 1,5% | Garlic,3% |
| Initial body weight, g   | 39.0     | 39.0        | 39.0      |
| Body weight at week 3, g | 842,03   | 840,15      | 830,24    |
| Body weight at week 5, g | 1809,15  | 1770,00     | 1749,83   |
| Body weight at week 6, g | 2250,26  | 2257,47     | 2229.39   |
| SD                       | 299,1918 | 334,3262    | 313.9847  |
| SE                       | 21,48072 | 24,38325    | 22,83903  |

Table 4. Body weight of chicken, g

In the first fattening period, at the end of the third week, the highest body mass was achieved in the control group (842,03g), while in the experimental groups (II, III) it was slightly lower and averaged at 840,15 and 830,24 g respectively. This was probably due to reduced food consumption, resulting from the intense smell of garlic, which required a period of adaptation of birds to this kind of feed. Horton et al. (1991a) had similar results. Tendency of chicks growth is recorded and at the end of the fifth week. However, at the end of the experiment which lasted 42 days, addition of garlic had influence of increase in body weight in group II (2257,47g) in comparison to the control group (2250,26g), but the differences between the groups were not statistically significant (Table 4). In the third group body mass of 2229.39g was achieved. Utilization of different additives levels in this experiment had different effects on the feed efficiency (Table 5). The lowest conversion rates of during the entire experiment of 1.83 kg/kg were recorded in group II with 1.5% garlic content in feed, which means by 3.18% less than in the control group. Third group also recorded low conversion rate (1.85 kg/kg), but for 2.12% less in comparison to the control group (1.89 kg/kg). The number of chick mortality and all loses were reduced, and the production index were increased (Table 6). The highest production index had group II (278.81), and the lowest group III (267.67).

|             | Group    |             |           |  |  |  |
|-------------|----------|-------------|-----------|--|--|--|
| Parameters  | Ι        | II          | III       |  |  |  |
|             | Control  | Garlic 1,5% | Garlic 3% |  |  |  |
| 13. week    | 1,55     | 1,52        | 1,53      |  |  |  |
| 45. week    | 1,77     | 1,75        | 1,76      |  |  |  |
| 16. week    | 1,89     | 1,83        | 1,85      |  |  |  |
| 1-6 Index,% | 100,00   | 96,82       | 97,88     |  |  |  |
| SD          | 0,029693 | 0,081398    | 0,055352  |  |  |  |
| SE          | 0,013279 | 0,036402    | 0,024754  |  |  |  |

Table 5. Feed conversion

Table 6. Chicks mortality and production index

|               | Group   |             |           |  |  |  |
|---------------|---------|-------------|-----------|--|--|--|
| Parameters    | Ι       | II          | III       |  |  |  |
|               | Control | Garlic 1,5% | Garlic 3% |  |  |  |
| Mortality %   | 3       | 1           | 2,5       |  |  |  |
| Culls, %      | 2       | 2,5         | 2         |  |  |  |
| Total loss, % | 5       | 3,5         | 4,5       |  |  |  |
| PI            | 270,70  | 278,81      | 267,67    |  |  |  |

## CONCLUSION

On the basis of presented results it can be concluded that chicks treated with 1.5% of garlic have achieved better production results than the control group, but the differences between the groups were not statistically significant. Body mass was increased and conversion rate was reduced by 3.18%.

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# EFEKAT RAZLIČITIH NIVOA BELOG LUKA U HRANI TOVNIH PILIĆA NA PROIZVODNE PARAMETRE

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#### Izvod

Korisno dejstvo belog luka (Allium sativum L.) na ljudski i životinjski organizam, poznato je od davnina, jer poseduje antimikrobna, antioksidativna i antihiperienzivna svojstva. Cilj ovih ispitivanja je bio da se utvrdi uticaj belog luka u hrani tovnih pilića na proizvodne parametre i zdravstveno stanje. Na početku tova formirane su tri grupe sa po 37 jednodnevnih pilića hibrida Ross 308, ujednačenih po masi, u pet ponavljanja. U eksperimentalnim grupama je bilo uključeno 1,5% komercijalnog belog luka u II grupi i 3% u III grupi. Na kraju eksperimenta, koji je trajao 42 dana, dodatak belog luka je uticao na povećanje telesne mase u II grupi (2257,47g) u odnosu na kontrolu (2250,26g), ali razlike nisu bile statistički signifikantne, dok je u trećoj grupi delovao depresivno (2229,39g) u odnosu na kontrolnu grupu. Konverzija hrane je bila bolja u grupama sa belim lukom, a najbolja u II grupi (1,83).

Ključne reči: Beli luk, pilići, proizvodni parametri.

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# DEVELOPMENT OF RICE WEEVILS (Sitophilus oryzae L.) AND LESSER GRAIN BORERS (Rhizopertha dominica F.) ON KERNELS AND SPIKES OF SPELT WHEAT\*

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SUMMARY: This research deals with the possibility of nutrition, development, survival and reproduction of primary wheat pests, rice weevils (Sitophilus oryzae L.) and lesser grain borers (Rhizopertha dominica F.) on kernels and spikes of spelt wheat. The rice weevils reproduced on all the three varieties of spelt wheat stored in kernels and the number of the offspring of F, generation was 141.6 imagoes on average. The average number of lesser grain borer offspring was 45.0. The number of lesser grain borer offspring on grains was smaller in comparison with the number of rice weevil offspring in the same storage variation. Rice weevils cannot feed and reproduce on any variety of spelt wheat when stored in spikes. In the same storage variation the lesser grain borers fed and reproduced although the average number of offspring was small (14.3 imagoes). On the spelt wheat in spikes the number of lesser grain borer offspring was smaller compared with those in kernels. *On all the three varieties of spelt wheat in the mixture of kernels and spikes* there were the offspring of both rice weevils and lesser grain borers. The average number of the imagoes of rice weevils was 135.3 and the number of lesser grain borers was 48.0. Based on the analysis of kernels and spikes, rice weevils developed only on kernels opposite to the lesser grain borers which normally developed on both kernels and spikes. The results show that the impossibility of rice weevil nutrition resulted from the physical impossibility of nutrition and not from the repellent spike effect.

Key words: rice weevil, lesser grain borer, development, spelt wheat

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Original scientific paper / Originalni naučni rad

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## INTRODUCTION

The modern agricultural production should provide sufficient amount of food for the needs of the population whose number is significantly increasing, and to observe ever rising requirements regarding the health safety. The protection of the stored wheat more and more relies on the protective measures and pests monitoring and on the application of chemicals only when necessary. The introduction of new - old varieties in the production as well as different storage methods can contribute to the successful pest control in storehouses.

The spelt wheat (*Triticum aestivum* spp. *spelta*) is an old wheat variety originating from Egypt, found in the valley of the Nile, in the fourth millennium BC. It was known to the ancient Romans. It was grown in Germany and the Pannonian plain (Molnar, 2009). The production was resumed in the seventies of the last century for more reasons than one. It is characterized by better digestion (high content of proteins, mineral matters, microelements especially zinc and vitamins). It is convenient for the organic food production (the application of fertilizers is not required). It is stored in spikes, therefore there are no problems with the insects which feed on the stored wheat (Johnson, 2008).

The fact is that about 100 species of insects can find food in our storehouses. The primary pests are capable of damaging the whole and sound kernels causing about 90% of all the damages in storehouses. Lately, rice weevils (*Sitophilus oryzae* L.) and lesser grain borers (*Rhizopertha dominica* F.), which have a high potential of reproduction, rapid development and a great number of generations within a year, are the most present regarding the number and the extent of damage. Both species feed on kernels (mouth nibbling apparatus) lay eggs on the surface, the complete development is within a kernel, an imago lives about 8 months. Imagoes of rice weevil fly out to the fields where they lay eggs on wheat spikes at high temperatures and the complete development is within a kernel. Lesser grain borers fly but do not leave the storehouse.

In the domestic entomology literature there are no data on the possibilities of nutrition and reproduction of rice weevils and lesser grain borers on the spelt wheat. That is why this research is aimed at the study of the development of the two mentioned important pest species if the spelt wheat is stored as kernels or spikes. As the kernels shatter during storage, the insects were also placed on the mixture of kernels and spikes with the objective to determine possible repellent spike effect for both species.

#### MATERIAL AND METHODS

Under the simulated conditions of practical storage, the possibility of nutrition, development, survival and reproduction of rice weevils and lesser grain borers was studied in the laboratory of the Faculty of Agriculture. Both species of insects used in these experiments were grown in a thermostat at  $27 \pm 1^{\circ}$ C and at the relative air humidity of 40-60%. The imagoes aged up to a week were used in the experiment. The experiment was done on the spelt wheat varieties: Eko 10, Ostro and Nirvana. The spelt wheat varieties were from Hungary, Austria and Serbia.

The experiments were done for each variety in three storage variations. The first variation comprised 200 kernels, the second 100 spikes and the third a mixture of 100 kernels and 50 spikes. Each variation was carried out in five replications. In each replication 20 imagoes of each insect species respectively were placed.

The offspring number of the  $F_1$  generation of rice weevils on kernels and spikes was monitored after 8 weeks i.e. 11 weeks for lesser grain borers. In the variation of the mixture of kernels and spikes the number of both species of insects was checked after 11 weeks.

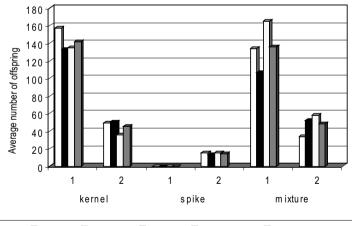
## **RESULTS AND DISCUSSION**

The variations will be presented separately in order to make the results more distinct.

#### a) Development of rice weevils and lesser grain borers on kernels of spelt wheat

In the experiment variation in which the development of both species of insects on the kernels of various spelt wheat varieties was monitored the results showed that the kernels of spelt wheat made the development of both species possible (Graph 1). In this variation rice weevils reproduced but the number of the offspring of the  $F_1$  generation (141.6 imagoes on average) was greater than the number of the offspring of lesser grain borers, but smaller that the obtained offspring of common wheat (Stojanović, 1966). The greatest number of the offspring of rice weevils was obtained on the Eko 10 variety (157 imagoes) and the smallest on the Nirvana variety (133 imagoes). In the same variation lesser grain borers developed worse (an average number of offspring 45.0 imagoes). The greatest number of offspring was obtained on the Ostro variety (50 imagoes) and the smallest on the Nirvana variety (36 imagoes).

The quality of kernels of spelt wheat (all varieties) for the nutrition of rice weevils was confirmed by the fact that none of the weevils died (Graph 2). The kernels proved to be good food for lesser grain borers as well. The average mortality was 2.1 imagoes. The lowest mortality was on the Eko 10 variety (0%) and the highest 4.2% on the Ostro variety.



□EKO 10 ■OSTRO □NIRVANA ■Average □1-rice weevil □2-lesser grain borer

Graph 1. Average number of offspring of F<sub>1</sub> generation of rice weevils and lesser grain borers depending on kernel variety and storage

Graf. 1. Prosečan broj dobijenih potomaka F<sub>1</sub> generacije pirinčanog žiška i žitnog kukuljičara u zavisnosti od sorte i načina čuvanja zrna

## b) Development of rice weevils and lesser grain borers on spikes of spelt wheat

Rice weevils cannot feed and reproduce on any variety of spelt wheat when stored in spikes (Graph 1). In the same variation lesser grain borers fed and reproduced although the average number of offspring was small (14.3 imagoes). On all the three studies varieties of spelt wheat the number of offspring was small and ranged from 13 to 15 imagoes (Ostro, Eko 10 and Nirvana). Kordan et al. (2008) came to the similar conclusions and reported that lesser grain borers developed worse on the spelt wheat in spikes.

During eight weeks all the imagoes of rice weevils placed on spikes died (Graph 2). The rice weevils did not feed on spikes, the mortality of imagoes was 100% whereas the mortality of lesser grain borers was 41.7 on average. The percentage of deaths per variety ranged from 40.4 (Ostro) to 43.2% (Eko 10).

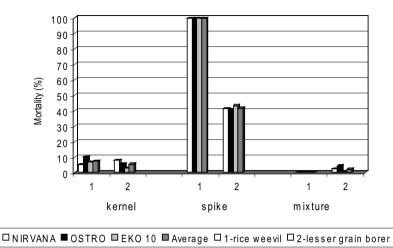
# c) Development of rice weevils and lesser grain borers on the mixture of kernels and spikes of spelt wheat

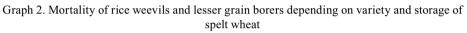
The fact that the spelt wheat is stored in spikes is extremely significant for storage. Still, in wheat storing and handling practice it is well known that it is impossible to prevent kernel shattering. The statement of Tramaterra et al., (1999) that the insects could not develop in storehouses if the spelt wheat was stored in spikes does not thoroughly agree with the results obtained in this experiment. The results of the third variation of the experiment with the mixture of kernels and spikes confirm this statement (Graph 1).

On all the three varieties if spelt wheat there were offspring of both rice weevils an lesser grain borers. The average number of the imagoes of rice weevils was 135.3 and of lesser grain borers 48.0. Based on the analysis of kernels and spikes in the third variation rice weevils developed only on kernels. This confirmed the fact that spikes had no repellent effect on the imagoes of rice weevils but that it was due to the physical impossibility of feeding on spikes. In addition, the statement that the storage method of spelt wheat in spikes makes the nutrition of insects in storehouses impossible is partially true, as there are always shattered kernels. In the mixture of kernels and spikes the rice weevils had three times more offspring than the lesser grain borers. The greatest number of offspring of rice weevils was obtained of the Nirvana variety (165 imagoes) and the smallest on the Ostro variety (107 imagoes). The greatest number of the offspring of lesser grain borers was obtained on the Nirvana variety and the smallest on the Eko 10 variety (34 imagoes).

## d) Mortality

The variation of the mixture of kernels and spikes of spelt wheat indicates a lower percentage of mortality of rice weevils but it also confirms that the weevils developed only on kernels and that no spikes were damaged. The average mortality was 7.4% for rice weevils and 5.4% for lesser grain borers (Graph 2). The rice weevils had the highest percentage of mortality on the Ostro variety (10.1%) and the smallest on the Nirvana variety (5.2%). The lesser grain borers had the highest percentage of mortality on the smallest on the Smallest on the Nirvana variety (7.9%) and the smallest on the Eko 10 variety (2.9%).





*Graf. 2. Smrtnost pirinčanog žižka i žitnog kukuljičara u zavisnosti od sorte i načina čuvanja spelte* 

The reason of the resistance of this old variety of wheat can be found in the structure of kernel and husk as well as in the storage conditions. If, after being harvested, kernels remain in husk (spikes) it is protected from pests to a great extent. The high protein content in the new varieties of spelt wheat may be higher than 20% and the glue content 45-55% higher than common wheat (Molnar, 2009) which contributes to the pest control. None of the species *Sitophilus granarius* L. and *Tribolium confusum* Duv., whose development was studied by Laszczak Dawid et al. (2006) does not prefer glue in spelt wheat. One of the explanations for the way in which the kernels of spelt wheat in spikes are protected from *Sitophilus oryzae* L. was given by Trematurra et al. (1999). According to these authors the evaporable matters of kernels, which incite the insects to feed, are not available to them if kermels are protected by husk.

## CONCLUSION

The results of this paper point at the advantages and disadvantages of storing the spelt wheat in spikes which has not been the practice in our country up till now.

Rice weevils do not feed and reproduce when the kernels are in spikes of spelt wheat. The husk protects kernels from the attack of rice weevils but not from lesser grain borers.

Lesser grain borers normally develop both on kernels and spikes of spelt wheat. The number of the offspring of lesser grain borers is significantly smaller than the number of the offspring of rice weevils on kernels.

After 11 weeks of feeding (per each species of insects respectively) on the mixture of kernels and spikes the number of both species increased considerably but the analysis clearly showed that, opposite to the lesser grain borers, rice weevils fed only on grains. The results show that the impossibility of the nutrition of rice weevils is the result of the physical impossibility of nutrition and not of the repellent spike effect.

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# RAZVIĆE PIRINČANOG ŽIŠKA (Sitophilus oryzae L.) I ŽITNOG KUKULJIČARA (Rhizopertha dominica F.) U ZRNU I KLASU SPELTE

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#### Izvod

Ova istraživanja ukazuju na mogućnost ishrane, razvića, preživljavanje i reprodukciju pirinčanog žižka (*Sitophilus oryzae* L.) i žitnog kukuljičara (*Rhizopertha dominica* F.) na zrnu i klasu spelte. Obe vrste su primarne štetočine žita i razvijaju se u zrnima žita.

Na sve tri sorte spelte uskladištene u zrnu pirinčani žižak se razmnožavao, a broj dobijenih potomaka  $F_1$  generacije u proseku iznosi 141,6 imaga. Prosečan broja potomaka žitnog kukuljičara je 45,0 imaga. Brojnost potomstva žitnog kukuljičara na zrnu je manja u odnosu na brojnost potomstva pirinčanog žižka na istoj varijanti.

Pirinčani žižak ne može da se hrani i razmnožava ni na jednoj sorti spelte kada je uskladištena u klasu. U istoj varijanti žitni kukuljičar se hranio i razmnožavao, mada je dobijen mali prosečan broj potomaka (14,3 imaga). Na spelti u klasu dobijeno je manje potomaka žitnog kukuljičara u odnosu na zrno.

Na sve tri sorte spelte u mešavini zrna i klasa, dobijeno je potomstvo i pirinčanog žižka i žitnog kukuljičara. Prosečan broj dobijenih imaga pirinčanog žižka je 135,3 a žitnog kukuljičara 48,0. Na osnovu pregleda zrna i klasa, pirinčani žižak se razvijao samo u zrnima, za razliku od žitnog kukuljičara koji se normalno razvijao i u zrnu i u klasu. Rezultati su pokazali da je nemogućnost ishrane pirinčanog žižka rezultat fizičke

nemogućnosti ishrane, a ne repelentno delovanje klasa.

Ključne reči: pirinčani žižak, žitni kukuljičar, razviće, pšenica spelta.

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# RESEARCH ON HIGH BUSH BLUEBERRY (VACCINIUM CORYMBOSUM L.) CULTIVATED IN MACEDONIA

TOSO ARSOV, MARJAN KIPRIJANOVSKI, VIKTOR GJAMOVSKI<sup>1</sup>

SUMMARY: Thanks to suitable and different soil and climate conditions, a large number of fruit species are grown successfully on the territory of Macedonia. Despite all the favourable conditions, only small parts of fruit species are cultivated on large areas and have higher economical significance. The high bush blueberry is a new fruit species and it is practically unknown to most potential producers. Blueberry fruit species have an incredible biological value, rich in vitamins and nutritive matters important to human health. A large number of positive characteristics put the blueberry in the group of most requested fruits. Although certain locations have excellent conditions for successful and profitable fruit growing, the high bush blueberry (Vaccinium corymbosum L.) is still not present in our production. With the purpose of researching the possibilities of cultivating the high bush blueberry, in 2005 three-vear old trees have been introduced from 4 blueberry varieties: Duke, Blue Crop, Legacy and Toro. During the research, we have examined the vegetative growth (height and width of the bush), the phenology (the start of the vegetation, blooming and ripening of the fruits), the yield, and the dynamics of the ripening and the quality characteristics of the fruits (fruit weight, dry soluble material, total acids, ash and vitamin C) Based on the preliminary results of the research, it can be determined that this new fruit species can be grown successfully in certain regions of the country.

*Key words*: *Vaccinium corymbosum L., variety, growth, phenology, yield, fruit quality.* 

## INTRODUCTION

The blueberry is a fruit species with fruits that have an excellent biologic value, rich in different nutritive matter important to human health. The fruits have a high con-

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tent of sugars, pro-vitamin A, vitamin B3 and B5, vitamin C (50-350 mg/ %), mineral matter, anthocyanins, enzymes, tannins, pectins, etc. They also contain relatively high quantities of potassium, magnesium and sodium. Due to all of these positive characteristics, the blueberry belongs to the group of fruit with high biological and economic value, and it is in great demand in the world. There are certain locations in the R. of Macedonia with excellent ecological conditions for growing. From an economical point of view, due to the relatively cheap labour work, the growing of blueberry fruit cultures here has significant comparative advantages. Therefore, we need to pay more attention to this fruit species in the future. A lot of blueberry species are being cultivated around the world, but the American or high bushed blueberry (Vaccinium corymbosum L.) is the most significant of all.

For successful growing of this cultivar, we need specific ecological conditions. It prospers on soils with an acid reaction and in areas with colder climate. These kinds of areas exist in various regions of Macedonia. The bushes early with harvest, and the quality varieties in full fecundity and proper appliance of agricultural and pomotechnical measures can give a yield of up to 15 t/ha. The first researches for this species regarding its growing and varieties were made in former Yugoslavia by Krgović (1983).

## MATERIAL AND METHODS

The trial orchard has been established in the region of the village Smolare, Strumica in spring 2005, with three-year old seedlings. The plantings were of 4 varieties, Blue Crop, Duke, Toro and Legacy, were taken from German nurseries and they were planted on deluvium soil with pH 5.9 and humus of 2.3 %. The soil was prepared with deep ploughing of 50 cm, and cattle manure of 50 t/ha. The trial orchard was established in 3 repetitions with 20 plants from each variety, or the total of 60 plants per variety was investigated. The seedlings were planted at the distance of 3 x 1.25 m or 2667 bushes per ha. In the trial orchard the distance between rows was maintained by clean cultivation, and the strip in the rows with width 1.5 m was mulched with bark of conifers. The orchard was under an irrigation system.

The research included: phenological characteristics (period of blossom and ripening of the fruits), vegetative growth of the bushes (high and width) and yield (per bush and per hectare), and quality characteristics of the fruits (pomological characteristics of the fruits, dry matters, total acids and vitamin "C").

#### **RESULT AND DISCUSSION**

**Phenological characteristics.** The duration of the blossoming is a significant characteristic of the variety because with the early blossoming varieties there is a danger that the flowers and fruits will be frozen by the late spring frosts. Averagely for the two years, the Duke variety started blossoming the earliest 22.03, whereas Legacy was the last 12.04 (Tab.1). The blossoming ended the earliest with the Blue Crop and Duke varieties (15.04), whereas Legacy ended it the last, as usual (11.05).

The time of ripening and the harvesting of the small fruit are important for planning of available manual labour, selling market for the fresh fruit and manufacturing capacities. In order to prolong the period of harvest, during the establishment of larger orchards, they need to be planted with varieties with different times of ripening. According to our research, the Duke variety ripens the earliest (06.06), and the Legacy ripens the last (22.06).

| Variety   | Year    |        | Blosson | 1        |        | g     |          |
|-----------|---------|--------|---------|----------|--------|-------|----------|
| variety   | rear    | Begin. | End     | Duration | Begin. | End   | Duration |
|           | 2007    | 28.03  | 16.04   | 19       | 16.06  | 15.07 | 29       |
| Blue crop | 2008    | 30.03  | 14.04   | 15       | 2.06   | 28.06 | 26       |
| Ĩ         | Average | 29.03  | 15.04   | 17       | 9.06   | 7.07  | 28       |
|           | 2007    | 26.03  | 18.04   | 23       | 15.06  | 10.07 | 25       |
| Duke      | 2008    | 19.03  | 12.04   | 24       | 28.05  | 25.06 | 27       |
|           | Average | 22.03  | 15.04   | 24       | 6.06   | 2.07  | 26       |
|           | 2007    | 26.03  | 14.04   | 19       | 22.06  | 20.07 | 28       |
| Toro      | 2008    | 22.03  | 18.04   | 27       | 5.06   | 14.07 | 39       |
|           | Average | 24.03  | 16.04   | 23       | 13.06  | 17.07 | 34       |
|           | 2007    | 10.04  | 6.05    | 26       | 28.06  | 27.07 | 29       |
| Legacy    | 2008    | 14.04  | 15.05   | 31       | 15.06  | 20.07 | 35       |
|           | Average | 12.04  | 11.05   | 29       | 22.06  | 24.07 | 32       |

 Table 1. Phenological characteristics of the varieties

 Tabela 1. Fenološke karakteristike sorte

Ciordia et al. (2002) acquired the data on the time of ripening of several varieties. The Duke variety ripens from the end of May to June 26. According to Oblak (1977), the Blue Crop variety in Slovenia conditions blossoms from 22.04 to 22.05, and ripens from 07.07 to 01.09.

**Vegetative characteristics.** The dimensions of the bush (Table 2) are important parameters which show the vigorousness of certain variety on which the bearing potential depends. The height and the width of the bush are different at different varieties and are from 63.3 cm height and 71.5 cm with the Blue Crop variety, to 130.3 cm and 120 cm with the Toro variety.

The number of suckers at the Blue Crop variety is the lowest and it is 4.3, whereas the Duke variety has the highest (7.5). According to Krgovic (1968) the number of suckers per bush is the smallest with the Blue Crop variety, and the smallest with Darrow.

| Table 2. Vegetative development of the bushes |
|---|
| Tabela 2. Vegetativni razvoj voćaka           |

| Variety  | Year  | Height, cm | Width, cm | Number of<br>suckers |
|----------|---|------------|-----------|----------------------|
|          | 2007  | 61.1       | 68.5      |                      |
| Bluecrop | 2008  | 65.5       | 74.5      | 4.3                  |
| *        | M         63.3           2007         106.9           Duke         2008         135.5 | 63.3       | 71.5      |                      |
|          | 2007  | 106.9      | 90.5      |                      |
| Duke     | 2008  | 135.5      | 122.5     | 7.5                  |
|          | М   | 121.2      | 106.5     |                      |
|          | 2007  | 111.6      | 105.0     |                      |
| Toro     | 2008  | 149.0      | 135.0     | 5.0                  |
|          | М   | 130.3      | 120.0     | 7                    |
|          | 2007  | 90.4       | 94.5      |                      |
| Legacy   | 2008  | 127.5      | 125.0     | 6.5                  |
|          | М   | 109.0      | 109.7     | 7                    |

**Pomological characteristics of the fruits.** The results for the pomological characteristics of the fruit are shown in Table 3. The height and the width of the fruit is a

variety characteristic, and it is in direct correlation with the fruit mass (Krgovic, 1983). This has been confirmed also in our research. Namely, the Legacy variety has the smallest fruit height and width (10.85 mm height and 14.15 mm width), whereas the Toro variety has the largest fruit dimension (13.25 mm height and 19.55 mm width).

| Variaty  | Vaar  | Pomolog    | gical characteristics of fru | iits      |
|----------|---|------------|------------------------------|-----------|
| variety  | 2007           Bluecrop         2008           M         2007           Duke         2007           M         2008           M         2007           Duke         2008 | Height, mm | Width, mm                    | Weight, g |
|          | 2007  | 11.9       | 17.3                         | 1.80      |
| Bluecrop | 2008  | 11.7       | 17.1                         | 1.85      |
| М        | М   | 11.8       | 17.2                         | 1.8       |
|          | 2007  | 12.0       | 18.6                         | 2.28      |
| Duke     | 2008  | 11.9       | 18.0                         | 2.30      |
|          | М   | 11.95      | 18.3                         | 2.29      |
|          | 2007  | 13.5       | 19.90                        | 2.90      |
| Toro     | 2008  | 13.0       | 19.20                        | 2.95      |
| Γ        | М   | 13.25      | 19.55                        | 2.92      |
|          | 2007  | 10.5       | 13.80                        | 1.31      |
| Legacy   | 2008  | 11.2       | 14.50                        | 1.33      |
| Γ        | М   | 10.85      | 14.15                        | 1.32      |

 Table 3. Pomological characteristic of fruits

 Tabela 3, Pomološke karakteristike plodova

The fruit weight also varied depending on the variety. The Legacy variety had the smallest fruit weight (1.8 g), and Toro had the largest (2.92 g). Krgovic (1983) points out that the fruit mass at different varieties for three years was averagely from 0.98 to 1.67 g. The authors say that the fruit mass drops with the aging of the bush. The fecundity of the variety and the fruit quality are an important element for growing of the high bush blueberry. The data for the yield per bush and per ha are shown in (Table 4). The Duke variety is the most productive with yield of 4.2 kg/bush and 11.1 t/ha, whereas the Blue crop variety has the lowest productivity (1.9 kg/bush and 5.07 t/ha). According to Oblak et al. (1984), the yield is from 3.6 t/ha with Early blue to 6 t/ha with Ran cocas.

Table 4. Yield per bush and hectareTabela 4. Prinos po žbunu i hektaru

| Variata             | Year | Yield   |      |  |  |
|---------------------|------|---------|------|--|--|
| Variety             | rear | kg/bush | t/ha |  |  |
|                     | 2007 | 1.8     | 4.80 |  |  |
| Bluecrop            | 2008 | 2.0     | 5.33 |  |  |
|                     | М    | 1.9     | 5.07 |  |  |
|                     | 2007 | 3.8     | 10.1 |  |  |
| Duke                | 2008 | 4.5     | 12.0 |  |  |
|                     | М    | 4.2     | 11.1 |  |  |
|                     | 2007 | 3.6     | 9.6  |  |  |
| Toro                | 2008 | 4.2     | 11.2 |  |  |
|                     | М    | 3.9     | 10.4 |  |  |
|                     | 2007 | 2.8     | 7.5  |  |  |
| Legacy              | 2008 | 3.5     | 9.3  |  |  |
|                     | М    | 3.2     | 8.4  |  |  |
| LSD <sub>0.05</sub> | 0.32 |         |      |  |  |
| 0.05                | 0.47 |         |      |  |  |

The chemical characteristics of the fruit are shown in Tab.5. According to Oblak et al. (1984), the chemical content of the grown high bush blueberry has higher content and more suitable composition than the wild blueberry. In our research, the total dry material is 9.8 % with Toro to 15.6 % with Legacy. The content of soluble dry material is 10.8 % for Blue Crop to 13.1 % for Legacy. The content of the total acid is an important parameter for the taste characteristics of the fruit, and according to our research, these values were from 0.71 % with Legacy to 1.17 % with Bluecrop and Toro. According to Stanisavljevic and Jokovic (1987), the content of the total acids with Bluecrop is 1.56 %. The content of vitamin C is 21.5 mg % with Legacy to 36 mg % with Bluecrop. Similar results for these parameters were also shown by the author Oblak et al. (1984).

| N/a mi a taa | V    | Year Dry matters, % |         |      |                   |  |
|--------------|------|---------------------|---------|------|-------------------|--|
| Variety      | Year | Total               | Soluble | %    | Vitamin "C", mg % |  |
|              | 2007 | 12.8                | 10.6    | 1.19 | 36.5              |  |
| Bluecrop     | 2008 | 13.2                | 11.0    | 1.15 | 35.5              |  |
|              | М    | 13.0                | 10.8    | 1.17 | 36.0              |  |
|              | 2007 | 9.9                 | 13.7    | 0.87 | 32.3              |  |
| Duke         | 2008 | 10.1                | 9.8     | 0.82 | 31.5              |  |
|              | М    | 10.0                | 11.75   | 0.84 | 31.9              |  |
|              | 2007 | 9.7                 | 8.8     | 1.18 | 28.2              |  |
| Toro         | 2008 | 9.8                 | 9.0     | 1.15 | 27.4              |  |
|              | М    | 9.8                 | 11.4    | 1.17 | 27.8              |  |
|              | 2007 | 15.3                | 13.0    | 0.74 | 22.0              |  |
| Legacy       | 2008 | 15.8                | 13.2    | 0.68 | 21.0              |  |
|              | М    | 15.6                | 13.1    | 0.71 | 21.5              |  |

 Table 5. Chemical characteristics of the fruit

 Tabela 5. Hemiske karakteristike plodova

### CONCLUSION

Based on this preliminary investigation we can conclude that high bush blueberry (Vaccinium corymbosum L.) can be successfully grown in some regions of the R of Macedonia. The improvement of soil structure and pH reaction can be influenced by incorporation of high amount of organic matters (bark of conifers). The varieties should be chosen very carefully because some varieties are developed very well and they are more productive (Duke and Toro), while some others are poorly developed and they have low productivity (Blue Crop). More detailed research about technology of production has to be performed for definitive conclusions and recommendations about growing this culture. Since the blueberry can be grown on soils with low pH, and with the purpose of a massive spread of the culture, it is necessary to perform research regarding the possibilities and manners of acidation of the soil.

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# ISPITIVANJE VISOKO ŽBUNASTE BOROVNICE (VACCINIUM CORYMBOSUM L.) GAJENA U MAKEDONIJI

## TOSO ARSOV, MARJAN KIPRIJANOVSKI, VIKTOR GJAMOVSKI,

### Izvod

Zahvaljuć povoljnim i različitim zemljišnim klimatskim uslovima veliki broj voćnih vidova uspešno mogu da se gaje u Makedonii. Pored dosta povoljnih uslova samo mali deo voćnih vidova se gaje na većim površinama i imaju veliko ekonomsko značenje.

Visoko žbunasta borovnica je nov voćni vid, skoro nepoznat za potencijalne proizvođaće. Plod ima veliku biološku vrednost, bogatu vitaminima i hranljivim materijama, značanje za čovečko zdravlje. Veliki broj pozitivnih karakteristika stavljaju borovnicu u grupi najtraženijih voćnih vidova. Mada pojedini lokaliteti u Makedonii imaju odlične uslove za profitabilnu proizvodnju, visoko žbunaste borovnice, (*Vaccinium corymbosum L.*) još nije dovoljno zastupljena u našu proizvodnju. U cilju ispitivanja visoko žbunaste borovnice u 2005 godini su introdukovane 3 godisnje sadnice, 4 sorte: Duke, Blue crop, Legacy i Toro. Za vreme istraživanja ispitivali smo vegetativni rast (visina i širina žbuna), fenologiju (početak vegetativne aktivnosti, cvetanje i sazrevanje ploda), produktivnost, dinamika sazrevanja ploda i kvalitetne karakteristike ploda (težina, rastvorlive suve materije, ukupne kiseline, pepeo i vitamin C). Bazirajući se na preliminarne rezultate ispitivanja, može da se zakljuci da ovaj voćni vid može uspešno da se proizvodi u određenim regionima u Republici Makedonii.

Ključne reči: Vaccinium corymbosum L., sorta, rast, fenologiju, prinos, kvalitet ploda.

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# COMPARATIVE INVESTIGATION OF SOME PEACH ROOTSTOCKS

## MARJAN KIPRIJANOVSKI, VIKTOR GJAMOVSKI, TOSO ARSOV<sup>1</sup>

SUMMARY: In the R. of Macedonia, the peach is cultivated at acreage of about 1400 ha. The peach orchards are mainly concentrated on the territory of the small rural municipality Rosoman in the Tikves region. Thanks to the favorable climate conditions in this region, the orchards give excellent results and the fruit has recognizable quality. The production includes a large number of varieties with different ripening time. The only rootstock in these orchards is the vineyard peach. The Rosoman region is rich in carbonate soils with a high pH. The high content of carbonates and the high sensitivity of the vineyard peach towards the alkaline soils are the reasons for frequent appearance of chlorosis. The chlorosis, as well as its negative consequences, is often a limiting factor for the peach being wide spread in this region. With the purpose of finding a solution for the chlorosis problem, in 1999 an experimental peach orchard was established on alkaline soils in the Rosoman region. The orchard was established with one year old trees from the red haven variety. Seedlings from vineyard peach and Nemaguard, and vegetative rootstock from the hybrid of peach x almond GF 677, were used as rootstocks. The trees were planted at the distance of  $4 \times 3 \text{ m}$ . The research was performed from planting until the end of the tenth vegetation. The research included the following: trunk diameter, occurrence of chlorosis on the leaves, premature mortality of the trees, as well as the yield per tree and yield on the acreage. According to the investigations, it has been concluded that the vegetative rootstocks from hybrid peach x almond GF 677 give the best results in adequate soil conditions. In the future orchards, the vineyard peach should be replaced by this rootstock.

Key words: rootstock, evaluation, peach, trees, growth, yield

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#### INTRODUCTION

The largest production region for peach in Macedonia is the Tikves region, where the peach is grown at the surface of almost 1000 ha. Traditionally, the seedlings from the vineyard peach are being used as rootstock. This rootstock is characterized by relatively easy plant production, great affinity towards the domesticated varieties, productivity and fruit quality (Kolekevski et al., 2004). But the vineyard peach has several flaws such as short lasting, poor development of fatigue soils, susceptibility to nematodes, poor growth on heavy, humid and cold soils and the high susceptibility to high content of carbonates. On the other hand, a large part of the Tikves region area is mostly with alkaline reaction and therefore the peach orchards often show leaf chlorosis (Ristevski, 1995). This causes low yields, low fruit quality, higher susceptibility to low temperatures etc. In order to avoid these problems, there are several solutions, and the most important is the choice of rootstocks which give better results under such unfavorable soil conditions.

The large number of newly created rootstocks requires their testing in different soil and climate conditions. The knowledge of their behavior in different conditions enables more adequate agricultural and pomotehnical measures, with the purpose of enabling optimal conditions for the growing of the orchards. A large number of authors have evaluated different peach rootstocks (Beckman et al. 2002; Dirlewanger et al. 2002; Lorety et al., 2002; De Salvador et al., 1993, 2002; Forlani et al., 1990; Paunovic et al., 1987; Yadava, 1990; Reighard, 2001; Ognjanov et al., 1996, 2008).

### MATERIAL AND METHODS

In the spring of 1999, a demonstrative orchard has been established in the region of Rosoman, on a limy soil with a pH of 7.9. For the establishment of the orchard we used one year old trees from the red haven variety, grafted on 3 different rootstocks: vineyard peach, as a control, GF 677 vegetative propagated and seedlings from Nemaguard. The trial orchard has been established in 4 repetitions in a random block system. Each repetition included 3 plants or 12 trees were examined in variant. The plants have been planted at the distance of  $4 \times 3 \text{ m}$ , or 833 fruit trees per ha. The crowns were formed by the system of an irregular palmete. The orchard has been maintained according to the standard technology for this region.

During the research, the following parameters were followed: at the end of each vegetation, the diameter of the trunk was measured and the trunk cross section area was calculated. The appearance of chlorosis of the leaves was marked based on the intensity of the yellow coloring of the leaves during the vegetation. The marking was performed on the scale from 0 to 5. The productivity of the trees was calculated based on the measurement of the weight of the fruit at the harvest. Due to the winter and late spring frosts, which caused damages of the buds and fruitlets in 2001,2002 and 2003, the yield is presented for the period from 2004-2008, when the trees were in their full fruitfulness.

### **RESULTS AND DISCUSSION**

Diameter and **TCSA.** the diameter of the trunk is an integral parameter for the whole vegetative potential of the trees. The large absorption of the root system results in

the increase of the production of organic matter in the crown, and they all contribute to the forming of more elements of xylem and phloem which at the end registers through the increase of the trunk diameter.

Analyzing the data from Table 1, we can notice that at the end of the 10<sup>th</sup> vegetation the trees grafted on rootstock GF 677 have the largest trunk diameter, whereas the rootstock Nemaguard is poorly vigorous. The trees grafted on rootstock GF 677 have the largest stem diameter respective to the trees grafted on vineyard peach by 37.2%, whereas the trees grafted on Nemaguard are poorly vigorous by 11.4% respective to the control.

Table 1. Trunk diameter of the trees, mm Tabela 1. Prečnik stabla

| Rootstock      |       | Year after planting |       |       |       |       |       |  |  |  |  |
|----------------|-------|---------------------|-------|-------|-------|-------|-------|--|--|--|--|
| KOOISIOCK      | V     | VI                  | VII   | VIII  | IX    | Х     | Index |  |  |  |  |
| Vineyard peach | 79.5  | 88.7                | 95.9  | 102.1 | 104.3 | 107.0 | 100.0 |  |  |  |  |
| Hydride GF 677 | 103.8 | 115                 | 125.2 | 133.9 | 140.2 | 146.9 | 137.2 |  |  |  |  |
| Nemaguard      | 68.6  | 75.9                | 82.8  | 85.1  | 90.2  | 94.6  | 88.4  |  |  |  |  |
|                |       | LSD 0.05            |       |       |       | 16.5  |       |  |  |  |  |
|                |       | 0.01                |       |       |       |       |       |  |  |  |  |

If the tree develops in better conditions for photosynthesis during the vegetation, it will have stronger cambial activity which will enlarge the growth and therefore the diameter (TCSA) (Figure 1). De Salvador et al., (1993) points out that the trees grafted on vegetative rootstock GF 677 in the 6<sup>th</sup> year have a larger TCSA by 47.5% respective to the trees grafted on vineyard peach seedlings. The decrease of the trunk diameter can cause reduction of the tree height which will have the positive effect for reducing the cost of the manual operations throughout the growing and harvest (DeJong et al., 2001).

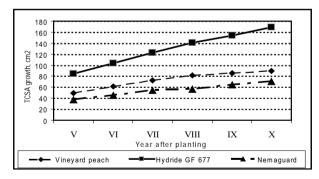


Figure 1. Dynamics of the TCSA growth Grafikon 1. Dinamika rasta poprečnog preseka stabla

Leaf chlorosis peach is very sensitive to leaf yellowing which occurs on soils with high content of carbonates. This affects negatively the process of photosynthesis which influences the vegetative development and fruitfulness, and sometimes leads to dying of the trees. The trees grafted on vineyard peach seedlings are especially sensitive to this. There are many solutions for this problem, but the most efficient is the choice of rootstock resistant to chlorosis. The data for the grading of leaf chlorosis during vegetation is shown in Table. 2. The vegetative rootstock GF 677 is the most resistant to the highest pH value of the soil, whereas the seedling of vineyard peach are the most sensitive. (Kolekevski et al., 2004) points out that the hybrid GF 677 has high resistance to the carbonates in the soil and recommends this rootstock for establishing peach orchards on soils rich in carbonates.

Table 2. Occurrence of chlorosis on the leaves (0-5)Tabela 2. Pojava hloroze lista (0-5)

| Rootstock      | Chlorosis (0-5) |
|----------------|-----------------|
| Vineyard peach | 4.0             |
| Hydride GF 677 | 0.0             |
| Nemaguard      | 2.0             |

0- no chlorosis

5-extremly yellowing of the leafs

Premature dying of the trees is one of the most common problems in the peach orchard growing. The early drying of the trees results in thinning of the orchards and therefore makes them non-rentable for growing. There are many reasons for the sudden death of the trees such as fatigue of the soil, presence of nematodes, bad soil conditions, pests and diseases, frost, high content of carbonates etc. According to Yadava (1990), the rootstock plays an important role in the physiology of the above-ground part and therefore it has its implication on the sudden death of the trees. During our research on the premature dying of the trees, the most sensitive ones were the trees grafted on Nemaguard, whereas the ones grafted on the hybrid GF 677 had no premature death (Table 3).

Table 3. Premature dying of the trees, % Tabela 3. Prevremeno sušenje voćaka, %

| Rootstock      | 4th year | 6th year | 8th year | 10th year |
|----------------|----------|----------|----------|-----------|
| Vineyard peach | 0.0      | 8.3      | 8.3      | 16.7      |
| Hydride GF 677 | 0.0      | 0.0      | 0.0      | 0.0       |
| Nemaguard      | 8.3      | 8.3      | 16.7     | 16.7      |

Productivity of the trees is the most important factor for successful growing of fruit orchards. The productivity depends on several factors such as genetic potential of the variety, ecological conditions, maturity and development of the tree, agricultural and pomotechnical measures, rootstock etc. During years 2001, 2002 and 2003, we had strong winter and late spring frosts which caused freezing of the buds and flowers and significantly reduced and disturbed the yield in the research orchard. Therefore, the data for the productivity of the trees is for the period from 2004-2008 (Table 4). For the researched 5 year period, the trees grafted on the vegetative rootstock GF 677 gave the highest yield (47, kg), whereas the trees grafted on the Nemaguard rootstock gave the lowest (29,7 kg). The trees grafted on the hybrid GF 677 gave higher yield by 29.1 % compared to the control.

| Rootstock      |      | Year after planting |      |      |      |      |       |  |  |
|----------------|------|---------------------|------|------|------|------|-------|--|--|
| KÖÖISIÖCK      | VI   | VII                 | VIII | IX   | X    | М    | Index |  |  |
| Vineyard peach | 33.7 | 38.1                | 39.6 | 33.4 | 37.5 | 36.5 | 100   |  |  |
| Hydride GF 677 | 37.8 | 52.7                | 54.9 | 43.4 | 46.7 | 47.1 | 129.1 |  |  |
| Nemaguard      | 30.9 | 31.8                | 31.4 | 24.2 | 26.3 | 29.7 | 80.0  |  |  |
|                |      | LSD 0.05            |      |      |      | 6.7  |       |  |  |
|                |      | 0.05                |      |      |      | 9.9  |       |  |  |

| Table 4. Yield per tree, kg, (2004-2008)    |
|---|
| Tabela 4. Prinos po stablu, kg, (2004-2008) |

The yield per hectare is also another important fact for the economical growing of the orchards. The average yield per hectare for a period of 5 years decreased by the percent of dead trees is shown in Tab. 6. These data show that the trees grafted on the vegetative rootstock GF 677 give the highest average yield per hectare (39.2 t/ha), whereas the trees grafted on the Nemaguard give the lowest average yield per hectare (20.9 t/ha).

0.01

Table 5. Yield per hectare, t/ha, (2004-2008) Tabela 5. Prinos po hektaru, t/ha (2004-2008)

| Rootstock      | Year after planting |      |      |      |      |      |       |  |
|----------------|---------------------|------|------|------|------|------|-------|--|
| KOOISIOCK      | VI                  | VII  | VIII | IX   | X    | М    | Index |  |
| Vineyard peach | 28.0                | 31.7 | 30.2 | 25.5 | 26.0 | 28.3 | 100   |  |
| Hydride GF 677 | 31.5                | 43.9 | 45.7 | 36.1 | 38.9 | 39.2 | 138.7 |  |
| Nemaguard      | 23.6                | 24.2 | 21.7 | 16.8 | 18.2 | 20.9 | 73.9  |  |
|                | LSD 0.05            |      |      |      | 5.8  |      |       |  |

8.9

# CONCLUSION

0.01

Based on the comparative research regarding the behavior of different peach rootstocks, the next conclusions can be presented:

- The rootstock has an important influence on the vigorousness of the trees. The most vigorous trees are those which were grafted on the vegetative rootstock GF 677. The trees grafted on this rootstock should be planted at a larger distance.
- The fruit trees grafted on the hybrid GF 677 do not show signs of chlorosis whereas the vineyard peach is extremely susceptible of high pH of the soil.
- The rootstock has an influence on the long lasting of the orchards. The vegetative rootstock GF 677 has shown best results.
- The fruit trees grafted on a vegetative rootstock GF 677, have the highest productivity, whereas the fruit trees grafted on the Nemaguard rootstock, which are poorly developed, showed the poorest productivity.
- The rootstocks from the hybrid GF 677, according to the vegetative development of the trees, the resistance to chlorosis, the premature dying, the productivity and quality of the fruits, shows excellent results and therefore is recommended for use as a peach rootstock planted on limy soils as a replacement for vineyard peach.

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# UPOREDNA ISPITIVANJA NEKIH PODLOGA ZA BRESKVU

### MARJAN KIPRIJANOVSKI, VIKTOR GJAMOVSKI, TOSO ARSOV

### Izvod

U R. Makedonii breskva se gaji na površini od oko 1400 ha. Uglavnom, zasadi su koncentrisani na teritoriji opštine Rosoman u Tikveškom regionu. Zahvaljući povoljnim klimatskim uslovima u ovom regionu zasadi daju izvanredne i kvalitetne plodove. U zasadima su zastupljene sorte sa različitim vremenom sazrevanja. Jedinstvena podloga na kojoj su podignuti proizvodni zasadi breskve je vinogradarska breskva. Rosomanski region je bogat karbonatnom zemljom sa visokom pH vrednošću. Visok sadržaj karbonata i velika osetljivost vinogradarske breskve na alkalna zemljišta je razlog za čestu pojavu hloroze u zasadima breskve. Hloroza kao i njene negativne posledice, česti su uzroci za limitiranje širenja proizvodnje breskve u ovom regionu. Sa ciljem za rešavanje ovog problema sa hlorozom u 1999. godini podignut je eksperimentalni zasad breskve na alkalnoj zemlji u Rosomanskom regionu. Zasad je podignut sa jednogodišnjim sadnicama sorte Redhaven, kao podloga korišteni su sejanci vinogradarske breskve, Nemagard i vegetativne podloge hibrida breskva x badem GF 677. Sadnice su sađene na rastojanju 4 x 3 m. Ispitivanje je vršeno od početka sađenja pa do desete vegetacije. Ispitivani su: dijametar stabla, hloroza listova, prevremeno sušenje voćaka kao i prinos po voćki i površini. Za vreme ispitivanja može se zaključiti da vegetativne podloge hibrida breskva x badem GF 677 daju najbolje rezultate na adekvatnim zemljišnim uslovima. Ova bi podloga mogla da zameni vinogradarsku breskvu.

Klučne reči: podloge, evaluacija, breskva, voćke, vegetativni rast, prinos

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# ALBA, MAYA AND ROXANA-NEWLY INTRODUCED| STRAWBERRY VARIETIES IN MACEDONIA

### MARJAN KIPRIJANOVSKI, TOSO ARSOV, VIKTOR GJAMOVSKI<sup>1</sup>

SUMMARY: The yield and the fruit quality in the strawberry orchards depend on the variety which is grown. In the world selection centers, there is a continuous creating of new varieties characterized with high productivity, fruit quality and resistance to unsuitable conditions. The growing of these varieties creates a higher profit and therefore is more requested amongst the producers. The genetic potential in the strawberries is higher if the ecological conditions suit the necessities of the adequate assortment. Therefore, it is necessary to do a detailed research in the ecological conditions of the region, before any mass production of a certain variety. The assortment in the strawberry production in Macedonia is very poor. With the purpose of modernization of the assortment, three promising strawberry varieties have been introduced: Alba, Mava and Roxana. Orchard has been established on September 23 2006 in the region of Kocani, in the eastern part of Macedonia, with imported green plants rooted in pots. The researches have been conducted in the period of 2006-2008. During the researches, the following parameters have been observed: height of the plant, number of leaves, period of blooming and ripening, number of flowers and fruits per plant, dynamic of the ripening, vield, average fruit weight, content of soluble drv matters, total acid, pH and vitamin C.

*Key words:* Fragaria ananasa Duch, evaluation, growth, productivity, quality

### **INTRODUCTION**

The strawberry has an important place in the structure of the complete fruit production in the Republic of Macedonia. In the last few years, the strawberry is being grown at an area of approx. 500 ha, and the total production is around 5000 t. The

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production includes different technologies of growing such as open field, growing in polyethylene tunnels, and vertical growing which is very common lately. The strawberry fruits are mostly used for fresh marked, and only a small part for different types of home processing.

The strawberry assortment in the developed fruit production countries is very dynamic. The growing of the Pocahontas variety prevails in our country.

Most world centers constantly create new strawberry varieties which characterize with good productivity, fruit quality, high resistance to unfavorable biotic and abiotic factors etc. We still do not practice creating varieties of the fruit species which will be adaptable for growing in adequate ecological conditions. Therefore, the introduction of varieties which had been bred in different selection centers is practiced for improvement of the assortment. The introduced varieties establish trial orchards in different production regions, their behavior in adequate ecological conditions is being observed, and the varieties that show good characteristics are recommended for further production.

In the strawberry production, the variety is the basic condition for creating higher profit. When choosing the assortment, one should take under consideration the biological-production characteristics of the varieties, climate-soil conditions in the region, system and technology of growing, post harvest technology, consumer's demands, purpose of the fruits, transport, vicinity of the market etc. (Kiprijanovski et al., 2003).

Numbers of authors have been researching the strawberry varieties (Stancecić et al., 1982, Tonkonozenko, 1985, Stanisavljević et al., 1997, Radajevska, 1997, Kaska, 1997, Dejvor et al., 1997)

These researches have been conducted with the purpose of examining the behavior of three introduced strawberry varieties in adequate ecological conditions and technology of growing.

### MATERIAL AND METHODS

In the period 2006/2008 in the region of Kocani (Eastern part of R. Macedonia), the researches of the some strawberry varieties have been conducted in orchards established with green plants rooted in pots. Alba, Maya and Roxana were the varieties of plants used, imported from the nursery New fruits from Cesena-Italy. The trial orchard was established in tree repetitions. Per 1000 plants from each variety have been used.

Two rows bed system was used with black plastic mulch and drip irrigation and fertirigation. Planting density was 47600 plants per ha. The trial orchard was established on September 23<sup>rd</sup>, 2006.

With the purpose of forcing their ripening on January 20th, the orchards have been covered with high polyethylene tunnels without heating.

The researches included the following parameters: vegetative development of the plants, the number of blossoms and marketable fruits per plant, the dynamics of fruit's ripening, fruit's average mass, total yield per plant and per ha, as well as the content of soluble dry matters and total acids, pH and vitamin "C".

### **RESULTS AND DISCUSSION**

Vegetative growth of the plants- The height of the plant is an important morphological characteristic for the whole vegetative activity, the quantity and quality of the yield. This characteristic of the strawberry is a specification of the variety but highly dependant on the maturity and the conditions of the orchard. The leaf is the basic organ of the fruit plants. It is the only producer of organic mater and distributed through the plant from there. Therefore, the whole vegetative and reproductive activity of the plant depends on the number of leaves, their health condition and lighting. The data for the vegetative development of the plant is shown in Table 1. The data shows that the development of the plant depends on the maturity as well as the variety. The Maya variety is the most vigorous by means of height and number of leaves, whereas the Alba variety is the less vigorous.

| Variety | Н    | leight of the pla | nts, cm | Number of leaves per plant |      |         |  |
|---------|------|-------------------|---------|----------------------------|------|---------|--|
| variety | 2007 | 2008 Average      |         | 2007                       | 2008 | Average |  |
| Alba    | 22,5 | 31,5              | 27,0    | 20,5                       | 25,6 | 23,1    |  |
| Maya    | 29,2 | 39,7              | 34,5    | 31,3                       | 38,3 | 34,8    |  |
| Roxana  | 30,6 | 37,9              | 34,3    | 29,3                       | 32,2 | 30,8    |  |
| Average | 27,4 | 36,4              | 31,9    | 27,0                       | 32,0 | 29,5    |  |
| LSD     |      | 0.05              | 1.85    | LSD                        | 0.05 | 2.42    |  |
| LSD     |      | 0.01              | 2,54    | LSD                        | 0.01 | 3.60    |  |

Table 1. Vegetative growth of the plantsTabela 1. Vegetativni rast bokora

**Phenology.** The most important phenophases for the annual development of the strawberries are the blossoming and the ripening. The beginning and the lasting of these phenophases depends on the inherited characteristics of the varieties, the geographic width, altitude, technology of growing as well as the climate conditions before and during this phenophase. The early ripening of the variety is an important factor for the strawberries for fresh consumption. This characteristic is very appreciated if the orchard is being grown in closed areas by forcing of the ripening. Usually, on our markets, the first fresh fruits reach a much higher price respective to the late ones. Therefore, the early ripening varieties have the potential for bigger financial effects. The Alba variety has the earliest development and ripening (Table 2). This variety ripens about 5-7 days earlier than the other varieties. Therefore, this variety is the most suitable for growing in protected culture orchards.

Table 2. Period of blossom and ripening of the fruitsTabela 2. Period cvetanja i zrenja plodova

| Variety | Blos       | som period  | Ripening period |             |  |  |
|---------|------------|-------------|-----------------|-------------|--|--|
| variety | 2007       | 2008        | 2007            | 2008        |  |  |
| Alba    | 3.03-15.04 | 5.03 -03.04 | 14.04-13.05     | 12.04-8.05  |  |  |
| Maya    | 6.03-23.04 | 9.03-16.04  | 20.04-22.05     | 18.04-19.05 |  |  |
| Roxana  | 7.03-20.04 | 9.03-14.04  | 19.04-19.05     | 17.04-15.05 |  |  |

The number of blossoms and fruits per plants. The number of flowers directly determines the potential yield of the strawberries. The basic motive for strawberry growing is the fruits. The strawberry yield depends on several factors, but mostly on the genetic potential of the variety, the development of the plants, the degree of pollination, ecological conditions, manner of growing, agro and pomotechnical etc. The number of fruits together with their size determines the total yield of the strawberries. Due to the poor development, in the first year of the research, all of the plant varieties have had less flowers and fruits compared to the second year. Averagely for the two years, the Alba variety had the lowest number of flowers and fruits per plant, whereas the maya variety had the largest number of flowers and Roxana had the largest number of marketable fruits (Table 3).

| Variety | Blo  | ossoms per | . plant | Number of marketable fruits per plant |      |         |  |
|---------|------|------------|---------|---------------------------------------|------|---------|--|
|         | 2007 | 2008       | Average | 2007                                  | 2008 | Average |  |
| Alba    | 28   | 68         | 48      | 25                                    | 43   | 34      |  |
| Maya    | 39   | 87         | 63      | 29                                    | 47   | 38      |  |
| Roxana  | 29   | 95         | 62      | 24                                    | 58   | 41      |  |
| Average | 32   | 82         | 57      | 26                                    | 49   | 38      |  |
|         | LSD  |            | 6.1     | LSD                                   | 0.05 | 2.5     |  |
|         | LSD  | 0.01       | 9.3     | LSD                                   | 0.01 | 3.7     |  |

Table 3. Number of blossoms and marketable fruits per plantsTabela 3. Broj cvasti i plodova po bokoru

**Fruit yield.** The yield in the strawberry orchards depends on a large number of factors amongst which are the variety, planting material quality, applied agro-measures, development of the plants, weather conditions etc.

Averagely for the two years, the Roxana variety had the highest yield (822,5 g/ plant), whereas the Maya variety had the lowest yield (512,5 g/plant) (Table 4). There isn't any statistical difference in the yield between Maya and Alba.

| Variety |       | g/ plant |       |      | t/ha |      |
|---------|-------|----------|-------|------|------|------|
|         | 2007  | 2008     | М     | 2007 | 2008 | М    |
| Alba    | 453   | 625      | 539,0 | 21,2 | 29,7 | 25,4 |
| Maya    | 435   | 590      | 512,5 | 20,6 | 28,1 | 24,3 |
| Roxana  | 660   | 985      | 822,5 | 30,2 | 46,9 | 38,5 |
| Average | 516,0 | 733,3    | 624,7 | 24,0 | 34,9 | 29,5 |
|         | LSD   | 0.05     | 55.4  | LSD  | 0.05 | 2.63 |
|         | LSD   | 0.01     | 84.6  | LSD  | 0.01 | 4.02 |

Table 4. Yield per plants and hectareTabela 4. Prinos po bokoru i hektar

**Time and dynamic of ripening** the time and dynamic of ripening at strawberries depends on a number of factors such as variety, climate conditions in the region of growing, growing technology etc.

The researched varieties show that the fruits from the Alba variety ripe earliest, whereas the fruits from the Roxana variety ripe the latest. The ripening of the fruits at Alba took place in a period of 28 days, average whereas at Maya and Roxana in a period of 30 days (Figure 1).

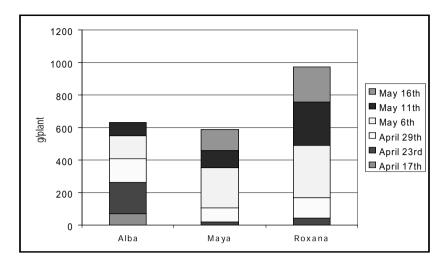


Figure 1. Distribution of fruits yield with strawberries varieties depending on fruits harvest, 2008 Grafik 1. Distribucija prinosa kod sorti jagode zavisno od datuma berbe, 2008

**Fruit quality** depends mostly on the variety, climate conditions, growing technology etc. The cultivar Roxana has the largest fruits of 28,4 g; whereas the fruits from the Maya variety have the lowest average mass of 15,3 g. Concerning the content of soluble dry material and total acids there isn't a large difference between the varieties. The lowest contain of vitamin c is at variety Alba of 35,0 mg/% and the highest at Roxana 47.4 mg/%.

| Variety |      | weight of<br>uits, g | Soluble<br>matte |      | Total acidity, % |      | рН   | Vitamin<br>"C"<br>mg/% |
|---------|------|----------------------|------------------|------|------------------|------|------|------------------------|
|         | 2007 | 2008                 | 2007             | 2008 | 2007             | 2008 | 2008 | 2008                   |
| Alba    | 18,1 | 14,5                 | 8,4              | 8.2  | 0,86             | 0,79 | 3,54 | 35,0                   |
| Maya    | 18,6 | 12,6                 | 7,9              | 7,8  | 0,72             | 0,69 | 3,80 | 45,4                   |
| Roxana  | 23,5 | 17,0                 | 8,0              | 7,3  | 0,71             | 0,82 | 3,62 | 47,4                   |
| Average | 20,1 | 14,7                 | 8,1              | 7,8  | 0,76             | 0,77 | 3,65 | 42,6                   |

Table 5. Quality characteristics of fruitsTabela 5. Kvalitetne karakteristike plodova

### CONCLUSIONS

Based on the researches, it is determined that:

- 1) The Roxana variety has good biological characteristics, high yield, quality and large fruits.
- 2) The Alba variety is good for its early ripening, uniformity and transportability, and good quality fruits. This variety can be recommended for growing in this region for forcing production
- 3) The Maya variety because of a bad transportability and lower yield did not give any good results in this region.

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# ALBA, MAYA I ROXANA-NOVO INTRODUCIRANE SORTE JAGODE U R. MAKEDONII

### MARJAN KIPRIJANOVSKI, TOSO ARSOV, VIKTOR GJAMOVSKI

### Izvod

Prinos i kvalitet plodova jagode u zasadima zavisi uglavnom od sorte koja se proizvodi. U svetskim selekcionim centrima stalno se kreiraju nove sorte koje se karakterišu visokom produktivnošću, kvalitetnim plodovima i otpornošću prema nepovoljnim uslovima. Gajenje ovih sorti donosi veliki profit i zato su tražene od strane proizvođača. Genetski potencijal kod jagode je veći ukoliko su ekološki uslovi dobri za adekvatni sortiment. Iz ovih razloga potrebna su detaljna ispitivanja za određene sorte u određenom regionu za dalju i veću proizvodnju. Sortiment jagode u R Makedoniji je veoma siromašan. U cilju modernizacije sortimenta introdukovane su tri sorte jagoda: Alba, Maya i Roxana. Zasad je podignut 23. Septembra 2006. godine u reonu grada Kočani, u istočnom delu Makedonije, sa uvezenim kontejnerskim sadnicama. Ispitivan-ja su sprovedena u periodu 2006-2008. godine sa sledećim parametrima: visina žbuna, broj listova, period cvetanja i sazrevanja ploda, broj cvetova i plodova po žbunu, dinamika sazrevanja, prinos, težina ploda, sadržina rastvorllivih suvih materija, ukupnih kiselina, pH vrednost i sadržaj vitamina "C".

Ključne reči: *Fragaria ananasa Duch*, ispitivanje, vegetativni rast, produktivnost, kvalitet ploda.

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## POSSIBILITIES OF ORGANIC MILK PRODUCTION IN SERBIA

# ANKA POPOVIĆ-VRANJEŠ, MIROSLAV GRUBAČIĆ , RADOVAN PEJANOVIĆ, ALEKSANDAR KRALJ, ANKA KASALICA, DRAGICA MIOČINOVIĆ, GORDANA NIKETIĆ<sup>1</sup>

SUMMARY: The research presented in this paper is aimed at comparing the quality of milk and milk products (based on the fatty acids content) produced based on the principles of organic production of Austria, Italy and Slovenia, with the conventional products produced in Serbia. It has been determined that the products of one country differ on the market of that country much according to the model of fatty acids, but that they also differ from country to country. It has also been determined that some conventionally produced products from Serbia have had higher content of fatty acids important for human health (omega 6 and omega 3 and arahidonic acids). UHT milk from Serbia (Subotica) has had higher percent (2.823%) of these fatty acids compared to the same quality milk from Italy (1.233%) and to the milk from Slovenia (0.615%). The fresh cheese mozzarella from Serbia (Guča) contained higher percentage of the named fatty acids (0.89%) compared to the same type of cheese from Italy (0.258%). The kefir produced in Serbia (Kraljevo) had almost a triple amount of the named fatty acids (2.823%) in comparison to the kefir produced in Slovenia. The quality of milk produced based on the principles of organic production on ten small farms of the hill of Fruška gora (with 8-10 cows) had some quality advantages in comparison to the conventional milk regarding certain parameters of its content (fat, dry matter) and number of somatic cells.

Key words: organic milk, conventional milk, parameters of quality.

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### INTRODUCTION

Modern aspirations of the agricultural development of Serbia inevitably include larger participation of organic agriculture in the total agricultural production. Serbia is one of the countries which still have the preserved agricultural ecosystem, and it also has small farms with the complete cycles of plant and animal productions. Organic agriculture is present in ecologically disturbed environments on various locations of Serbia. One of such environments is the National Park of Fruška Gora located in the Province of Vojvodina, Serbia, in the vicinity the town of Novi Sad. The pastures and fertile soil, vineyards and orchards, deciduous forests, all show that there is a huge space potential for the organic production to be organized in a very short transitional period of time there. By the development of the organic agriculture, the customers are to be given the possibility to get higher quality food products from Serbia, the products that are in compliance with the strict quality standards of food and environment protection, while the producers will have a new market-export through eco-tourism. Small households are to be given a chance to be promoted through sustainable (organic) agriculture, through labor-intensive production system and by cheap inputs, as well.

## MATERIAL AND METHODS

The content of fatty acids in milk and in milk products from Italy, Austria, Slovenia and Serbia has been determined by the method of gas chromatography (Butte, 1983). The researches have been made on raw milk produced in the conditions of both organic and conventional production in the area of Vojvodina. Organic milk was collected from small farms located in the area of the hill of Fruška gora, while conventional milk from the big farm located in the area of the town of Vrbas. The milk samples were tested in relation to their physical-chemical composition and to the total number of bacteria and the number of somatic cells, once a month in the period from February to December 2009. The parameters of physical-chemical quality of milk produced according to organic and conventional systems ("The Official Gazette of the SFRY, nos. 32/83; IDF 20B: 1986; IDF 29:1964; IDF 27:1964) were tested by the standard methods of analysis. The total number of bacteria was determined by adding them on the agricultural surface for the total number and by incubation on 30°C/72h, and the number of somatic cells by the method of IDF 148A:1995, with the Coulter Counter device. In the organic system, from early May till early September the feeding of the Simmental breed of cows was based on pasturing with added concentrate, while during other months they were fed with concentrate, hay and grains (corn and barley). In the conventional system applied on the farm near the place of Vrbas, during the research period the cows were fed on the mix feed which consisted of corn meal, silage, molasses, whey and hay.

#### **RESULTS AND DISCUSSION**

The content of fatty acids important for human health (omega 3, omega 6 and arachidonic acids) in milk and in some milk products from o3rganic production from Italy, Austria and Slovenia as well as in milk and some milk products from Serbia from conventional production have been given in Table 1.

Table 1. Content of fatty acids in milk products of conventional production (%); (Serbia, Austria) *Tabela 1. Sadržaj masnih kiselina u mlečnim proizvodima iz konvencionalne proizvodnje (%);* (Srbija, Austrija)

|     |                      |  |   |                                | SER                           | BIA                      |  |                            | AUSTRIA                |
|-----|----------------------|--|---|--------------------------------|-------------------------------|--------------------------|--|----------------------------|------------------------|
| No. | Fatty acids          | No of C atoms and<br>unsaturated bonds | Pasteurized milk,<br>2.8 % mm, Dukat,<br>Sombor | UHT milk<br>3.5 % mm, Subotica | Yoghurt, 3.2 % mm<br>Subotica | Kefir, 2.5 % mm<br>Imlek | Katschkaval cheese<br>Sava Kovačević,<br>Vrbas | Mozzarella<br>Valeta, Guča | Emmentaler,<br>Austria |
| 1   | Capric               | 10:0                                   | 1.222   | 1.515                          | 0.292                         | 3.060                    | 0.438  | 0.691                      | 0.739                  |
| 2   | Udecanoic            | 11:0                                   | 0.628   | 0.843                          | 0.335                         | 1.053                    | 0.541  | 0.488                      | 0.342                  |
| 3   | Lauric               | 12:0                                   | 13.925  | 13.096                         | 5.357                         | 14.485                   | 7.398  | 8.374                      | 8.485                  |
| 4   | Tridecanoic          | 13:0                                   | 0.094   | 0.309                          | 0.073                         | 0.166                    | 0.108  | 0.369                      | 0.070                  |
| 5   | Miristinic           | 14:0                                   | 15.395  | 17.460                         | 17.132                        | 15.404                   | 17.947   | 13.825                     | 12.154                 |
| 6   | Miristoleate         | 14:1                                   | 0.303   | 0.436                          | 0.147                         | 0.538                    | 0.321  | 0.232                      | 0.231                  |
| 7   | Pentadecenoic        | 15:0                                   | 0.420   | 0.649                          | 0.161                         | 0.861                    | 0.209  | 0.295                      | 0.282                  |
| 8   | cis-10-pentadecenoic | 15:1                                   | 1.195   | 1.861                          | 0.543                         | 1.318                    | 0.837  | 0.900                      | 1.139                  |
| 9   | Palmitic             | 16:0                                   | 54.160  | 43.197                         | 63.562                        | 38.600                   | 57.419   | 60.093                     | 59.821                 |
| 10  | Palmitoleic          | 16:1                                   | 0.504   | 0.637                          | 0.178                         | 1.025                    | 0.295  | 0.320                      | 0.332                  |
| 11  | Heptadecanoic        | 17:0                                   | 0.287   | 1.018                          | 0.121                         | 0.386                    | 0.166  | 0.221                      | 0.253                  |
| 12  | cis-10-heptadecanoic | 17:1                                   | 0.337   | 1.188                          | 0.220                         | 0.808                    | 0.312  | 0.314                      | 0.429                  |
| 13  | Stearin              | 18:0                                   | 5.017   | 8.040                          | 4.638                         | 9.473                    | 4.900  | 6.093                      | 7.218                  |
| 14  | Olein                | 18:1n9c                                | 5.134   | 7.567                          | 6.504                         | 9.763                    | 7.789  | 6.579                      | 7.151                  |
| 15  | Linolelaidic         | 18:2n6t                                | 0.255   | 0.287                          | 0.138                         | 0.237                    | 0.352  | 0.315                      | 0.175                  |
| 16  | Linoleic             | 18:2n6c                                | 0.450   | 0.921                          | 0.412                         | 0.858                    | 0.617  | 0.392                      | 0.374                  |
| 17  | Linolenic            | 18:3n3                                 | 0.181   | 0.335                          | 0.062                         | 0.846                    | 0.142  | 0.154                      | 0.248                  |
| 18  | gamma Linoleic       | 18:3n6                                 | 0.248   | 0.194                          | 0.074                         | 0.473                    | 0.117  | 0.171                      | 0.320                  |
| 19  | Arachidic            | 20:0                                   | 0.245   | 0.449                          | 0.050                         | 0.646                    | 0.093  | 0.173                      | 0.239                  |
| 20  | Totally %            |  | 100   | 100                            | 100                           | 100                      | 100  | 100                        | 100                    |

Table 1. Content of fatty acids in milk products of organic production (%); (Austria, Italy, Slovenia) *Tabela 1. Sadržaj masnih kiselina u mlečnim proizvodima iz organske proizvodnje (%); (Austrija Italija, Slovenija)* 

|    |                      |  | AUST                          | FRIA                       |                      | ITALY                   |                   | :                    | SLOVE                            | NIA                   |
|----|----------------------|--|-------------------------------|----------------------------|----------------------|-------------------------|-------------------|----------------------|----------------------------------|-----------------------|
| No | Fatty acids          | No of C atoms and<br>unsaturated bonds | Bio Emmentaler<br>45% mm u SM | Bio Lancase<br>25% mm u SM | Bio milk<br>3.6 % mm | Bio UHT milk<br>3.6 %mm | Bio<br>Mozzarella | Bio milk<br>3.5 % mm | Vita yoghurt<br>ser. 5. 3,2 % mm | Bio kefir<br>3.5 % mm |
| 1  | Capric               | 10:0                                   | 0.980                         | 0.418                      | 1.022                | 3.640                   | 0.131             | 0.317                | 0.723                            | 1.085                 |
| 2  | Udecanoic            | 11:0                                   | 0.583                         | 0.328                      | 0.739                | 1.361                   | 0.131             | 0.136                | 0.395                            | 0.453                 |
| 3  | Lauric               | 12:0                                   | 10.200                        | 5.556                      | 10.567               | 15.271                  | 2.672             | 4.068                | 8.488                            | 11.239                |
| 4  | Tridecanoic          | 13:0                                   | 0.133                         | 0.062                      | 0.552                | 0.200                   | 0.096             | 0.055                | 0.204                            | 0.423                 |
| 5  | Miristine            | 14:0                                   | 13.511                        | 20.205                     | 17.331               | 14.979                  | 10.229            | 15.017               | 14.000                           | 15.577                |
| 6  | Miristoleate         | 14:1                                   | 0.175                         | 0.138                      | 0.229                | 0.280                   | 0.082             | 0.129                | 0.205                            | 0.285                 |
| 7  | Pentadecanoic        | 15:0                                   | 0.343                         | 0.212                      | 0.248                | 0.512                   | 0.074             | 0.254                | 0.230                            | 0.330                 |
| 8  | cis-10-pentadecanoic | 15:1                                   | 1.151                         | 0.686                      | 0.850                | 1.369                   | 0.281             | 0.891                | 0.819                            | 1.198                 |
| 9  | Palmitic             | 16:0                                   | 54.632                        | 58.648                     | 54.668               | 37.253                  | 78.881            | 64.524               | 63.645                           | 58.111                |
| 10 | Palmitoleic          | 16:1                                   | 0.388                         | 0.232                      | 0.322                | 0.631                   | 0.092             | 0.249                | 0.304                            | 0.452                 |
| 11 | Heptadecanoic        | 17:0                                   | 0.364                         | 0.152                      | 0.266                | 0.843                   | 0.064             | 0.181                | 0.230                            | 0.239                 |
| 12 | cis-10-heptadecanoic | 17:1                                   | 0.349                         | 0.320                      | 0.297                | 0.787                   | 0.108             | 0.333                | 0.303                            | 0.299                 |
| 13 | Stearin              | 18:0                                   | 8.083                         | 5.192                      | 5.344                | 10.244                  | 3.075             | 5.840                | 5.105                            | 4.130                 |
| 14 | Olein                | 18:1n9c                                | 7.550                         | 7.024                      | 6.055                | 9.793                   | 3.824             | 7.290                | 4.438                            | 4.804                 |
| 15 | Linolelaidic         | 18:2n6t                                | 0.261                         | 0.121                      | 0.277                | 0.333                   | 0.000             | 0.099                | 0.274                            | 0.385                 |
| 16 | Linoleic             | 18:2n6c                                | 0.417                         | 0.272                      | 0.659                | 1.464                   | 0.165             | 0.237                | 0.185                            | 0.289                 |
| 17 | Linolenic            | 18:3n3                                 | 0.295                         | 0.099                      | 0.252                | 0.339                   | 0.023             | 0.106                | 0.206                            | 0.249                 |
| 18 | gamma Linolenic      | 18:3n6                                 | 0.399                         | 0.229                      | 0.221                | 0.372                   | 0.029             | 0.176                | 0.140                            | 0.287                 |
| 19 | Arachidic            | 20:0                                   | 0.186                         | 0.104                      | 0.101                | 0.328                   | 0.041             | 0.096                | 0.108                            | 0.165                 |
| 20 | Totally, %           |  | 100                           | 100                        | 100                  | 100                     | 100               | 100                  | 100                              | 100                   |

UHT milk from Serbia (Subotica) produced in conventional conditions had higher level (2.823%) of these fatty acids compared to the same type of milk from Italy (1.233%) and from Slovenia (0.615%). The fresh cheese mozzarella from Serbia (Guča) had a higher percent of the named fatty acids (0.89%) compared to the same type of cheese from Italy (0.258%). The kefir from Serbia (Kraljevo) had almost three times higher percentage of the named fatty acids (2.823%) in comparison to the kefir from Slovenia. The named acids are important for human health because they lessen the risks of diabetes, cardiovascular diseases, and of coronary heart diseases and of brain stroke, as well as of many forms of cancer (Pariza, 2003; Connor, 2000).

The composition and physical-chemical quality of milk produced on the small farms of the organic systems located in the area of Fručka gora and those from the big farm of the conventional system in the area of the municipality of Vrbas have been shown in Table 2. Raw milk from both organic and conventional productions had good chemical composition and physical-chemical properties. The differences were determined regarding the milk fat and dry matters because organic milk was richer than conventional milk in the content of these parameters, which agrees with the researches

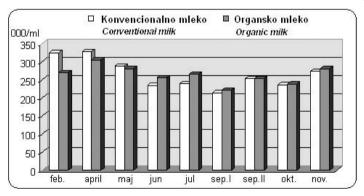
performed by Huszti (2009). Average content of proteins in both conventional and organic milk was within normal limits, with some insignificant differences which agree with the researches performed by Toledo and Björck (2002), and Huszti (2009). In regard to the other tested parameters (casein, lactose, ash, dry matter without fat, freezing point and pH value), no important differences between conventional and organic milk were determined.

Table 2. Composition and physical-chemical properties of raw milk from organic and conventional production

| Parameter                 | Org     | anic milk      | Conventional milk |                |  |  |
|---------------------------|---------|----------------|-------------------|----------------|--|--|
| Parameter                 | Average | min. – max.    | Average           | min max.       |  |  |
| Milk fat, %               | 3.80    | 3.30 - 4.21    | 3.07              | 2.78 - 3.76    |  |  |
| Dry matter, %             | 12.22   | 11.73 - 12.88  | 11.46             | 11.00 - 12.25  |  |  |
| Dry matter without fat, % | 8.42    | 8.13 - 8.67    | 8.42              | 8.21 - 8.54    |  |  |
| Protein, %                | 3.20    | 2.92 - 3.48    | 3.14              | 3.01 - 3.34    |  |  |
| Casein, %                 | 2.61    | 2.13 - 2.87    | 2.60              | 2.49 - 2.76    |  |  |
| Lactose, %                | 4.46    | 4.32 - 4.46    | 4.53              | 4.30 - 4.64    |  |  |
| Ash, %                    | 0.75    | 0.72 - 0.81    | 0.75              | 0.73 - 0.88    |  |  |
| Freezing point, °C        | -0.522  | -0.514 - 0.528 | -0.520            | -0.494 - 0.531 |  |  |
| pH value                  | 6.64    | 6.44 - 6.77    | 6.65              | 6.47 - 6.85    |  |  |
| Acidity, °SH              | 6.70    | 5.63 - 7.33    | 6.27              | 5.81 - 7.07    |  |  |

Tabela 2. Sastav i fizičko hemijske osobine sirovog mleka iz organske i konvencionalne proizvodnje

The total number of bacteria in raw milk from small farms of the area of Fruska gora amounted approximately 210,000/ml, while it was 143,000/ml in raw milk from the farm of Vrbas. The larger total number of bacteria in organic milk in comparison to bacteria in conventional milk shows that farmers from small farms should be taught on the fact that hygiene affects the quality of milk. The average number of somatic cells in organic milk was 259,000/ml, while it was 268.000/ml in conventional milk (Graph 1).



Graph. 1. Number of somatic cells in organic and conventional milk Graf I. Broj somatskih ćelija u organskom i konvencionalnom mleku

The number of somatic cells in milk is directly related to the health condition of cow udders, and it affects the production, composition and quality of milk to be processed. (Timms, 1990). We have concluded that a lower number of somatic cells in organic milk than in conventional milk results from better health condition of cow udders of organic production.

## CONCLUSION

According to the performed research, the following can be concluded:

- Based on the comparative tests on the content of fatty acids important for human health (omega 3, omega 6 and arachidonic acids) in milk and products from the markets of Italy, Austria and Slovenia, and from conventional production from Serbia, a higher quality of some products from conventional production was determined.
- Raw milk from organic production was richer in the content of fat and dry matter in comparison to raw milk from conventional production, while in regard to other parameters, no differences were noticed.
- Larger number of bacteria present in organic milk in comparison to conventional milk shows that the level of hygiene in the process of milk production has to be higher.
- Smaller number of somatic cells in organic milk in comparison to the number in conventional milk shows that that the health condition of cow udders in organic production is better.
- Milk production on smaller farms in the area of Fruška gora can serve as a model for the organic production of our autochthonic cow breeds with consuming pasture during whole year, thus making a good balance between plant and animal production.

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TOLEDO, P., BJÖRCK, A. L.: Composition of raw milk from sustainable production systems. International Dairy Journal, 12:75-80, 2002

# ISPITIVANJE MOGUĆNOSTI ORGANSKE PROIZVODNJE MLEKA U SRBIJI

# POPOVIĆ-VRANJEŠ ANKA, MIROSLAV GRUBAČIĆ, RADOVAN PEJANOVIĆ, ALEKSANDAR KRALJ, ANKA KASALICA, DRAGICA MIOČINOVIĆ, GORDANA NIKETIĆ

#### Izvod

Istraživanja u ovom radu su imala za cilj da se napravi komparativna analiza mleka i nekih mlečnih proizvoda u pogledu masnih kiselina važnih za ljudsko zdravlje (omega 3, omega 6 i arahidonske ) sa tržišta Italije,Austrije i Slovenije iz organske proizvodnje i mleka i nekih mlečnih proizvoda sa tržišta Srbije iz konvencionalne proizvodnje. Rezultati su pokazali da su neki mlečni proizvodi proizvedeni u Srbiji boljeg kvaliteta u pogledu sadržaja navedenih kiselina , i ako su iz konvencionalne proizvodnje ,što govori da je Srbija ekološki očuvana i da ima potencijal za razvoj organske proizvodnju. Istraživanja izvršena u Vojvodini su obuhvatala dva sistema proizvodnje mleka , jedan po principima organske proizvodnje i drugi sisitem konvencionalne proizvodnje.Organska proizvodnja je locirana na Fruškoj Gori i obuhvatala je 10 malih farmi sa prosečno 8-10 krava domaće simentalske rase .Konvencionalna proizvodnja je bila sa velike farma sa 700 krava crno-bele Holštajn rase sa teritorije opštine Vrbas.Pored sastava mleka i fizičko-hemijskog kvaliteta mleka, ispitan je Ukupn broj bakterija i broja somatskih ćelija, u posmatranom periodu od februara do decembra meseca 2009. godine.

Kjučne reči: organsko mleko, konvencionalno mleko, parametri kvaliteta

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# THE NUMBER OF AZOTOBACTER IN THE RHIZOSPHERE SOIL OF DIFFERENT ALFALFA GENOTYPES\*

## SNEŽANA ANDJELKOVIĆ, MIRJANA JARAK, JASMINA RADOVIĆ, TANJA VASIĆ, JASMINA MILENKOVIĆ, SNEŽANA BABIĆ<sup>1</sup>

SUMMARY: To make the cultivating of alfalfa and other plant species more successful, the microbial inoculums containing single or combined microorganism cultures are used. The purpose of these inoculants is to expedite useful microbiological processes and to increase the amount of plant assimilatives. This study concentrates on the influence of monovalent and polyvalent inoculums on the quantity of A. chrococcum in the rhizosphere soil of different alfalfa cultivars. Depending on the inoculant type and on the genotype of used alfalfa, the number of azotobacter differed in studied soils.

Key words: soil, azotobacter, microbial inoculums, alfalfa.

### **INTRODUCTION**

The microorganisms are one of the most important biological component of soil. They are the indicator of soil fertility (Mandić et al., 2004) or of soil degradation. Their ratio in the total metabolical activity in the soil is 60-90% (Lee, 1994). All biologic changes in the soil occurs under the influence of enyzmes. These enyzmes are mostly of organic origin (Djuric et al., 2008). The free nitrogen-fixing microorganisms have the most important role in the providing plants with nitrogen and in increasing the nitrogen balance in the soil. The most significant among these microorganisms are the bacteria from *Azotobacter* strain (Jarak, Čolo, 2007). Besides fixing the elemental nitrogen, *Azotobacter sp.* also produces biologically active matters that have positive effect on plant growth and development, such as auxins, gibberellins, pyridoxine, biotin and nicotinic acid (Dobbelaere et al., 2005).

The number of *Azotobacter sp.* is used as the indicator of biological value of the soil. The bacteria of *Azotobacter* genus give advantage to productive, neutral soils and

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are very succeptable to the moisture deficit (Djukić et al, 2007). The acid reaction has negative effect on the number and activity of *Azotobacter sp*.

Alfalfa (*Medicago sativa* L.) is, economically, the most important forage legume. The green mass of alfalfa is, also, used as the organic fertilizer (Manojlović et al., 2007). In addition to high potential for yield and quality of biomass, this species is characterized by intensive process of biological nitrogen fixation. For successful alfalfa and other plant species growing, the microbial inoculums are applied. These inoculants contain individual or mixed microorganism cultures, whose purpose is to expedite useful microbiological processes and increase the amount of plant assimilates. Rhizosphere microorganisms live on the root, and in rhizosphere soil (azotobacter, actinomycetes, etc.), while rhizobium lives in the root tissue.

The aim of this research is to examine the influence of rhizobium, azotobacter and actinomycetes, individually (monovalent inoculums) or in combination (polyvalent inoculums) on the number of *Azotobacter sp.* in the different types of soil.

#### MATERIAL AND METHODS

Two types of soil, with different physical and chemical characteristics, taken from two locations: Mačkovac (A) and Gaglovo (B) were used. The chemical analyses of the soil samples were done using the standard methods (Tab. 1).

Table 1. The chemical composition of the soil *Tabela 1. Hemijski sastav zemljišta* 

| Parameter /<br>Parametar<br>Soil /<br>Zemljište | Total nitrogen /<br>Ukupni azot % | P <sub>2</sub> O <sub>5</sub> mg/100g | K <sub>2</sub> O mg/100g | Humus, % | pH/KCl | pH/H <sub>2</sub> O |
|---|-----------------------------------|---------------------------------------|--------------------------|----------|--------|---------------------|
| A   | 0.138                             | 6.60                                  | 24.05                    | 2.62     | 5.96   | 6.44                |
| В   | 0.204                             | 10.20                                 | 51.0                     | 4.30     | 4.77   | 5.83                |

The inoculation was done using the different inoculums: 1. Rhizobium; 2. Azotobacter; 3. Actinomycete; 4. Rhizobium + azotobacter; 5. Rhizobium + actinomycete; 6. Azotobacter + actinomycete; 7. Rhizobium + azotobacter + actinomycete; 8. Control – no inoculation. The varieties of *Rhizobim meliloti* (varieties L7 and L17), *Azotobacter chrococcum* and the varieties of *Streptomyces sp.* Actinomycete (varieties 5, 7 and 9K) were used. These microorganisms originated from the Departmant of microbiology of the Faculty of agriculture in Novi Sad. The rhizobium cultures were grown on the YM (yiest and manitol) medium using the method by Vincent, azotobacter cultures were grown by Fedorov method and actinomycetes on the medium by Krasiljnikov (Jarak and Djurić, 2006).

Two alfalfa genotypes, K-28 (Serbia) and Synteza 1 (Slovakia), were used for this study. The alfalfa seed was sterilized using the 0.2% HgCl<sub>2</sub> solution and 70% alcohol and rinsed several times with sterile tap water and afterwards submerged in the correct inoculum. After this, the seed of each variant was planted in the pots with correct soil type. Two months after sowing, in the blossoming phase of alfalfa, the soil samples were taken for the microbiological analysis. Soil samples were taken after second and third cut, too.

The number of azotobacter was assessed using the fertile drops method on the

medium by Fedorov. The inoculation was done using the 0,2ml of soil suspension diluted by 10<sup>-2</sup>. The incubation lasted for 48h on 28°. The number of grown colonies was calculated per 1g of absolutely dry soil (Jarak and Djurić, 2006).

The results were calculated by ANOVA and the differences between genotypes were tested by the LSD test.

### **RESULTS AND DISCUSSION**

*Azotobacter sp.* is actively multiplied well in cultivated soils, which are rich in organic matter, calcium, phosphorus, a lot of moisture and in which the pH is above 5 (Djukić et al., 2007). In the neutral soils, the number of this bacteria ranges from several hundred to several thousand. In both alfalfa cultivars in the soil A in a treatment where the *Azotobacter sp.* inoculums were not applied, its number was higher than in the soil B, which had a lower pH value. This is in accordance with the results listed by Miličić and Jarak (2008) and Mandić et al. (2004). In the control and in the treatments with soil B in which the *Azotobacter sp.* was not applied, its absence or insignificant number was recorded.

The abundance of *Azotobacter sp.* in the rhizosphere of both alfalfa genotypes in treatments where inoculation was not carried out is significantly different from the treatments where the inoculation in both soils was performed.

Activity of *Azotobacter sp.* was higher in genotype K-28 then in genotype Synteze in soil A, with respect to its significantly higher number in most treatments with K-28. Between alfalfa genotypes in soil A, only three treatments showed no statistically significant difference in the number of *Azotobacter sp.* (Table 2).

In the rhizosphere of genotype K-28 in the second treatment on soil B number of *Azotobacter sp.* was significantly higher than in other varieties. In second and third cuts, in treatments 4, 5 and 7, activity of *Azotobacter sp.* were higher in Synteze 1 (Table 3). The effect of alfalfa genotype on the number of *Azotobacter sp.* on land B shows no uniformity.

The results showed that in all treatments with soil with increased acidity, in which the inoculation was performed, there is a trend of number increasing of these bacteria in both cultivars, so that in the third cut the number of bacteria was the highest (Tab. 3). In the soils with neutral reaction such regularity in alfalfa genotypes was not recorded (Tab. 2). Spedding et al. (2004) in their researches imply that there are seasonal changes in microbial composition and quality of soil.

| Cut / | Culting   | r / C            |       |        |        | Variants / | Varijante | ?       |        |       |
|-------|-----------|------------------|-------|--------|--------|------------|-----------|---------|--------|-------|
| Otkos | Cultiva   | Cultivar / Sorta |       | 2      | 3      | 4          | 5         | 6       | 7      | 8     |
|       | K-        | 28               | 45.92 | 803.64 | 24.20  | 733.07     | 14.13     | 807.1   | 469.65 | 25.29 |
| Ι     | Synt      | eza 1            | 61.95 | 360.04 | 34.76  | 298.05     | 45.98     | 164.89  | 92.79  | 23.82 |
| 1     | LSD       | 0.05             | 10.35 | 69.19  | 6.28   | 87.17      | 5.52      | 29.09   | 24.58  | 7.56  |
|       | LSD       | 0.01             | 17.16 | 114.74 | 10.41  | 94.81      | 9.14      | 49.57   | 57.36  | 12.54 |
|       | K-28      |                  | 24.94 | 244.52 | 30.77  | 290.30     | 26.65     | 518.70  | 197.11 | 9.2   |
| П     | Synt      | eza 1            | 69.59 | 357.45 | 15.52  | 350.44     | 16.43     | 281.42  | 111.32 | 33.13 |
| 11    | LSD       | 0.05             | 9.74  | 29.11  | 6.33   | 33.32      | 6.77      | 23.82   | 16.81  | 4.33  |
|       | LSD       | 0.01             | 16.16 | 48.28  | 10.50  | 55.25      | 11.32     | 39.51   | 27.88  | 7.17  |
|       | K-        | 28               | 21.51 | 409.19 | 130.85 | 1187.58    | 45.81     | 1828.5  | 903.25 | 29.21 |
| III   | Synteza 1 |                  | 28.81 | 410.64 | 45.13  | 406.01     | 18.87     | 1565.97 | 836.98 | 22.09 |
|       | LSD       | 0.05             | 6.57  | 47.16  | 14.57  | 39.35      | 4.57      | 176.76  | 67.27  | 6.58  |
|       | LSD       | 0.01             | 10.89 | 78.19  | 24.16  | 65.26      | 7.59      | 293.11  | 111.57 | 10.92 |

Table 2. The number of azotobacter  $(x10^2g^{-1})$  in soil A *Tabela 2. Brojnost azotobaktera*  $(x10^2g^{-1})$  *u zemljištu A* 

In what number will the introduced microorganisms survive in the new conditions depends largely on the properties of soil (Jošić, 2004). In our research, due to the presence of organic compounds that are used as an energy source, *Azotobacter sp.* are well adapted to soil with lower pH value.

In the soil A, its neutral reaction is the benefit for introduced microorganisms. But this soil is poorer in nutrients and there is competition between introduced microorganisms and the indigenous population of microorganisms. The *Azotobacter sp.* reacts very violently by reducing its number to the changes in habitat conditions or other environmental factors. This is used in soil microbiology as the one of the indicators of soil fertility (Djurić, 2010).

| Cut / | Culting   | (Carta    |      |         |       | Variants / | Varijante | ?       |         |      |
|-------|-----------|-----------|------|---------|-------|------------|-----------|---------|---------|------|
| Otkos | Cultiva   | r / Sorta | 1    | 2       | 3     | 4          | 5         | 6       | 7       | 8    |
|       | K-        | 28        | 0    | 546.84  | 0     | 270.9      | 5.61      | 435.18  | 546.71  | 3.88 |
| I     | Synt      | eza 1     | 3.73 | 385.47  | 0     | 231.71     | 5.62      | 359.11  | 477.27  | 4.39 |
| 1     | LSD       | 0.05      | 0.25 | 17.70   | 0     | 11.44      | 2.47      | 17.39   | 14.07   | 0.48 |
|       | LSD       | 0.01      | 0.42 | 29.36   | 0     | 18.90      | 4.10      | 28.84   | 23.34   | 0.79 |
|       | K-        | 28        | 0    | 1364.26 | 0     | 322.15     | 2.03      | 1615.22 | 1128.18 | 1.96 |
| П     | Synt      | eza 1     | 0    | 1209.08 | 3.83  | 889.71     | 5.95      | 994.66  | 1351.38 | 1.96 |
| 11    | LSD       | 0.05      | 0    | 28.12   | 0.20  | 16.73      | 0.31      | 26.57   | 36.61   | 0.29 |
|       | LSD       | 0.01      | 0    | 26.26   | 0.33  | 27.75      | 0.52      | 44.07   | 60.83   | 0.40 |
|       | K-        | 28        | 3.85 | 3977.60 | 3.71  | 587.89     | 5.57      | 1976.15 | 1798.51 | 0    |
| III   | Synteza 1 |           | 1.82 | 311.99  | 31.86 | 1110.83    | 7.67      | 4239.77 | 2807.89 | 0    |
| 111   | LSD       | 0.05      | 0.14 | 135.14  | 4.38  | 37.73      | 0.72      | 40.24   | 63.37   | 0    |
|       | LSD       | 0.01      | 0.22 | 224.0   | 7.27  | 59.26      | 1.19      | 66.73   | 105.09  | 0    |

Table 3. The number of azotobacter  $(x10^2g^{-1})$  in soil B Tabela 3. Brojnost azotobaktera  $(x10^2g^{-1})$  u zemljištu B

### CONCLUSION

The results of the study of monovalent and polyvalent inoculum which contains *Azotobacter sp.*, imply that the bacterial inoculation is justified. This is due to the fact that there was significant increase both in number and in microbiological activity of this free nitrogen fixator in the rhizosphere soil on which the alfalfa cultivars K-28 and Synteza 1 were grown. This primarily applies to the soil B, because there was a significant increase in the number of these bacteria in the treatments with the inoculation in the relation with the treatments without inoculation, in both studied alfalfa cultivars. In the most cases, there are significant differences in the number of *Azotobacter sp.* in the rhizosphere of alfalfa genotypes within the same treatment.

Based on the results of this study, there is the need for the introduction of microorganisms in the soil with low microbiological activity in order to increase nitrogen balance in the soil, crop yield and production of ecologically safe food.

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# ZASTUPLJENOST AZOTOBAKTERA U RIZOSFERNOM ZEMLJIŠTU RAZLIČITIH GENOTIPOVA LUCERKE

# SNEŽANA ANDJELKOVIĆ , MIRJANA JARAK, JASMINA RADOVIĆ, TANJA VASIĆ, JASMINA MILENKOVIĆ, SNEŽANA BABIĆ

#### Izvod

Kako bi se gajenje lucerke pa i drugih biljnih vrsta što uspešnije obavilo, sve više se primenjuju mikrobni inokulanti koji sadrže pojedinačne ili združene kulture mikroorganizama, a čija je svrha pospešiti korisne mikrobiološke procese i povećati količinu biljnih asimilativa. U radu je vršeno ispitivanje uticaja monovalentnih i polivalentnih inokuluma na zastupljenost azotobakera u rizosfernom zemljištu različitih sorti lucerke (*Medicago sativa* L.). Brojnost azotobaktera u rizosferi je zavisila, kako od vrste inokulanta, tako i od tipa zemljišta i genotipa lucerke.

Ključne reči: zemljište, azotobacter, mikrobni inokulanti, lucerka.

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# *IN VITRO EUPHORBIA CYPARISSIAS L.* EXTRACT'S ACTIVITY AGAINST POULTRY ARGASIDES<sup>\*</sup>

## ROMEO T. CRISTINA, FLORIN MUSELIN, EUGENIA DUMITRESCU, SNEJANA PETROVICI<sup>1</sup>

SUMMARY: In vitro efficiency study of Euphorbia cyparissias extracts, against Argas persicus, a specific poultry tick, in order to bring new data about bio control and acaricidal effect on parasites is proposed. Argas persicus (nymphs and adults) were initially maintained for 48 hours before testing at 21±1°C, to 55±5% relative moisture, and 14h:10h (light/dark) photoperiod. After this, they were put in contact with 20 (initial concentration), 10, 5, 2, 1, and respectively 0.5 % solutions. For each extract dilution were fulfilled six tests: three for sear (dry) herb (SHE) and three for herb's inflorescence (HIE). Watching accomplished at 15, 30, 45 and 60, minutes after contacts, followed parasite's viability, locomotion, appendices' amplitude and frequency, ortho and versostasys and death after an original key. Statistics after Anova determined arithmetic mean, standard error, and deviation, respectively variance values. Registered mean values of ticks in versostasys after 15 minutes of contact with alcoholic solutions were of 6.16±1.49 for SHE and of 5.83±1.32 for the HIE. After 30 minutes of exposure values were of 4.16±1.13 for SHE, and 3.83±1.04 for HIE, at 45 minutes registered mean values have fall to  $2.50\pm0.84$ , respectively  $2.33\pm0.84$ , and finally, after 60 minutes values were 1.00±0.36 for SHE, respectively of 0.83±0.40 for HIE. In conclusion, extracts of Euphorbia showed a significant in vitro acaricidal effect on Argas, inclusively for smaller concentrations. After 15-90 minutes from the exposure to different dilutions all in vitro studied ticks have died.

**Key words:** *Euphorbia cyparissias, extract, Argas persicus, in vitro, efficacy.* 

Original scientific paper / Originalni naučni rad

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### INTRODUCTION

Among mites frequently identified in the Romania's South-Western part, most often were found: *Argas persicus* <sup>(Oken, 1818)</sup> in poultry and *Argas reflexus* <sup>(Fabricius, 1794)</sup> in pigeons, most common species (Cosoroaba, 2000).

The *Argas* mites host specificity is feeble, ticks sucking on numerous wild and domestic animals species, they attack especially during the night, their stings causing itches and severe local inflammations.

Nutrition of larval ixoidian hexapods, respectively of the octopoded nymphs and adults leads to severe anaemia especially in young poultry categories.

Among hosts, the most sensitive are chickens and turkey poults, where mortality can be severe. *Argas* mites transmit numerous pathogen agents like: *Spirocheta gallinarum, Pasterurella multocida, Salmonella pullorum*, stadium transmitted, or through the ovarian way (Cosoroaba, 2000).

Present work describes an efficiency *in vitro* study about *Euphorbia cyparissias* alcoholic extracts against nymphs and adults of *Argas persicus*.

The study proposes to bring certain data towards direct acaricidal effect of the up mentioned herb extracts on different stages of parasite viability and does not emphasise the intimate action or toxic mechanisms (theme of another study).

### MATERIALS AND METHODS OF THE STUDY

#### Ticks

Nymphs and adults gathering were realised from traditionally aviary, in south-western part of Romania.

Parasites were placed in paper bags and brought into laboratory where they were transferred into plastic recipients with sieve cowls.

Argas persicus specimens (both nymphs and adults) were identified after key conceived by Cosoroabă (2000) and maintained 48 hours before testing at  $21\pm1^{\circ}$ C to  $55\pm5\%$  relative moisture to a 14h:10h (light / dark) photoperiod.

### Alcoholic extract obtaining

*Euphorbia cyparissias* (common name - *Cypress spurge*) is easy to find and recognize in Romania's spontaneous flora.

For extracts, 20 grams of sear (dry) herbal part, (SHE) respectively herbal inflorescence (HIE) was weighted and minced. Herb parts were macerated after the *Romanian Pharmacopoeia* X Edition (1993) in 2:10 (m/m) ratio in 100 ml alcohol 96 (v/v) in brown bottles for 10 days and shaking three times a day.

After the alcoholic extraction and pressing of the vegetal residuum, the homogenised solutions were set to sedimentation at 5-10°C for 6 days and after the filtration, the obtained solutions were limpid, yellowish with a characteristic aromatic scent. Through water dilution, extracts became milky opalescent, sediments appearing in short time.

Used solutions were 20 (initial solution), 10, 5, 2, 1, and respectively 0.5 %, for each alcoholic dilution being fulfilled six tests: three for dry herb's alcoholic extract (SHE), and three for herb's inflorescence extracts (HIE).

### **Bio** analysis

Filter papers placed in classic Petri plates where uniformly moistened with 1 ml of testing extract dilutions, on each paper, being scattered 10 ticks.

A control lot was constituted in the similar conditions filter paper being moistened with a 20% hydro alcoholic solution.

## Readings

Ticks watching accomplished (with the stereomicroscopic magnifier at  $20 \times 2.5$  resolution) at 15, 30, 45, and respectively 60 minutes after contact with extracts, observed the parasite viability following the locomotion activity, the locomotors appendices amplitude, and frequency activities, the tick's ortho and versostasys, respectively death after the key presented in Table 1.

Table 1. Evaluation of Argas viability

| LARGE         | LARGE        |    | VERSOSTASYS    | EXITUS   |
|---------------|--------------|----|----------------|----------|
| MOVEMENTS     | MOVEMENTS    |    | VERY SLOW LIMB | MOTILITY |
| HIGH MOTILITY | LOW MOTILITY |    | MOVEMENTS      | ABSENCE  |
| ++++          | +++          | ++ | +              | -        |

Conceived key was to establish the right moment when *Euphorbia* extract penetrates parasite's cuticle, the appearance of primary nervous signs and the evolution of paralysis, the limit of filter paper evaporation being considered at 30 minutes from the start of experiments (Cristina et al., 2007).

## Statistical analysis

Efficacy analysed after, descriptive statistics and ANOVA *test* (SPSS-7.5) determined values of: arithmetic mean, standard error, and standard deviation, respectively variance for this study.

## RESULTS

Results showed that there are not notable differences between of *in vitro* studied alcoholic extract solutions therapeutic efficacies against the *Argas persicus* ticks.

Mean values of ticks observed in versostasys after 15 minutes after contact with *Euphorbia*'s extract solutions was of  $6.16\pm1.49$  for SHE and of  $5.83\pm1.32$  for the HIE.

After 30 minutes of exposure were registered values of  $4.16\pm1.13$  for SHE, and  $3.83\pm1.04$  for HIE.

At 45 minutes from the beginning of experiments, the registered mean values have fall to  $2.50\pm0.84$ , respectively  $2.33\pm0.84$ , and finally, after 60 minutes values were  $1.00\pm0.36$  for SHE, respectively of  $0.83\pm0.40$  for HIE (see table 2).

Table 2. Statistic analysis results for the bioactivity of dry herb (SHE) and herb's inflorescence (HIE) alcoholic extracts

| Minu | tes   | N    | Minimum | Maximum | Mean (± Std. error) | Standard Deviation | Variance |
|------|-------|------|---------|---------|---------------------|--------------------|----------|
| SHE  | 15    | 15 6 | 0.00    | 10.00   | 6.1667 (1.4926)     | 3.6560             | 13.367   |
| HIE  | 15    |      | 1.00    | 9.00    | 5.8333 (1.3271)     | 3.2506             | 10.567   |
| SHE  | 30    | 6    | 0.00    | 8.00    | 4.1667 (1.1377)     | 2.7869             | 7.767    |
| HIE  | 30    |      | 1.00    | 7.00    | 3.8333 (1.0462)     | 2.5626             | 6.567    |
| SHE  | 45    | 6    | 0.00    | 6.00    | 2.5000 (0.8466)     | 2.0736             | 4.300    |
| HIE  | 43 0  | 0    |         | 5.00    | 2.3333 (0.8433)     | 2.0656             | 4.267    |
| SHE  | 60    | 60 6 | 0.00    | 2.00    | 1.0000 (0.3651)     | 0.8944             | 0.800    |
| HIE  | HE 00 |      |         |         | 0.8333 (0.4014)     | 0.9832             | 0.967    |

Analysing variance after ANOVA *test*, it was concluded that are no significant differences between studied solutions (F=5.8, p=0.07).

The inner action mode of *Euphorbia cyparissias* extracts is not clarified yet but there are current preoccupations in this field (Cristina et al., 2008 a, Cristina et al. 2009).

However, on the base of known toxicity of *quercetin-3-glucuronide* and *campho-rol-3-glucuronide*, the main flavonoids of *Euphorbia cyparissias*, it is very probable to ascertain that the lethal effect is producing after the tick's cuticle and his nervous system passage (Gassmann et al., 1995).

Results confirm collective's obtained data until now and encourage new investigations, both *in vitro* and *in vivo*, about the efficacy of *Euphorbia cyparissias* extracts, on other parasites (Cristina et al., 2007, Cristina et al., 2008 b).

### DISCUSSION

Control of the acarian populations is multifarious and includes numerous therapeutic techniques and chemical substances (Nordenfors et al., 2001).

Although initially substances had proven their efficiency, after repeated use, resistance has installed and from this point of view, introduction of new bio control alternatives is necessary (Beugnet et al., 1997, Genchi et al., 1984, Zeman, 1987).

In this respect, herb extracts of *Euphorbia* can be an important resource for the poultry's mite control, knowing the fact that those are a rich reservoir of efficient bioactive compounds (Cristina et al., 2009, Gassman et al., 1995).

For the time being, though herbs are ready to hand, generally, there is not known very much about the acaricidal activity of vegetal extracts against poultry ticks.

Investigations linked by antiparasitary bio control diversified, numerous control sources being studied (e.g. fungus, essential oils, and vegetal extracts) (Cristina et al., 2007, Kim et al., 2004).

For example, the effect of *Beauveria bassiana* and *Metarhizium anisopliae* entomopathogen fungus against argasides was studied.

Authors have suggested that up mentioned plants had a good potential in the control of *Argas*, showing that, in natural conditions the efficacy was of 100% after three weeks post applications (Sewify et al., 2001).

In another study, was examined the acaricidal effect of essential oil of *Melaleuca alternifolia* (tea tree oil) at different doses and exposures on nymphs of *Ixodes* and the results showed that the tea tree oils enhanced acaricidal proprieties and could be extremely useful in ticks controlling (Iori et al., 2005).

In an extensive research work, the acaricidal activity of 56 plants essential oils showed great potential as control agents against poultry red mites *Dermanyssus gallinae* (Kim et al., 2004).

### CONCLUSIONS

The experiment pointed out the followings:

1) *Euphorbia cyparissias* sear herb and inflorescence alcoholic extracts have a significant acaricidal *in vitro* effect, inclusive to their tested smallest concentration

(0.5%).

2) After 15-90 minutes of exposure to different dilutions of alcoholic solution, (20, 10, 5, 2, 1, and 0.5%) all *in vitro* studied mites have died.

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# AKTIVNOST *IN VITRO* EKSTRAKTA *EUPHORBIA CYPARISSIAS L*. PROTIV ŽIVINSKIH ARGASIDA

## ROMEO T. CRISTINA, FLORIN MUSELIN, EUGENIA DUMITRESCU, SNEJANA PETROVICI

#### Izvod

Ispitivana je in vitro efikasnosti ekstrakta Euphorbia ciparissias protiv Argas persicus, specifične krpelja živine, kako bi se dobili novi podaci o kontroli uticaja acaricida kod živine. Argas persicus (nimfe i odrasle) su u početku održavani 48h pre testiranja na  $21 \pm 1^{\circ}$ C, u  $55 \pm 5\%$  vlage, i u režimu14h:10h (svetlo / tamno) photoperiod. Ovi paraziti su bili izloženi uticaju rastvora 20, 10, 5, 2, 1 i 05% ekstrakta Euforbia. Zaključeno je da ekstrakti Euphorbia, u in vitro uslovima, pokazuju značajan acaricidni efekt na Argas, uključujući i minimalne primenjene koncentracije. Posle 15-90 minuta od početka izlaganja različitim koncentracijama ovog ekstrakta, svi krpelji su uginuli.

Ključne reči: Euphorbia cyparissias, ekstrakt, Argas persicus, in vitro, efikasnost.

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# *EUPHORBIA CYPARISSIAS L.* EXTRACT'S ACTIVITY AGAINST SHEEP IXODIDES\*

#### ROMEO T. CRISTINA, EUGENIA DUMITRESCU<sup>1</sup>

SUMMARY: Euphorbia cyparissias extract activity in one in vitro and one in vivo, experiments against specific sheep's Dermacentor and Haemaphysalis ixodides is proposed. Ticks identification was done after known identification keys in parasitology. Euphorbia extract alcoholic dilutions of 10, 5, 2, 1, 0.5 and 0.25% were used. Ixodides' in vitro viability monitorized to 30, 60, 90 and 120 min., revealed that applications effect after 30 and 60 min. from exposures was a reduced one. Difference between ticks' average to 30 and 90 min. was significantly greater 3.28 (p<0.001), and between 30 and 120 min. of 5.65 (p<0.001). Between 60 and 90 min. from exposure, ticks' average was significant 2.68 (p<0.001), also between 60 and 120 min., 5.05 (p<0.001). After 90 and 120 min. the surviving rate was reduced and significant differences between ticks' average being ascertained 2.36 (p<0.001). ANOVA test showed that, there are differences between ticks' which survived according to exposure time and also to the used concentration. All ticks from in vitro experiment have died after three hours from exposure. It was also ascertained that the hungry female ticks are more resilient than the hungry ones. In the in vivo experiment, ticks' mortality percentage was between 9 and 13 for D. marginatus and 0 to 23 for H. punctata, issuing that the studied extract can be used only as a bio-control measure against ticks and not as a therapy mean. In this case Euphorbia's extract efficacy was significantly distinct according to exposure (p < 0.001) and used concentrations (p < 0.001).

Key words: Euphorbia cyparissias, extract, ixodides, sheep, efficacy.

Original scientific paper / Originalni naučni rad

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#### INTRODUCTION

Use of acaricidal substances against ixodides remains main terapeutica to follow for the practicians, but chemical methods has at least two great disadvantages: are costly and have an unrefined action.

To these is adding in time, the risk of resistant populations and drug residues appearance.

Motivated by these reasons, researchers are striving to find new alternatives, more economic, non pollutant even if them efficacy is far to be satisfying

## **Objective**

The study want to make a comparative observation of acaricidal effect of differed alcoholic extracts concentrations of *Euphorbia cyparissias* L., on sheep's' specific ixodides in one *in vitro* and one *in vivo* experiment.

#### MATERIALS AND METHOD OF THE STUDY

#### Extract obtaining

Alcoholic extract was obtained after *Romanian Pharmacopoeia* X<sup>th</sup> Edition (1993).

By 10 grams of minced dry plant, was macerated to 1:10 (m/m) ratio for 10 days, being stirred three times per day, in 100 ml brown bottles with alcohol 98 (v/v). After extraction and residue pressing each of extractive liquids reunited and homogenised were let to sediment to the 5-10 °C and after this strained, being avoided the evaporation losses. Obtained tincture has a limpid aspect, yellowish, with aromatic smell, corresponding to Pharmacopoeia's instructions about tinctures. By dilution with water, tincture becomes turbid and it's changes in withy opalescent sediment.

#### Animals

Experiment was accomplished in 32 sheep, divided in eight lots. To the each lot constituted were one *Euphorbia* extract dilution distributed Used concentrations were: 10% (initial dilution), 5%, 2%, 1%, 0.5%, respectively 0.25% and a placebo lot treated only with alcohol (70°). The tincture concentrations were dipped to the sternal region, were found the largest ticks number. Both treated and placebo lots animals were kept in the same conditions.

#### **Bio** analysis

For *in vitro* testing classic Petri dishes were used in which were placed filter paper. In each dish was poured uniformly 1ml of dilution and placed 50 ticks / Petri dish (100 totals/each dilution). Control lot was made in the same conditions but scattered on filter paper poured only with hydro alcoholic solution.

#### Readings

The species identification was made after known morphologic characteristics keys of Feider (1965), Babos (1964) and Estrada-Peña (2004) and monitoring was made to stereomicroscopic magnifier (res.  $20 \times 2.5$ ) to the intervals of: 30, 60, 90 and 120 minutes ticks' viability, manifested through mobility, amplitude and frequency of appendices, ortho and versostasys and exitus being followed. The moment of statistic evaluation was considered those before exitus (namely versostasys +) after model described by Cristina and al. (2006). For the *in vivo* study, from 24 hours from extracts application, all remained ticks from sternal region were gathered to follow in lab condition the ticks'

eventual exitus. For the *in vivo* study all ticks were collected and evolutions observed for each temporal case in part.

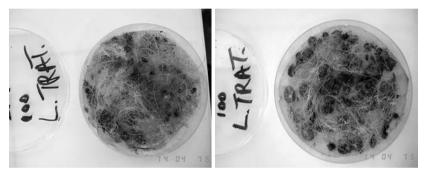
Statistic analysis

Efficacy analysed after ANOVA *test* (SPSS-7.5) determined values of: arithmetic mean, standard error, standard deviation, respectively variance.

#### RESULTS

## In vitro testing

Tincture's acaricidal activity on *D. marginatus* and *H. punctata* females (photo) was tested by direct contact. The testing only on females was considered enough, because them are mainly responsible by the direct or indirect pathogen activity, because only a very few males are feeding (even any) and so their pathogenic activity is insignificant.



**Photo.** Dermacentor marginatus and Haemaphysalis punctata females (original) Tincture's efficacy in differed concentrations correlated with contact time is showed in Tables 1 and 2, who revealing the average number of surviving *D. margina*tus and de *H. punctata* females.

Table 1. *Dermacentor marginatus* females that survived after the contact with the extract (Average  $\pm$  D.S. to five tests/each concentration)

| Concentration % | Exposition time (minutes) |             |             |             |  |  |  |
|-----------------|---------------------------|-------------|-------------|-------------|--|--|--|
| Concentration % | 30                        | 60          | 90          | 120         |  |  |  |
| 0 (Control)     | 50                        | 50          | 50          | 50          |  |  |  |
| 10              | 49(9.8±0,4)               | 47(9.4±0.8) | 31(6.2±1.3) | 6(1.2±0.8)  |  |  |  |
| 5               | 49(9.8±0,4)               | 46(9.2±0.4) | 37(7.4±1.1) | 14(2.8±0.8) |  |  |  |
| 2               | 50(10±0)                  | 49(9.8±0.4) | 36(7.2±0.8) | 24(4.8±1.6) |  |  |  |
| 1               | 50(10±0)                  | 49(9.8±0.4) | 32(6.4±1.5) | 24(4.8±1.7) |  |  |  |
| 0.50            | 50(10±0)                  | 50(10±0)    | 39(7.8±1.7) | 26(5.2±1.9) |  |  |  |
| 0.25            | 50(10±0)                  | 50(10±0)    | 32(6.4±1.1) | 30(6.0±0.7) |  |  |  |

| Concentration | Exposition time (minutes) |             |             |             |  |  |  |  |
|---------------|---------------------------|-------------|-------------|-------------|--|--|--|--|
| %             | 30                        | 60          | 90          | 120         |  |  |  |  |
| 0 (Control)   | 50                        | 50          | 50          | 50          |  |  |  |  |
| 10            | 50(10±0)                  | 44(8.8±0.4) | 27(5.4±1.1) | 17(3.4±1.1) |  |  |  |  |
| 5             | 50(10±0)                  | 44(8.8±0.4) | 31(6.2±1)   | 21(4.2±1.3) |  |  |  |  |
| 2             | 50(10±0)                  | 45(9.0±1)   | 32(6.4±2.1) | 23(4.6±0.8) |  |  |  |  |
| 1             | 50(10±0)                  | 45(9.0±0.7) | 33(6.6±1.1) | 23(4.6±1.3) |  |  |  |  |
| 0.50          | 50(10±0)                  | 46(9.2±0.8) | 35(7.0±1.2) | 26(5.2±0.8) |  |  |  |  |
| 0.25          | 50(10±0)                  | 47(9.4±0.5) | 36(7.2±0.8) | 25(5.2±1.2) |  |  |  |  |

Table 2. *Haemaphysalis punctata* that survived after the contact with the extract (Average  $\pm$  D.S. to five tests/each concentration)

Analyzing the data from tables and also the variability test's results it can be observed that there are not significant differences in what concerns the efficacy and the tick's specia. Acaricidal effect appears after 30 minutes after exposure and it can be recognized after the movement ability reduction in almost all ticks, the effect being more visible and thus, after 120 minutes from the exposures few ticks being mobile.

#### **Statistics**

It was ascertained that are significant differences in what concerns the average number of surviving ticks in correlation with exposition time: F = 376.71 df = 3, p < 0.001 and  $R^2 = 88.6\%$ . Also there are significant differences also in correlation with used concentration: F = 6.51, df = 5, p < 0.001.

*Iori* and *al.* (2005) have tested the acaricidal effect of *Melaleuca alternifolia* extract (tea tree oil, TTO) to differed doses (4, 6, 8 and 10  $\mu$ l) and exposition times (30, 60, 90 and 120 minutes) on *Ixodes ricinus* nymphs. Authors observed that the 8  $\mu$ l used dose was lethal for 70% of the ticks and mortality raised up to 80%, when 10  $\mu$ l doses were used. The effect was correlated with exposure time, this being significant after 90 minutes after exposures.

#### In vivo testing

After 24 hours from the sheep dipping, all ticks found in sternal region were harvested, numbered and studied in lab conditions and modified behaviour in terms of mobility and vitality were observed. Also colour and consistency of tick's cuticle was modified comparatively with the control individuals. The identified species were *Dermacentor marginatus* and *Haemaphysalis punctata* with the predominance of the first one. From other related studies of the collective it was also ascertained that *D. marginatus* was a dominant tick specia in the areas. Death percentage at 24 hours was between 9 and 13% for *D. marginatus* and respectively, between 0-23% *H. punctata*.

From data found in Table 3 it can be observed that there are not significant differences in what means mortality in correlation with the extract's concentration, the difference being made by the attached in sternal region ticks' number.

| Concentration | Specia        | Ticks number | Alive | Dead | Efficacy % |
|---------------|---------------|--------------|-------|------|------------|
| 10            | D. marginatus | 304          | 293   | 38   | 11         |
| 10            | H. punctata   | 11           | 11    | 0    | 0          |
| 5             | D. marginatus | 332          | 297   | 35   | 11         |
| 5             | H. punctata   | 31           | 24    | 7    | 23         |
| 2             | D. marginatus | 448          | 376   | 72   | 16         |
| 2             | H. punctata   | 10           | 7     | 3    | 9          |
| 1             | D. marginatus | 389          | 339   | 50   | 13         |
| 1             | H. punctata   | 2            | 0     | 0    | 0          |
| 0.5           | D. marginatus | 381          | 340   | 41   | 11         |
| 0,5           | H. punctata   | 1            | 0     | 0    | 0          |
| 0.25          | D. marginatus | 282          | 258   | 24   | 9          |
| 0,25          | H. punctata   | 10           | 8     | 2    | 20         |
| Control lot   | D. marginatus | 478          | 478   | 0    | -          |
| Control lot   | H. punctata   | 213          | 213   | 0    | -          |

Table 3. Number of ticks gathered from the dipped sheep with *Euphorbia cyparissias* extract to 24 hours from the first application

The extract's acaricidal effect it is not a spectacular one, like *"knock-down"*, death being slow and late, in almost all cases after 48-72 hours from the applications.

The hungry or partial fed ticks were more sensitive than those that are the satiated ones. This observation was made also for the *in vitro* model.

The action way, apparently not spectacular of the Euphorbia extracts can be considered at a first glance a drawback.

To a second application at seven days interval, to the dipped sheep with the 10%, 5%, 2% and 1% concentrations there were not identified any satiated tick.

The number of the attached ticks was small (2-3 to maximum 5), all famish, what means that they are recently attached what suggest the repellent effect. To smaller concentrations of 0.5 and 0.25% close satiated ticks females were seen.

After the second dipping the ticks gathered at three days is presented in Table 4. A significant reduction of the ticks was observed, but with no correlation to the used concentration

| Concentration | Specia   | Ticks number |
|---------------|--|--------------|
| 10            | D. marginatus  | 33           |
| 10            | H. punctata  | 0            |
| 5             | D. marginatus  | 36           |
| 3             | H. punctata  | 1            |
| 2             | D. marginatus  | 157          |
| 2             | H. punctata  | 15           |
| 1             | D. marginatus  | 51           |
| 1             | D. marginatus<br>H. punctata<br>D. marginatus<br>H. punctata<br>D. marginatus<br>H. punctata<br>D. marginatus<br>H. punctata<br>D. marginatus<br>H. punctata<br>D. marginatus<br>H. punctata | 1            |
| 0,5           | D. marginatus  | 42           |
| 0,5           | H. punctata  | 2            |
| 0.25          | D. marginatus  | 66           |
| 0,25          | H. punctata  | 1            |
| Control lot   | D. marginatus  | 176          |
|               | H. punctata  | 67           |

Table 4. Number of ticks gathered from the dipped sheep with *Euphorbia cyparis*sias extract to 72 hours from the first application

#### DISCUSSION

The studies demonstrate the great importance of the results obtained *in vitro* versus *in vivo* comparison, which often can be significantly different.

If in the *in vitro* tests the ticks are dying after one or two hours due to the permanent contact, beside the used concentration, in the case of field application the situation is totally different.

*In vivo* the contact time being shorter (being a alcoholic solution it's evaporating much faster), the ticks have a greater moving autonomy. This aspect must be analyzed much closely in the aim of a proper excipient choosing, with a greater remanence on ticks' cuticle. Thus in the results interpretation is compulsory to evaluate the mode how ixodides – acaricidal contact was accomplished.

In our opinion the studies must be continued to ascertain precisely that *E. cyparis*sias can be used currently against ticks' populations.

Until now researches revealed that the alcoholic extract can be used only as an alternative method in the fight against ticks, due it's repellent and low acaricidal resistance risk. In our view is to extent the researches, using oily and glycerinated *E. cyparissias* extracts, for a better efficacy and also the effects on food conversion, reproduction and larval hatch parameters will be monitorized.

For example *Borges* et *al.* (2005), observed that 0.25% *Melia azedarach* alcoholic extract solutions, produced *in vitro* 100% mortality to *Boophilus microplus* but in the field conditions the evinced results were modest (tough was observed a significant reduction of fed ticks at 21 days after dipping).

*Webb* and *David* (2002) making treatments on naturally infested Tswana, Brahman and Simmental cattle with a 5% aqueous extract from seeds of *Azadirachta indica*, applied through dipping in doses of 5g x b.w.<sup>-1</sup> have observed a parasite reduction, but not them total elimination.

*Cristina* et *al.* (2006) have tested the *E. cyparissias extract* on *Argas persicus* proving it's efficacy in correlation with the concentration, contact time, dose and used concentration. The obtained results as well of other authors' reports suggest that often the studied plants efficacy is very dependent of type of the plant extract, concentration, exposure and parasites' life stages.

#### CONCLUSIONS

- 1) There are not significant differences of the acaricidal effect of *Euphorbia cyparissias* alcoholic extract according to ticks' specia
- 2) The extract efficacy is significantly different in correlation with the exposition time (p<0,001) and dose (p<0,001).
- 3) The *E. cyparissias* extract can be used as alternative control method of ixodides, being a natural acaricid, an economic and eco friendly alternative, harmless to humans and ecosystem.

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## AKTIVNOST EKSTRAKTA *EUPHORBIA CYPARISSIAS L.* PROTIV IXODIDA OVACA

## ROMEO T.CRISTINA, EUGENIA DUMITRESCU

#### Izvod

Ispitivan je uticaj aktivnosti ekstrakta Euphorbia ciparissias, u in vitro i in vivo uslovima, na terapiju ixodida ovaca. Identifikacija krpelja izvršena klasičnim parazitološkim metodama. Korišten je alkoholni rastvor ekstrakta Euphorbia, u razređenjima 10, 5, 2, 1, 0,5 i 0,25%. Monitoring preživljavanja iksodesa, u in vitro uslovima, je vršen u intervalima od 30, 60, 90 i 120 minuta, posle ekspozicije. Pokazalo se da efikasnost delovanja ovog ekstrakta na mortalitet krpelja, značajno zavisi od vremena izloženosti (p <0,001) i koncentracije primenjenog rastvora (p <0,001). U in vivo uslovima, svi krpelji su uginuli unutar 3h posle početka izlaganja ovom ekstraktu, bez obzira na primenjenu koncentraciju.

Ključne reči: Euphorbia cyparissias, ekstrakt, ixodides, ovce, efikasnost.

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# EFFECT OF *MENTHA PIPERITA* ON ANTIOXIDATIVE STATUS IN BROILER CHICKENS\*

## LJILJANA KOSTADINOVIĆ, JOVANKA LEVIĆ, SAVA PAVKOV, GORDANA DOZET, TAMARA GALONJA-COGHILL<sup>1</sup>

SUMMARY: This report describes an investigation on the effects of Mentha piperita on antioxidative status in hemolysed blood and liver homogenate of broiler chickens (glutathione peroxidase-GSH Px, superoxide dismutase-SOD, concentration of malondialdehyde-MDA and lipid peroxidation-TAOC). Three levels of whole Mentha piperita: 0; 100; 150 and 200 g/kg were incorporated into basal diet of 240 broilers for 42 days. Blood and liver were collected for the subsequent evaluation of antioxidant status. Feeding of diet supplemented with 200g/kg Mentha piperita significantly decreased the concentration of malondialdehyde (MDA) in plasma in comparison with the control group. The activities of glutathione peroxidase (GSH Px) were significantly higher in blood of chicks fed the diet containing 200 g/kg of Mentha piperita. Other diets containing 100 i 150 g/kg of Mentha piperita had no effect on lipid peroxidation and activity of antioxidative protection enzyme in the liver homogenate and blood hemolysate of broilers. The present investigation shows that Mentha piperita exibits a significant antioxidant activity in fattening chickens and can be used as a source of antioxidant in dietary supplement.

Key words: Mentha piperita, antioxidative status, broilers, feed.

#### INTRODUCTION

*Mentha* species- of the family labiatae- are well known in traditional medicine (Lewis and Elvin-Lewis, 1977). *Mentha piperita* – like many other members of this

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genus, is often used in domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effects on the digestion (Foster and Duke, 1999). For diagnostics of blood and organ diseases catalytic activity of enzymes in erythrocytes and liver is most comonly monitored.

Erythrocytes are directly exposed to molecular oxygen; their plasma membrane has high levels of polyunsaturated fatty acids and an anionic channel specific for single oxygen. Furthermore, erythrocytes contain a high concentration of hemoglobin that is prone to autooxidation. Erythrocytes have a highly effective system in protecting from free radicals: they contain all the enzymes of antioxidative protection and a high level of glutathion (Jovanović, 1993). The liver is an organ with a central metabolic role in the organism, often referred to as ,,the main laboratory" since it performs the major detoxification tasks. Diverse mechanisms are involved. For this reason the liver is the prime target for the study of the metabolism of xenobiotics and other substances (Popović, 1988). Lipid peroxidation is a reaction between polyunsaturated fatty acids and oxygen which is initiated by radical intermediates and active oxygen species produced by normal metabolic reactions or during metabolization of chemicals. Antioxidant enzymes counteract excessive formation and deleterious effects of reactive oxygen metabolites (Cotgreave et al., 1988). For example, superoxide dismutase (SOD) catalyzes the conversion of superoxide anion radical to H2O2, catalase reduces H2O2 to water, while glutathine peroxidase (GSH Px) acts in conjuction with other enzymes to reduce H<sub>2</sub>O<sub>2</sub> and to terminate lipid peroxidation.

Active components from herbal plants have been explored as possible antioxidants (Halvorsen et al., 2002; Dragland et al., 2003). *Mentha piperita* is an herbal antioxidant shown to possess strong antibacterial, antioxidative and antiinflammatory activities (Mimica-Dukić et al., 2003).

However, the role of *Mentha piperita* as a dietary antioxidant source in broiler chicken has not been investigated. The objective of this study was to determine supplemental effects of *Mentha piperita* on antioxidant system in broiler chickens.

#### MATERIAL AND METHODS

Animals, diets and treatments. The experimental protocol was approved by the local Ethics Committee; the principles of animal protection were strictly followed. Experiments under *in vivo* conditions were performed on broilers of the heavy line Arbor Acres, of both sexes. One –day-old broilers, randomly selected, were divided into four groups, each numbering 60 individuals. Bird fed a standard basal diet. All birds had free access to water and feed. Temperature and lighting regimens were in accordance with the recommenditation of the breeder. The initial room temperature 32-33° C was reduced weekly by 1°C to a final temperature of 28°C. The control group fed a basal diet; the second, third and fourth groups were fed the some basal diets supplemented with 100, 150 and 200g/kg of chosen herb: *Mentha piperita*, respectively.

Levels of hemoglobin, necessary for the expression of the enzymatic activities in hemolysed blood, were determined using commercial test ("Dialab", Vienna, Austria) on a spectrophotometer ("MultiscanMCC340, Finland). Protein content was determined by the modified method of Gornall and Bardwall (1949).

**Praparation of blood hemolysate.** Blood was drawn from the hearts of broilers into heparinized test tubes. After centrifugation (10 min. at 3500 rpm and 4°C) and

plasma removal, the pellet was rinsed 3 times in saline. The resulting erythrocyte pellet was suspended in an equal volume of double distilled of water and vortexted. After incubation for 1 hour at room temperature, the hemolysate was centrifuged for 15 min. at 3500 rpm and the supernatant aliquoted for further analysis.

**Preparation of liver homogenate.** The exised liver was perfused to eliminate blood and the total mass determined. One gram of the tissue was minced with scissors and homogenized in an ultratorax in 3 volumes of isotonic buffer (0,05 M Tris-HCl, 0,25M sucrose, pH=7,5). The homogenate was filtered through gauze into ice-cold tubes and aliquoted for further analysis.

**Determination of enzymatic activity.** The SOD (EC 1.15.1.1) activity was determined by the spectrophotometric method based on the inhibition of adrenaline reduction to adrenochrome at pH= 10,2 (Jovanović, 1993). The GSH Px (EC 1.11.1.9) activity was determined by spectrophotometric measurement of absorbance at 412 nm with cumenhydroperoxide as the substrate (Chin et al., 1976).

Tissue samples of liver for malondialdehyde (MDA) determination were homogenised with deionised distilled water and 50 ml of butylated hydroxytoluene. The MDA concentrations in homogenates were measured by the mofified fluorimetric method in accordance with Jo and Ahn (1998).

Lipid peroxidation was determined with thiobarbituric acid. The oxidation of cellular membrane lipids was measured via reaction of lipid peroxides with thiobarbituric acid (Buege and Aust, 1978).

*Statistics.* Differences between means were evaluated at different levels of significance (p<0,05; p<0,01; p<0,001) using repeated measures analysis of variance (Sokal and Roulf, 1981). The results are given as means  $\pm$  SEM.

#### RESULTS

Chickens for fattening did not show any visible clinical changes during the whole experiment. The results of antioxidant indices in hemolysed blood are shown in Table 1. The MDA activity in blood and plasma was significantly lower (p<0,05) only in the group supplemented with 200g/kg of *Mentha piperita* compared to the control group. Birds of group supplemented with 200g/kg *Mentha piperita* had greater (p<0,05) lipid peroxidation (TAOC) and SOD than broiler chicken in control group. The activity of GSH Px did not differ among treatments.

Table 1. Concentration of malondialdehyde (MDA) and content of lipid peroxidation (TAOC) and enzymatic activity of glutathione peroxidase (GSH Px) and superoxid dismutase in hemolysed blood of broilers fed diets supplemented with different concentrations of Mentha piperita. (mean±SEM; n=10 in each group)

Tabela 1. Koncentracija malondialdehida (MDA) i lipidnih peroksida (TAOC) i enzimska aktivnost superoksid dismutaze (SOD) i glutation peroksidaze (GSH Px) u hemolizatima krvi brojlera hranjenih različitim koncentracijama nane (Mentha piperita)(vrednost $\pm$ SD; n=10 u svakoj grupi)

|                               | Treatment / Tretman                     |  |  |  |  |  |  |  |
|-------------------------------|---|--|--|--|--|--|--|--|
| <i>Parameter</i><br>Parametar | <i>Control group</i><br>Kontrolna grupa | 100g/kg Mentha<br>piperita<br>100g/kg nane | 150g/kg Mentha<br>piperita<br>150g/kg nane | 200g/kg Mentha<br>piperita<br>200g/kg nane |  |  |  |  |
| TAOC, U/ml                    | $15,93 \pm 0,32b$                       | $18,70 \pm 0,35b$                          | $19,16 \pm 0,41a$                          | $19,33 \pm 0,46a$                          |  |  |  |  |
| SOD, U/ml                     | $139,44 \pm 3,73$                       | $155,44 \pm 4,10$                          | $143,12 \pm 4,43$                          | $140,74 \pm 4,96$                          |  |  |  |  |
| GSH Px, U/ml                  | $184,20 \pm 5,08a$                      | $184,90 \pm 3,10a$                         | $185,\!10\pm 4,\!20$                       | $193,64 \pm 3,90a$                         |  |  |  |  |
| MDA, nmol/ml                  | $7,32 \pm 0,04a$                        | $6,24 \pm 0,01$                            | $6,25 \pm 0,01$                            | $6,23 \pm 0,01b$                           |  |  |  |  |

Significant differences within a row are indicated by the same superscript letter (p<0,05) Statistički značajne razlike između vrednosti u istom redu označene su slovom u superskriptu (p<0,05)

Table 2. Concentration of malondialdehyde (MDA) and content of lipid peroxidation (TAOC) and enzymatic activity of glutathione peroxidase (GSH Px) and superoxid dismutase in liver of broilers fed diets supplemented with different concentrations of Mentha piperita.(mean $\pm$ SEM; n=10 in each group)

Tabela 2. Koncentracija malondialdehida (MDA) i lipidnih peroksida (TAOC) i enzimska aktivnost superoksid dismutaze (SOD) i glutation peroksidaze (GSH Px) u jetri brojlera hranjenih različitim koncentracijama nane (Mentha piperita) (vrednost $\pm$ SD; n=10 u svakoj grupi)

|                               | Treatment / Tretman                     |  |  |  |  |  |  |
|-------------------------------|---|--|--|--|--|--|--|
| <i>Parameter</i><br>Parametar | <i>Control group</i><br>Kontrolna grupa | 100g/kg Mentha<br>piperita<br>100g/kg nane | 150g/kg Mentha<br>piperita<br>150g/kg nane | 200g/kg Mentha<br>piperita<br>200g/kg nane |  |  |  |
| TAOC, U/mg of protein         | $1{,}68\pm0{,}06$                       | $1,\!86\pm0,\!06$                          | $1{,}92\pm0{,}07$                          | $1,\!87\pm0,\!08$                          |  |  |  |
| SOD, U/mg of protein          | $215,82 \pm 3,70b$                      | 236,33 ± 3,35a,b                           | 235,68 ± 3,60a,b                           | $240,96 \pm 3,42a$                         |  |  |  |
| GSH Px, U/mg of protein       | $2,73 \pm 0,16a$                        | 4,51 ± 0,25a,b,c                           | $2,18 \pm 0,30c$                           | $2,\!93\pm0,\!33b$                         |  |  |  |
| MDA, nmol/mg of protein       | $4,17 \pm 0,09a$                        | $3,\!88\pm0,\!05$                          | $4,\!10\pm0,\!07$                          | $3,77 \pm 0,11b$                           |  |  |  |

Significant differences within a row are indicated by the same superscript letter (p<0,05) Statistički značajne razlike između vrednosti u istom redu označene su slovom u superskriptu (p<0,05)

Table 2 shows hepatic antioxidant indices of birds. The concentration of MDA was significantly lower (p<0,05) in the group supplemented with 200g/kg *Mentha piperita* than control group. MDA concentration in liver were not significantly affected by diets. The GSH Px activity in the liver was significantly higher only in the group of birds fed the diet supplemented with 150 g/kg of *Mentha piperita* compared to the control and both experimental groups.

#### DISCUSSION

We found significantly lower lipid peroxidation in blood and plasma of chicks fed the diet supplemented with 200g/kg of *Mentha piperita*. Other diets containing 150 and 100g/kg of *Mentha piperita* had no effect on lipid peroxidation.

Additionally, effects on antioxidant enhacement of Mentha piperita in vivo were shown for some antioxidant parameters such as SOD, GSH Px, TAOC and MDA. These measurements were used, because they reflect the effect of Mentha piperita on antioxidant activities. First, MDA can endogenously reflect lipid peroxidation, which is the consequence of diminshed antioxidant protection as levels of ROS (reactive oxygen species) increase. Also, SOD and GSH Px are the main parameters used to assess oxidative status in the enzymatic system. Finally, we assayed the TAOC level, which reflects the nonenzymatic antioxidant defense system. Several enzymatic factors such as SOD and GSH Px can scavenge formed ROS to function as antioxidants. Superoxide can first be degraded into H<sub>2</sub>O<sub>2</sub> by SOD and subsequently catalyzed to convert water by a series of enzymes including GSH Px. This could be beneficial for the birds because increased antioxidant activity ensures proper and rapid elimination of ROS. In the present study, Mentha piperita in broiler feed increased SOD levels across serum and liver but GSH Px levels were not altered. Supplementation with Mentha piperita, therefore, may enhance the ROS scavenging by elevating the SODlevel rather than the GSH Px level. SOD were not significantly affected in chickens fed diet supplemented with Mentha piperita. Lower GSH Px activity is generally accompained with an increase of the MDA concentration. The digestive tract itself is considered to be a major site of free radical production in animals and some of them might be delivered via portal blood system into the liver.

In our study, results shows that *Mentha piperita* has an effect on the liver function. Our results indicate that this herb has hepatoprotective effects.

#### CONCLUSION

The present investigation shows that chosen herb *Mentha piperita* exibits significant antioxidant activity in broiler chickens. Thus, it appears that this spice exerts antioxidant protection through its ability to activate the antioxidant enzymes. Finding of the present study establish that *Mentha piperita* has appreciable immunostimulatory activity.

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# UTICAJ NANE (*MENTHA PIPERITA*) NA ANTIOKSIDATIVNI STATUS BROJLERA

## LJILJANA KOSTADINOVIĆ, JOVANKA LEVIĆ, SAVA PAVKOV, GORDANA DOZET, TAMARA GALONJA-COGHILL

## Izvod

U radu su prikazani rezultati ispitivanja uticaja lekovite biljke *Mentha piperita* na antioksidativni status hemolizata krvi i homogenata jetre 240 brojlerskih pilića, teške linije Arbor Acres, oba pola (glutation peroksidaze-GSH Px; superoksid dismutaze-SOD, lipid peroksidacije-TAOC i koncentracije malondialdehida-MDA). Cela biljka *Mentha piperita* je dodata u osnovnu hranu za brojlere u količinama: 0, 100, 150 i 200 g/kg u toku 42 dana tova. Hrana sa dodatkom od 200g/kg nane dovela je do statistički značajnog smanjenja koncentracije malondialdehida (MDA) u krvi u odnosu na kontrolnu grupu. Aktivnost glutation peroksidaze (GSH Px), takođe je bila statistički značajno povećana u krvi brojlera koji su dobijali hranu sa 200g/kg nane. Ova istraživanja pokazuju da se *Mentha piperita* može primeniti kao izvor antioksidanata u hrani za brojlere.

Ključne reči: Mentha piperita, antioksidativni status, brojleri, hrana za živinu

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## VALUE ESTIMATION OF CEREAL STRAW\*

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SUMMARY: At the current rate of consumption, fossil fuels will very soon become deficient belies greater utilization of other sources of energy. Biomass, due to its great potential and ability of renewal, is especially favourable for energetic exploitation. Considering the ratio of the exchange rate of 1.38 tones of straw per 1 ton of coal, with the purchase price of coal calculated according the coal market price of 48.38  $\in$ , it is possible to conclude that the value of straw as an energy source, makes 22.41  $\in$ /ton (on a parcel). Average value of straw per one hectare amounted to over 80  $\in$  in 2007, or slightly below 68  $\in$  in 2010 year, which clearly shows the direct impact of fuel price increase.

Key words: valorisation, straw, agriculture

#### INTRODUCTION

Limited sources of fossil fuels, with the reserves estimated to a consumption period of 30-40 years, guide mankind to look for alternative energy sources. The lack, or actually the plenitude of energy, is conditioned by the course of development in technology and economy. This is especially apparent if we know that the energy obtained by organic conversion, or the energy of biomass, available from time immemorial, is nowadays drastically neglected in our area, as well as in the world.

Original scientific paper / Originalni naučni rad

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<sup>\*</sup> The research has been conducted as a part of a project "Sustainability of the chain of the food mass production" financed by the RS Ministry of Science and Technological Development, TR-20066.

#### MATERIAL AND METHOD

Regardless of the broad studying of the aforementioned issues, it is very difficult to determine the value of the straw as a by-product. In the evaluation of the straw value, two basic principles can be applied: 1) Principle of replacement and 2) Harvest principle. In the first case, it is the replacement price of the observed material by another material, or in the other case processing (utilisation) price which shows the increase of the value achieved by the use of the observed material.

Since it is very difficult to talk about the increase of the value in production which uses biomass as energy, it is possible to apply only the first principle, the principle of the replacement. In other words, it is possible to make a comparison with the coal, where all the expenses of the straw preparation should be subtracted from the calculated straw value. System of round cylindrical bales has been chosen as a method of preparation.

The research was done on several selected farms in Vojvodina. The calculation of expenses for the use of operating and binding machines has been carried out based on the previously developed model (Zekić, 2000) implemented through the computer programme. The work is done comparing the results of research during 2007 (Zekić, Tica, 2007) and the calculation performed during 2010.

#### **RESULTS AND DISCUSSION**

Te calculation of the fuel value which is to be replaced was derived with the use of coal from local resources. When comparing two potentially replaceable sources of energy, heat power represents one of the main indicators of the observed material usability as fuel. Basic characteristics of different kinds of coal are given in Table 1<sup>2</sup>.

Table 1 Characteristics of certain kinds of coal Tabela 1 Karakteristike pojedinih vrsta uglja

| Coal class / Klase uglja        | Group within the class<br>Grupe unutar klasa | Energy value<br>Energetska vrednost (MJ/kg) |
|---------------------------------|--|---|
| Lignite                         | Lignite A / Lignit A                         | <14,6                                       |
| Lignit                          | Lignite B / Lignit A                         | 14,6-19,3                                   |
| Brown coal / Mrki ugalj         |  | 19,3-26,7                                   |
| Hard coal / <i>Kameni ugalj</i> |  | 24,4-32,5                                   |
| Anthracite / Antacit            |  | >32,5                                       |

Main energetic features of different kinds of biomass with standard humidity contents, when the very same is suitable for energetic utilisation (Zubac, Bubalo, 1995) are shown in the following table (Table 2).

The average value of the cereal straw is 14 MJ (Janjić, Brkić, 1998); if lignite from local resources (19,3 MJ kg) is used as the energy source to be replaced it is possible to reach the exchange rate of 1.38 tones of straw for 1 tone of lignite.

The first step in estimating expenses of the straw preparation is to make a pre-

<sup>2</sup> http://hypertextbook.com/facts/Energy Density of Coal.htm

liminary estimation for the use of operating and binding machines. In both cases the calculation includes use of a tractor MTZ- 820 for exploitation in the transportation and manipulation process, John Deere – 592, model 1.81 M regular press, Zmaj – 401 trailers, pick – up device and unbind device Zmaj – 402. Expenses of the straw exploitation include expenses of straw preparation, transportation to the storage location, manipulation and storage expenses.

Table 2 Energy value of certain biomass kinds Tabela 2 Energetska vrednost pojedinih vrsta biomase

| Material / Materijal          | Energy value / Energetska vrednost (kJ/kg) |
|-------------------------------|--|
| Cereal straw / Slama žitarica | 16.200                                     |
| Cornstalks / Kukuruzovina     | 17.100                                     |
| Soy-bean straw / Sojina slama | 18.200                                     |

Based on the expense calculation towards certain categories and given efficiency (Renijus, 1985; Schmid, 1995), it is possible to come to the final expenses of preparation, transportation, and manipulation of the straw packed in cylindrical bales. The calculation includes all the expenses established in the previous stage of work, while the expenses of storage were calculated through the assumed loss of the used straw estimated to 20%. The overview of total expenses for preparation of the straw in shape of cylindrical bales is given in table 3.

Table 3 Calculation of total expenses for preparation of the straw in shape of cylindrical bales in 2007 i 2010 years

Tabela 3 Obračun ukupnih troškova slame spremljene u obliku valjkastih bala u toku 2007 i 2010. godine

| Category of expenses  | 2007.                                | years                         | 2010.                                | years                         |
|---|--------------------------------------|-------------------------------|--------------------------------------|-------------------------------|
| (by operations)<br>Kategorija troškova<br>(prema operacijama) | Expenses<br><i>Troškovi</i><br>(€/t) | Structure<br>Struktura<br>(%) | Expenses<br><i>Troškovi</i><br>(€/t) | Structure<br>Struktura<br>(%) |
| Baling<br>Baliranje   | 5,08                                 | 31,21%                        | 5,61                                 | 29,81%                        |
| Material- connecting materials<br>Materijal – vezivo          | 0,62                                 | 3,79%                         | 0,53                                 | 2,82%                         |
| Loading and transportation<br>Utovar i transport              | 5,45                                 | 33,44%                        | 6,59                                 | 35,02%                        |
| Manipulation<br><i>Manipulacija</i>                           | 0,97                                 | 5,94%                         | 1,21                                 | 6,44%                         |
| Unwrapping<br>Odmotavanje                                     | 1,46                                 | 8,95%                         | 1,74                                 | 9,25%                         |
| Storage<br>Skladištenje                                       | 2,72                                 | 16,67%                        | 3,14                                 | 16,67%                        |
| Total<br>Ukupno   | 16,29                                | 100,00%                       | 18,82                                | 100,00%                       |

#### CONCLUSION

At current rate of consumption, fossil fuels will very soon become deficient belies greater utilization of other sources of energy. Due to its great potential and possibility of renewal, agricultural biomass is particularly favourable for energetic exploitation. The most available parts of the biomass are wastage and by-products of the forestry, wood industry, agriculture, and primary treatment of agricultural products.

It has been ascertained, by the calculation of the expenses, that the price of preparation for one tone of cereal straw by the system of cylindrical bales costs 18,82  $\in$ . Considering the ratio of the exchange rate of 1.38 tones of straw per 1 ton of the coal and that the purchase price of the coal is calculated with the market price of 48.38  $\in$ , it is possible to conclude that the value of the straw, as an energy source, makes 22,41  $\in$ / ton (on a parcel). Average value of straw on one hectare amounted to over 80  $\in$  in 2007, or slightly below 68  $\in$  in 2010 year.

In the development of technological solutions, advantage has to be given to those technological solutions that are already commonplace in practice. This is conditioned, firstly, by very low investment power of agriculture, as well as by the low qualification structure of the manpower in agriculture. Achievement of such complex goals can only be accomplished with stimulating measures from adequate state institutions. On the other hand, an increase of the energy utilisation of the straw would have positive influence on the development of rural areas and employment of the local population. The assumption is that with the increase in production of energy in rural areas, due to the exploitation of the biomass, conditions would be created to keep inhabitants capable for work, yet latently unemployed in provincial and industrially insufficiently developed regions. The state should encourage domestic industry to produce equipment for energy utilisation of the biomass. Apart from that, it is necessary to enable for as large number of potential biomass beneficiaries as possible to learn about the potential use of the biomass as energy source.

The starting point in energy utilisation of biomass is creating conditions where agricultural households will use their own by-products to produce energy, in as large number as possible, in other words, stimulating organisation of more independent farms in terms of energy production. In this case, transportation expenses for the remaining biomass are relatively low, which presents important condition of the prudence of the entire process.

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# PROCENJIVANJE VREDNOSTI SLAME STRNIH ŽITA

## VLADISLAV ZEKIĆ, NEDELJKO TICA, VELJKO VUKOJE

#### Izvod

Izvesnost da će fosilna goriva, pri ovakvoj dinamici potrošnje, veoma brzo postati deficitarna nalaže veće korišćenje drugih izvora energije. Zbog svog velikog potencijala i obnovljivosti biomasa je posebno zanimljiva za energetsku eksploataciju. Ako se u obzir uzme odnos odnosa zamene od 1,38 tone slame za 1 tonu uglja i nabavna cena uglja obračunava po tržišnoj ceni od 48,38  $\in$  moguće je doći do zaključka da je vrednost slame kao energenta iznosi 22,41  $\in$ /tona (na parceli). Prosečna vrednost slame u jednom hektaru iznosila je oko 80  $\in$  u 2007, ili nešto ispod 68  $\in$  u 2010. godini što jasno pokazuje direktan uticaj rasta cene pogonskog goriva.

Ključne reči: valorizacija, slama, poljoprivreda.

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# IRREVERSIBLE ERYTHROCYTE'S MEMBRANE RUPTURE CAUSED BY HYPERBARIC OXYGENATION

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SUMMARY: Hyperbaric oxygen is often used for therapeutic purposes, but also as a model for investigation of oxidative stress of erythrocytes. The aim of this study was to investigate why 40 minutes after exposure to hyperbaric oxygen caused irreversible damage to red blood cells. In trial was attended by 60 mice strain Mus musculus, which are exposed to oxygen under pressure of 3.5 ATA. Blood was taken by vein-puncture and prepared to observe the scanning (SEM) and transmission electron microscopy (TEM). The reason of serious irreversible damage is a red blood cell membrane rupture, which occurs in the area with thin wall membrane. At that point the content of red blood cells pour out in the external environment, and diameter of damage was about 1µm.

Key words: hyperbaric oxygenation, erythrocyte, membrane damage.

## INTRODUCTION

Erythrocytes, which play an important role in the delivery of oxygen and maintenance of energy metabolism in the body. An important feature of red blood cells is their flexibility. This ability to depend on cell types (the ratio of surface and volume of cells), cytoplasmic viscosity (governed by MCHC) and stability of membranes. For the proper performance of the functions in the body, it is necessary to have preserved form of erythrocytes, the structure of the wall and preserved metabolically modified iron and hemoglobin. Erythrocytes have no nucleus, as well as cytoplasmic bodies.

Oxygen under pressure has long been used in many therapeutic purposes, and the negative impact of hyperbaric oxygenation studied through history (Dharmeshkumar et al, 2003). It is known that acute oxygen poisoning causes damage to the CNS, while

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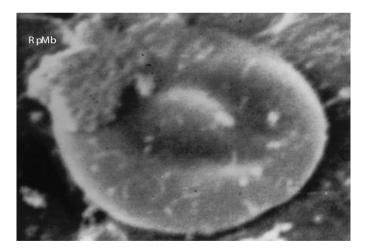
chronic exposure of hyperbaric oxygen damages the respiratory organs. Researches related to the impact of hyperbaric oxygenation (HBO) on erythrocytes indicate that the expression of animals according to HBO comes through: increased osmotic fragility, increased MCV, Hb concentration, Ht% (Etlik and Tomur, 2006), increased red blood cells oxidatively wall burden (Otter et al, 2005). However, prolonged normobaric hyper-oxia could caused haemolysis (Torbati et al., 2006).

Taking into account all the pathophysiological changes, based on previously obtained results on red blood cells shape change during hyperbaric oxygenation (Belic and Cincović, 2009), could be presupposed that basically all the changes are caused by changes in wall ultrastructure of erythrocytes.

The aim of this paper is to show changes in the ultrastructure of erythrocytes leading to irreversible deterioration of red blood cells during exposure to hyperbaric oxygen.

#### **MATERIAL AND METHOD**

Blood for analyses was collected by venepunction in 40 minutes after starting of exposure to hyperbaric condition (3,5 ATA),. Blood was heparised, and washed in isotonic-physiology solution. Next step was drying in alcohol and acetone series. Prepared samples were imaging by scaning and transmission electronic microscopes, class JSM-T20 and Philips SEM500. RBC were observed and pictured in 1000 and 2000X amplification, with angle of view 0-30°. It's important to mention that samples prepared for transmission microscopy were put into resin solution after drying.



#### RESULTS

Figure 1. 40.min. of hyperbaric oxigenation – perforated RBC membrane (RpMb), lose of RBC matter, SEM. 10000X

Slika 1. 40. minut ekspozicije hiperbaričnom kiseoniku – ruptura membrane eritrocita (RpMb) sadžaj izlazi iz eritrocita, SEM, 10000X

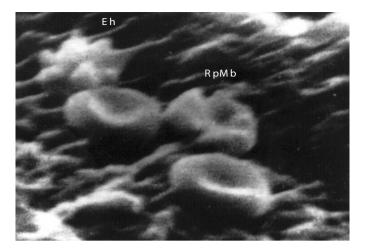


Figure 2. Experimental sample – 40.min of hyperbaric exposition, Eh-echinocyte, RpMb-perforated RBC membrane, SEM, 2500X

Slika 2. Eksperimentalni uzorak - 40. minut. Eh - ehinocit, RpMb - ruptura membrane, SEM, 2500X

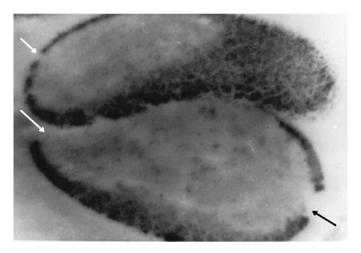


Figure 3. 40. min.- Intersection of two RBC with ruptured membrane, TEM, 10000X. *Slika 3. 40. minut. - Presek dva eritrocita sa rupturama membrane, TEM,10000X.* 

#### DISCUSSION

Previous research has shown that hyperbaric oxygenation leads to changes in the shape of erythrocytes (Belic and Cincović, 2009). These changes can be recurrent and non-refundable. Increase in the number of echinocyte, with the development of convulsion in laboratory mice reduces the reversibility of damage to red blood cells.

The reasons for the rapid accidence of erythrocytes' wall during hyperbaric oxygenation are not yet sufficiently clear. The erythrocytes' wall is composed of lipid bilayer (40%), protein (52%) and carbohydrates (8%). Phospholipids are asymmetrically distributed in the wall, arranged in two layers, between which is free cholesterol. The outer layer is phosphatidyl choline (PC) (30%) and sphingomyelin (SM) (25%). The inner layer of phospholipids include phosphatidyl-etanolamine (PE) (28%) and phosphatidyl-serine (PS) (14%). Red blood cell wall proteins may be integral and peripheral. Integral proteins (group 3, glycophorins, aquaporins) are those that are embedded in the membrane by hydrophobic connection with the lipids. Peripheral proteins (spectrin, actin, protein 4.1, pallidin, ankyrin, adducin, tropomycin, tropomodulin) are on the cytoplasmic side and contact with the integral proteins, forming flexibility and stability of the membrane (Palek and Sahr, 1992).

The basic unit of membrane skeleton structure is hexagonal network with six molecules of spectrin (Liu et al, 1987). The basic units are interconnected with multiple spectrin tetramers. Spectrin, actin and protein 4.1 are complex, and the ankyrin binds to lipid membranes through interaction with group 3 (Liu et al, 1990). Lipid part and its phospholipide components are related to protein of red blood cells and have a role in energy metabolism (Sheetz and Casaly, 1980). Change the shape of erythrocytes (anysocytosis) occurs in different disease of bone marrow, heart, kidney, liver, and the poisoning and autointoxication. In particular, the red blood cells characteristic of hereditary diseases, when the violation of hexagonal structure (Palek and Sahr, 1992).

Recent research puts the change in the relationship of form and function of the wall red blood cells (Wu et al 2008; Park et al, 2009). Because of all this would be interesting to do the separation and quantification of the building elements of the wall of red cells, but also examine the energy metabolism of different cell types, thus the reasons for the occurrence of rupture become clearer. The fact that many proteins can be present in red blood cell's wall of the less differentiated forms, indicating that it is possible to predict the protein material and the viability of erythrocytes during their life (Koury et al, 1987; Hanspal and Palek, 1987).

#### CONCLUSION

Irreversible changes and loss of erythrocyte functions during hyperbaric oxygenation result in red blood cells membrabe ruptures localized in one or more openings, few nanometers in diameter, through which the content is leaving the cell, which loses its function.

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# IREVERZIBILNI NASTANAK RUPTURE MEMBRANE ERITROCITA IZAZVAN HIPERBARIČNIM KISEONIKOM

## BRANISLAVA BELIĆ, MARKO R.CINCOVIĆ, JELENA BELIĆ, BOJANA VIDOVIĆ

## Izvod

Hiperbarični kiseonik često se koristi u terapeutske svrhe, ali i kao model za ispitivanje oksidativnog opterećenja eritrocita. Cilj ovog rada bio je da ispitamo zašto 40 minuta posle izlaganja hiperbaričnom kiseoniku nastaju nepovratna oštećenja crvenih krvnih zrnaca.U ogledu je učestvovalo 60 miševa soja Mus musculus, koji su izlagani kiseoniku pod pritiskom od 3,5 ATA. Krv je uzimana venepunkcijom i pripremana za posmatranje skening (SEM) i transmisionim elektronskim mikroskopom (TEM). Razlog teških ireverzibilnih oštećenja jeste ruptura membrane eritrocita, koja nastaje na mestu istanjenja zida membrane. Na tom mestu izlazi sadržaj eritrocita u spoljnu sredinu, a oštećenje je promera oko 1 mikrometra.

Ključne reči: hiperbarična oksigenacija, eritrociti, oštećenje membrane.

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## HEMATOLOGY PARAMETERS AND PHYSICAL RESPONSE TO HEAT STRESS IN DAIRY COWS

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SUMMARY: Heat stress in dairy cows occurs during July and August (THI> 72). The trial was attended by 10 cows in summer and the same 10 cows in the winter season. Physical response: Increase in rectal temperature and pronounced diurnal variations can be observed during the summer (38.4:39, p < 0.01), a significant increase in the respiration per minute (46.5:65, p < 0.01) and decrease rumen contraction (10.75:5.5, p < 0.01). Haematology: heat stress leads to certain hematological changes, such as: decrease in the number of erythrocytes (7,38:6,51, p < 0.01), decrease in MCV (53.25:47.31, p > 0.05), decrease in hematocrit (39.3:30.8, p < 0.01), decrease of white blood cells (9.18:7.75, p < 0.01) and platelets (425:351, p < 0.05), as well as decrease in the concentration of hemoglobin (111.5:107, P > 0.05). Hematological changes indicates overhydration signs, indicating active body cooling evaporation. Correlation between physical and hematology response were significant.

*Key words*: heat stress, dairy cow, hemathology parameters, rectal temperature, respiratory rate, rumen contraction.

#### **INTRODUCTION**

Heat stress occurs when cows are exposed to high ambient temperatures and their body starts to overheat. The main type of heat is evaporation, whose intensity depends on the temperature and humidity. Index of temperature and humidity (THI index) is a good indicator of stressfullness of ambient temperature. Critical value of THI which starts stress response in cows is 72 (Dikmen and Hansen, 2009). In our geographic area during the summer to realize the conditions for the development of heat stress.

Original scientific paper / Originalni naučni rad

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Penetration of the wave of African climate and global warming leads to an increase in the number of tropical days and nights in the territory of Vojvodina (HMZS). In heat stress productive part of the energy cows spent on cooling. Therefore, cows react with reduced production and reduced milk quality (Cincović and Belic, 2009) with reduced reproductive performance and udder health disorder.

Heat stress leads to changes in body temperature, breath, heart, and rumen contraction frequency. Forced breathing that occurs during heat stress leads to respiratory alkalosis and compensatory rumen acidosis. Because evaporative cooling may be the emergence of dehydration, but compensatory increase in water intake can cause increased blood volume. Tendency of animals to a state of oxidative stress and damage to organs due to the withdrawal of blood to the periphery of the body's shown during stress. Transport of oxygen and free radicals depends on the concentration of hemoglobin and erythrocyte indexes. Functional status of erythrocytes depends on the maintenance of acid-base homeostasis of the organism. Prolonged exposure to heat stress leads to the tendency of animals to a variety of inflammation, which would be the analysis of white blood lineage could assume (Kadzere et al, 2002; West, 2003; Murphy, 1992).

The aim of this study was to investigate the movement of cows, body temperature, respiration rate, heart rate and rumen contraction (physical response), along with the determination of complete blood count (CBC).

#### MATERIAL AND METHODS

The experiment has entered 20 cow Holstein Friesian breed, which were in lactation. Cows were in stalls with drop. 10 cows was observed during January and February, and 10 during July and August. Rectal temperature measurements in cows and auscultation of the heart, lungs and rumen performed four times daily, at 9, 13, 17 and 21h, and then the ambient temperature and humidity was measured. Calculate THI was done using the formula: THI = TVF-[0.55 - (0.55 xRVP/100)] x (TVF-58, 8); TVF-air temperature in Fahrenheit (° F), RVP-relative humidity in percentage, and measurements were performed synchronously with other.

Blood samples were taken between 7.00 and 12.00 AM by jugular venipuncture using disposable syringes. Blood anticoagulated with disodium-EDTA was used for blood cell count, and with citrate for haemoglobin. All tubes were placed immediately on ice and were transferred to the laboratory. Anti-coagulated blood was used for blood cell determination using automated veterinary hematology analyzer (Hemavet 950, UK).

For statistic analyzes were used Z and t test.

#### RESULTS

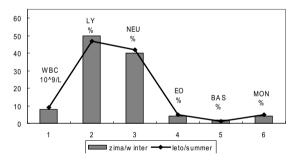
Table 1. THI value during experiment periodTabela 1. Kretanje vrednosti THI u oglednom periodu

|             | THI min | THI max | THI average |
|-------------|---------|---------|-------------|
| Winter/Zima | 53.2    | 63.1    | 58.15       |
| Summer/Leto | 65.6    | 77.2    | 71.4        |

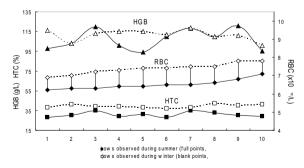
Table 2: Rectal temperature, respiration and heart rate and rumen contraction in different season

| Rectal temp. (°C)<br>Rektalna temp.          | 9h   | 13h  | 17h  | 21h  | Prosek<br>Average | Xmax-Xmin | SD     | Z       |
|--|------|------|------|------|-------------------|-----------|--------|---------|
| Winter/ Zima                                 | 38.2 | 38.5 | 38.6 | 38.3 | 38.4              | 0.4       | 0.183  | D <0.01 |
| Summer/ Leto                                 | 38.6 | 39.2 | 39.5 | 38.7 | 39                | 0.9       | 0.424  | P<0,01  |
| Resp.rate (/min)<br>Frekvenca respiracije    | 9h   | 13h  | 17h  | 21h  | Prosek<br>Average | Xmax-Xmin | SD     | Z       |
| Winter/ Zima                                 | 46   | 39   | 49   | 52   | 46.5              | 13        | 5.568  | D <0.01 |
| Summer/ Leto                                 | 49   | 69   | 74   | 68   | 65                | 25        | 10.984 | P<0,01  |
| Heart rate (/min)<br>Srčana frekvenca        | 9h   | 13h  | 17h  | 21h  | Prosek<br>Average | Xmax-Xmin | SD     | Z       |
| Winter/ Zima                                 | 52   | 63   | 56   | 61   | 58                | 11        | 4.967  | NG      |
| Summer/ Leto                                 | 62   | 68   | 75   | 66   | 67.75             | 13        | 5.439  | NS      |
| Rumen contract. (/min)<br>Kontrakcija buraga | 9h   | 13h  | 17h  | 21h  | Prosek<br>Average | Xmax-Xmin | SD     | Z       |
| Winter/ Zima                                 | 10   | 13   | 11   | 9    | 10.75             | 4         | 1.708  | D <0.01 |
| Summer/ Leto                                 | 7    | 4    | 5    | 6    | 5.5               | 3         | 1.291  | P<0,01  |

Tabela 2: Kretanje vrednosti rektalne temperature, frekvence disanja, kontrakcije srca i buraga



Graph 1: Absolute and relative number of white blood cells in differential season Grafikon 1: Apsolutni broj leukocita i procentualna zastupljenost pojedinih ćelija bele loze



Graph 2: RBC number, hematocrit value (HT) and Hb concentration (HGB) in winter and summer Grafikon 2: Kretanje broja eritrocita (RBC), hematokrita (HT) i koncentracije Hb (HGB) u sezonama

#### DISCUSSION

Mild heat stress were indicated during summer months (Table 1). In the history of the study of thermal stress, the body temperature of cows, in addition to rectal temperature, measured in various ways: by measuring timpanic temperatures (Mader et al, 2002), infrared thermography (Stewart et al, 2007) or subcutaneous implantation telemeter connected to the software (Beatty, 2005). In most experiments confirmed diurnal rhythm of body temperature in dairy cows, and rectal temperature variations over 1°C can cause serious decrease in cows productivity. Results show a significant increase in body temperature and respiration rate in heat stress (Table 1). Episodic periods of heat stress may present the cow with greater challenges in the short-term because physiological homeothermic adaptations take weeks rather than days to occur (Collier et al., 2006). 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 ode to excess heat (Gaughan et al, 2000). In order to reduce thermogenesis in dairy cows leads to reduced food intake and increased consumption of water and consequently hemodilution

Evaporation requires increased volume of water circulating in the blood. All this leads to a decrease in the number erythrocytes (7,38:6,51, p <0.01) and decreased hematocrit (39.3:30.8, p <0.01), as well as decrease in the value of MCV (53.25:47.31, p> 0.05). Heat stress occurs nonsignificant decrease in concentration of hemoglobin (111.5:107, P> 0.05) (Graph 2). The number of white blood cells decreases significantly during the summer (9.18:7.75, p <0.01), but no significant change relative to certain elements of white cells (Graph 1). Number of platelets (425:351, p <0.05) also shows significant decrease. Related results were obtained (Beatty, 2005) in a strictly controlled experiment.

Numerous results indicate that heat stress leads to creation hemoconcentration (Koubkova et al, 2002; Toharmat et al, 1998), increase in hematocrit and the number of blood elements. These experiment was performed in the heat chambers or periparturient period. This can further activate the stress axis with temporary cortisol concentration increase, which increases diuresis in cattle (Parker et al, 2004). Significantly reduced frequency of rumen contraction (10.75:5.5, p <0.01) arises as a consequence of reduced food intake, altered pH in the rumen and changes in the endocrine profile (Silanikove, 1992). Changes in the characteristics of red blood cells, reduced hemoglobin concentration altered metabolism during heat stress may predispose cows to oxidative stress. Erythrocyte antioxidants are important indicators of oxidative stress in dairy cows (Bernabucci et al, 2002). Between hematology parameters and physical response (rectal temperature, respiration rate and rumen contraction) exist statistic significant correlation (no showed results).

#### CONCLUSION

Increase in rectal temperature and pronounced diurnal variations can be observed during the summer, a significant increase in the respiration per minute and decrease rumen contraction. Heat stress leads to certain hematological changes, such as: decrease in the number of erythrocytes, decrease in MCV, decrease in hematocrit, decrease of white blood cells and platelets. Hemoglobin concentration decrease, also. Hematological changes indicate overhydration signs, indicating active body cooling evaporation.

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# HEMATOLOŠKI PARAMETRI I FIZIČKI ODGOVOR MLEČNIH KRAVA NA TOPLOTNI STRES

## BRANISLAVA BELIĆ, MARKO R. CINCOVIĆ, DRAGICA STOJANOVIĆ, ZORANA KOVAČEVIĆ, STRAHINJA MEDIĆ, VLADAN SIMIĆ

#### Izvod

Toplotni stres kod mlečnih krava dešava se tokom jula i avgusta (THI>72). U ogledu je učestvovalo 10 krava u letnjoj i 10 krava u zimskoj sezoni. Fizički odgovor: Porast rektalne temperature i izraženije diurnalne varijacije se mogu zapaziti tokom leta (38.4:39, p<0.01), a signifikantan je i porast respiracija u minuti (46.5:65, p<0.01) i pad buražne kontrakcije(10.75:5.5, p<0.01). Hematologija: Tokom toplotnog stresa dolazi do određenih hematoloških izmena, a to su: pad broja eritrocita (7,38:6,51; p<0.01), pad MCV (53.25:47.31, p>0.05), pad hematokrita (39.3:30.8, p<0.01), pad broja leukocita (9.18:7.75, p<0.01) i trombocita (425:351, p<0.05). Opada i koncentracija hemoglobina (111.5:107, p>0.05). Hematološke izmene ukazuju na znakove hiperhidratacije, što je znak aktivnog rashlađivanja organizma evaporacijom. Postoji korelacija između fizičkog i hematološkog odgovora.

**Ključne reči**: toplotni stres, mlečne krave, hematološki parametri, rektalna temperatura, frekvenca disanja, frekvenca buražne kontrakcije.

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## METABOLIC PROFILE OF BLOOD AND MILK IN DAIRY COWS DURING HEAT STRESS

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SUMMARY: Hot weather, which is characteristic during summer in Vojvodina region, has adverse effects to performance and well being of dairy cows. Experimental model included 20 Holstein-friesian cows, 10 cows were observed during winter, and 10 cows during summer (stress period). The reduction in plasma glucose (2.97:3.58 mmol/L, p<0.001) and cholesterol (5.97:6.46 mmol/L, p<0.01) during the hot periods were significant. Total protein concentration shown significant increase (70.41:73.59 g/L, p<0.001). Milk fat (3.59:3.228, p>0,05), protein (3.305:3.004%, p<0.001) and dry mater (8,612:8,157%, p<0.001) decrease during hot period. Changes in blood and milk were in statistic significant correlation (except milk fat vs. blood cholesterol). Decrease of feed efficiency, and change in gluconeogenesis is the main reason for these results.

Key words: heat stress, dairy cow, blood parameters, milk composition.

#### **INTRODUCTION**

Hot weather, which is characteristic during summer in Vojvodina region, has adverse effects to performance and well being of dairy cows. Heat stress with THI (temperature-humidity index) over 72 induces many physiological and pathophysiological responses from organism. Behaviour adaptation to heat stress allude: decrease in dry matter consume, increase of water consume, increase rectal temperature and respiration rate, prolonged laying or standing, decrease sleep time, decrease sexual behaviour, change in blood and rumen pH, decrease in milk production, etc (Cincović i Belić, 2009, Kadzere et al, 2002; West et al, 2003; Silanikove, 1992). Cortisol, T3 and T4 (thyroxine

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and tri-iodothyronine) levels decrease to lower heat production, assist in acclimation. Heat stress increases energy maintenance requirements which leads to decreased feed efficiency (Collier and Zimbelman, 2007). Decrease of feed efficiency with homeostatic change in heat stress give rise to change in metabolic profile of blood and milk in dairy cows.

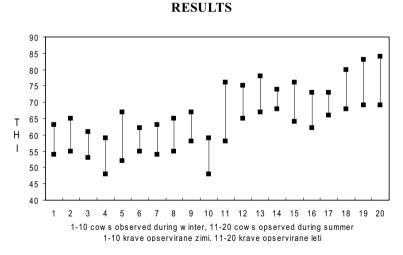
The aim of this paper was to research total protein, glucose and cholesterol concentration in blood and change in milk composition during heat stress.

#### MATERIAL AND METHODS

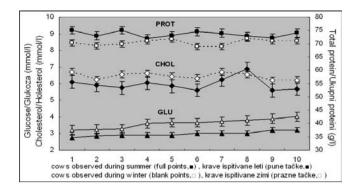
Experimental model included 10 Holstein-friesian cows, observed during winter, and again during summer (stress period). Ambiental temperature and relative humidity value obtained from regional meteorology station, and by direct measurement on the farm. THI index calculated by standard formulas (Ingraham et al, 1974).

Minimum restraint, jugular venipuncture, and blood collection were accomplished as rapidly as possible between 06:00 and 07:00 h before the morning milking. Blood samples from the same 10 cows, were collected from jugular vein-puncture into two evacuated tubes; one tube containing anticoagulant and the other tubes was plain for plasma and serum separation. Plasma samples were later analyzed for total protein (biuret reaction), cholesterol (enzymatic), and glucose (glucose-oxidase test), using special kits for each analysis. The following measures of blood serum were obtained directly from a Rayto RT-1904 C analyzer. Measures of milk ingredients were obtained from Milcoscan analyzer (Gerber). These methodology are spectrophotometric.

Statistic analyze imply t- and Z-tests and analyze of correlation, by Microsoft Office Excel program.

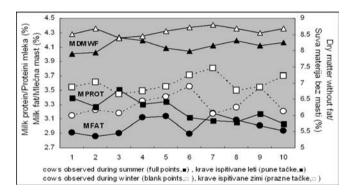


Graph 1. THI value in experimental day for each cow Grafik 1. Vrednost THI indeksa u danu uzorkovanja krvi i mleka za svaku kravu



Graph 2. Biochemical parameters of dairy cow's blood in thermal comfort and heat stress (summer)

Grafika 2. Biohemijski parametri krvi mlečnih krava u termoneutrolanoj zoni i toplotnom stresu (leto)



Graph 3. Milk parameters in thermal comfort and heat stress (summer) Grafik 3. Parametri mleka u termoneutrolanoj zoni i toplotnom stresu (leto)

Table 1. Correlation and statistic significance of dependence between blood and milk parameters *Tabela 1. Korelacija i statistički značaj zavisnosti promenljivih parametara krvi i mleka* 

|                              | Glucose<br>Glukoza | Total prot.<br>Ukupni prot | Cholesterol<br>Holesterol | Milk protein<br>Proteini ml. | Milk fat<br>Mlečna mast | DM in milk<br>Suva mat.ml |
|------------------------------|--------------------|----------------------------|---------------------------|------------------------------|-------------------------|---------------------------|
| Glucose<br>Glukoza           | 1                  | -0.63 **                   | 0.418 ns                  | 0.746**                      | 0.636**                 | 0.807**                   |
| Total prot.<br>Ukupni prot   | *                  | 1                          | -0.58**                   | -0.685**                     | -0.704**                | -0.772**                  |
| Cholesterol<br>Holesterol    | *                  | *                          | 1                         | 0.521*                       | 0.422 ns                | 0.574**                   |
| Milk protein<br>Proteini ml. | *                  | *                          | *                         | 1                            | 0.563 **                | 0.706**                   |
| Milk fat<br>Mlečna mast      | *                  | *                          | *                         | *                            | 1                       | 0.774**                   |
| DM in milk<br>Suva mat.ml    | *                  | *                          | *                         | *                            | *                       | 1                         |

ns - nonsignificant \*p<0.05 \* \*p<0.01

#### DISCUSSION

Heat stress with maximum THI over 72 was detected during summer (Graph 1.). The reduction in plasma glucose (2.97:3.58 mmol/L, p<0.001) and cholesterol (5.97:6.46 mmol/L, p<0.01) during the hot period were significant. Total protein concentration was significantly increased (70.41:73.59 g/L, p<0.001) (Graph 2.). These reduction during heat stress can be explained by reduce of feed efficiency, reduction of dry matter intake, increase of glucose utilisation in contraction of respiratory muscle, hemodilution, endocrine change etc (Kadzere et al, 2002). Lower values of glucose in cows in hot conditions were also observed by Ronchi et al. (1999), who found a reduction of liver activity, so that the decrease in plasma glucose concentration was explained by the negative effects of that reduction on gluconeogenesis. Many paper showed increase of urea concentration, because urea concentratio indirectly show utilization of aminoacids as energy source.

Decrease in milk quality was significant, except for milk fat (Graph 3). These change were in statistic significant correlation with change in blood (except milk fat vs.blood cholesterol) (Table 1). Decrease dry matter intake, change in rumen pH and change in production of milk precursor, decrease of blood flow thru udder, reduce permeability of mammary tight junctions in the lactating bovine mammary epithelium, change in potassium chanel and increase mastitis prevalence as reason for decrease in milk quality and quantity (Lough et al, 1990; Stelwagen et al, 1998, Silanikove et al, 2009). Milk production not only decreases as a result of HS but milk fat is also low-ered with a relative increase in long-chain fatty acids as a result of decreased circulating acetate (a volatile fatty acid) levels, resulting from decreased fiber intake. Cortisol and growth hormone may influence mammary metabolism, cortisol by altering glucose supply to the mammary epithelial cells while GH may alter the circulation of long-chain fatty acids to the udder (Thompson, 1985).

Cholesterol is important precursor in cortisol production, and was mentioned that cortisol concentration decrease during heat stress. Reist and colleagues (2003) proposed that cholesterol and glucose are closely related metabolites. Glucose has been shown to promote the uptake of cholesterol for steroidogenesis.

#### CONCLUSION

Basal glucose and cholesterol concetracion were lower during heat stress, but total protein concentration was higher. These phenomenon can be explained by change in feed efficiency and gluconeogenesis. Decrease in milk protein, fat and dry matter is in significant corelation with change in blood.

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# METABOLIČKI PROFIL KRVI I MLEKA KOD KRAVA U TOPLOTNOM STRESU

## MARKO R. CINCOVIĆ, BRANISLAVA BELIĆ, DRAGICA STOJANOVIĆ, ZORANA KOVAČEVIĆ, STRAHINJA MEDIĆ, VLADAN SIMIĆ

#### Izvod

Toplo vreme koje je karakteristično za region Vojvodine iam negativan efekat na produktivnost i dobrobit mlečnih krava. Eksperimentalni model je podrazumevao 20 krava Holštajn-frizijske rase, od čega je 10 krava observirano tokom zimskog, a 10 tokom letnjeg (stresnog) perioda. Redukcija koncentracije glukoze u plazmi (2.97:3.58 mmol/L, p<0.001) kao i holesterola (5.97:6.46 mmol/L, p<0.01) tokom toplog perioda statistički je značajna. Koncentracija ukupnih proteina u krvi signifikantno raste tokom toplotnog stresa (70.41:73.59 g/L, p<0.001). Procenat mlečne masti (3.59:3.228, p>0,05), proteini mleka (3.305:3.004%, p<0.001) i suva materija (8,612:8,157%, p<0.001) opadaju tokom toplog perioda. Parametri krvi i mleka su u statistički značajnoj korelaciji (osim mlečne masti i holesterola krvi). Smanjeni unos i iskorišćavanje hrane i izmenjena glukone ogeneza mogu biti razlog ovakvih promena tokom toplotnog stresa.

Ključne reči: toplotni stres, mlečne krave, parametri krvi, sastav mleka.

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## PROMEMORIA

## ACADEMICIAN SLAVKO BOROJEVIĆ (1919 - 1999) FAMOUS BREEDER

## JELENA BOŠKOVIĆ<sup>1</sup>

For his material and spiritual progress and transformation, humanity has to thank first of all science, scientific creativity and research. A special gift for creativity, is what determines telented man, not only a scientist, but also writer, artist, musician, even military leader and statesman. One of the most prominent scientists from the former Yugoslavia is the academicianian prof. Dr. Slavko Borojevic, which had a glorious life and continued to live past his physical death, on 19th September 1999 in Novi Sad.

Academician Borojevic was born on 21st November 1919, on Banija mountain, in Knezovljani village near Kostajnica, where the mornings were breaking quickly, lightened by the green light and as man's soul, each moment reflected happenings in the sky. In such place, person's growing is determined by gratitude to hills that are plowed and wheat that is planted, for fruit fall under the weight of the autumn rain and fog, and to human hand, that accepts them and brings joy to children in the winter. Landscapes, which create dark green fields, have become close and intimate in dreams and reality of this extraordinary man.

In such place, the work of academician Borojevic began, persistent, lasting and valuable. His educational path started in Petrinja, and he finished high school in Sisak, and then enrolled the Agricultural-Forestry University of Zagreb, where he studied until 1941, when the occupation started. He joined partizans, with his brother and other family and stays in NOP until the end of the war. The beauty of living, education, thoughts was broken with that war. He stayed with his people, listening to missiles from night to night, flying over rooftops of houses and foliage of trees. He watched uncomparable devastation of settlements every day.

Academician Borojevic was painfully surviving the horrors of war with his family, in time when two ways of life collided as two un-winning wills. The ruins of that battlefield were not dead for this thoughtful man, who felt it like the heatstroke with the power of destroying the soul and heart. He was aware of the will of the people, who knew the reason and the cost of its victory, as well as what to do with it. All his memories are described in the war diary, from which emerged the book. He comes out of the war as a senior officer, not wishing to continue the military profession, but choosing agriculture and continuing studies begun.

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He graduated in 1947, and because of its prominent interest in science in 1948 he was elected an assistant in Genetics on The Faculty of Agriculture, University of Zagreb, with prof. dr Alois Tavčar, the most influential scientist in the field of genetics of that period. At the same faculty he did his PhD thesis titled "Heterosis in rye produced by crossing local populations and cultivars" and successfully defended it in 1953, being elected as lecturer of Genetics in 1956. He was one of the first young scientists who received a scholarship from the Rockefeller foundation(1951-52) for wheat genetics and breeding in the USA. It was a period of intense development of new scientific approaches in genetics and breeding of organisms that were contrary to Lisenko's theory.

During his stay in the scientific institutions of the United States he met the most prominent scientists in the field of genetics and plant breeding, such as Barbara McClintock, 1983 Nobel Prize winner, for the detection of mobile genetic elements in 1958. It is important to mention Dr Milislav Demerec, who was also born in Kostajnica, Republic of Croatia, working in the United States since 1923 and was director of the Carnegy Genetic Institute in Cold Spring Harbor from 1943-1960, was one of the leading scientists in the field of genetics of corn, wine flies, bacteria and bacteriophage and other.

This period of academicians Borojevic's stay in the US was an important beginning of establishing scientific cooperation with famous scientists and launching of his initiatives for the development of scientific thoughts in genetics and plant breeding in his own country. His mindfulness, with no residue, was present in every moment of his work. He knew what a sharp sword was worth in the hands of that who can not deliver him from the cover. Prof. Borojevic learned the refraine of his life-long anthem: "Think, feel and work". He was embraced by the optimism and continuous speech: it is worthwhile to think, if there is anything worthy of the Olympic gods, it is thinking, said the ancient Greeks.

With the return to his country, some epochal discoveries happen in the research of the American biochemist James Dewey Watson (Watson JD) and the British physicist and molecular biologist Frances Crick who discovered the molecular structure of DNA as a double coil with cross-hydrogen bonds and determined the role of DNA in heredity in 1953. Maurice Wilkins (Wilkins IHF) confirmed the validity of Watson-Crick model the very same year, using X-rays. For this discovery, Watson, Crick and Wilkins received the Nobel Prize for Physiology and Medicine in 1962. This important study provided a great stimulus for the young scientist Borojevic in creating his own scientific directions.

Scientific pathway of academician Borojevic is exclusively linked to his exceptional and great personality, which corresponds to the time and situation. His scientific creativity, as well as what we learned from his contemporaries about him personally, leave us with no doubt that there is plenty of light and directions for everyones research, light that does not fade, and the road signs that do not deceive.

In the period between 1950-1960 a network of academician institutions in Yugoslavia was formed. In Novi Sad, prof. Lazar Stojkovic organized studies of agricultural sciences in Vojvodina and invites professor Slavko Borojevic, already well-known scientist from Zagreb to come to Novi Sad and take a leading position in the Department of genetics and plant breeding. After his arrival, he raised above most, intellectually and scientifically, publishing numerous scientific papers, which was extremely important for a newly formed educational institution, and in 1957 he was elected Associate professor of Genetics.

The power of scientific personality of academician Borojevic comes to full expres-

sion through the prolific published experimental work, which resulted in his appointment as full professor of Genetics. In those years, nothing happened in agronomic science if is hasn't originated from professor Borojevic. This was the time when he already had connections and scientific cooperation with the worldwide institutions, especially in the US.

Since the Italians were the pioneers in the wheat selection, prof. Borojevic went to Italy, a country of famous professor Nazareni Strampelia to achieve scientific cooperation and new cooperative programs in wheatselection. It will later have a huge impact on the development of scientific thought and great progress in the selection work, and also the practical application of scientific achievements. Science was in his blood, movement and life thoughts. It was a world with which he shared the day and night, year after year.

Among other scientists academician Borojevic occupied most hierarchical place with his intellect, results and work. He enjoyed working, constantly creating new results that were accepted by national and international scientific community and rewarded. He was the organizer of numerous conferences both in his country and abroad. He chaired numerous conferences, symposia, congresses, working groups, gave introductory talks, and that all contributed to his scientific reputation. His correspondence with prominent scientists from the world of genetics and plant breeding, reflect the continuous contacts.

Exchanging opinions, considerations of new scientific directions were complemented by his research opus. His letters with most interesting scientific content he read to his colleagues and students. He loved to hear and listen to other people's opinions and to take tham into account. After coming back from numerous world-wide meetings at which he participated, he used to organise discussions and meetings with other scientists in the field of genetics and breeding of organisms.

Academician Borojevic was able and willing to lecture the whole day and more, if necessary, to expose all the new results in the science of genetics and breeding, emphasizing world's dominant research where our can scientists could participate. Through numerous international projects, study stays, visits of scientists, he brought together scientists and students and taught everyone how to develop scientific thought and implement it in practice. His candid look spoke for him, even when he was quiet. It was a rare and unique man with impressive analytical thinking about each problem. He generously passed his research to all his colleagues and wished them success and enjoyment in that success.

Academician Slavko Borojevic created a selection of winter wheat varieties that have played important role in global efforts to promote the production of cereals. The greatest achievement lies in tha fact that his former country of Yugoslavia was transformed from an importer to an exporter of wheat. Academician Slavko Borojevic created 54 winter wheat cultivars insensitive to fotoperiodsm. In his program 120 varieties of winter wheat were created, having yield capacity over 10 t / ha. He achieved this by transferring Rth8 and PPD genes from the Italian mediterranean species in the continental variety, mostly in the US winter wheat cultivars.

For example, his winter wheat variety Sava was created from the intersection of the Italian variety Fortunato 2 and the American Red Coat. Sava was greatest yielding wheat variety in the world in international trials (nurseries) of wheat organized in 33 locations in the world by Dr. Virgil Johnson, USA, in the period between 1971-1973. It was recognized as a new variety of wheat in 1976 in Hungary, 1977 in Czechoslovakia, in 1978 in Romania and in 1980 in Austria. It was used in breeding programs worldwide.

Academician Borojevic's success in the selection of winter wheat varieties was the highlight of his contribution, as pointed out by many scientists in the world. The maxi-

mum yield of the Sava variety was 10t/ha in the area of 30 hectares in Galanta Sale in Slovakia (Spaldon, 1986). It was the maximum yield ever achieved in Central Europe.

Yugoslav high-yield cultivars Sava and other were used in crossings as carriers of Rht8 and Ppdl genes in Bulgaria. These varieties were bred with the varieties of Bezostaja type, and later with cultivars Aurora and the Caucasus. More than 70 varieties in Bulgaria were created and officially recognized in the period between 1969 and 1988. Most of these varieties possessed Rht8 and Ppdl genes. The average yield in that period increased more than three times according to Panayotova (1988 and 2000).

After introduction of academician Borojevic's winter wheat varieties, the average yield of wheat increased from 1.25 t/ha to 4.19 t/ha in 1988, in the former Yugoslavia. In Vojvodina, the yield of wheat increased from 1.65 to 5.61 t/ha. Former Yugoslavia as a country with population of about 22 million, has changed from an importer to significant exporter of wheat. Neighboring countries, like Hungary, Bulgaria and Romania have also become successful producers and partially even exporters of wheat.

Globally, academician Borojevic has created winter wheat varieties that are recognized and cultivated in many countries, and that have impact on the production of wheat in Europe. He created a gene bank of winter wheat, which is still successfully used in the selection by Institutes around the world. His theoretical concepts of new varieties have been successfully applied not only by wheat breeders, but also by breeders dealing with other plant species, such as soybean and other cultivated species in the country and in the world.

During his long career as a university professor, Slavko Borojevic took part in the education of about 3,500 agronomists. He was the advisor in 14 senior specialist studies, 32 master studies and 42 doctoral theses, including students from abroad even as far as Egypt, Bangladesh, Algeria and Pakistan.

With a special devotion he passed on his knowledge with pleasure, spending hours and hours working with students. Every year, he received more national and international visitors, ranking from students to top scientists in the field of genetics and plant breeding. Talking to them, he always discussed and considered all the possibilities for increasing wheat production by innovated wheat modeling. A truly wise man, like academician Borojevic was, never dismissed endless opportunities science can offer, bearing in mind its impact on modelling human life and social relations.

As a university professor, Slavko Borojevic rejected Lisenko's theory in genetics and selection both at the University and the Institute for the selection of Novi Sad. The 1968, 1971 and 1976 editions of his book, Genetics, Slavko and Katarina Borojevic, were used as textbook at 16 universities in the former Yugoslavia by 200.000 students, and had the greatest impact on the introduction of modern genetics in Serbia and Yugoslavia. Writing and openly criticizing Lisenko's theory in international scientific meetings he directly influenced mainstream genetic thought in Hungary, Bulgaria, Czechoslovakia, even in the former Soviet Union.

Slavko Borojevic is also the author of Principles and methods of breeding plants, Novi Sad, 1981 and 1992 editions. That book was used as a textbook in undergraduate and postgraduate studies at eight universities and agricultural faculties around the former Yugoslavia, as well as the manual for selection, by many scientists and experts.

Also, Serbo-Croatian editions of this book were soon translated into Russian - Kolos, Moscow in 1984 and used as textbooks at universities and many institutions of the former Soviet Union. Another translation of this book was in English by Elsevier Scientific Publishing Company, Amsterdam in 1990, and is still used throughout the world.

Academician Slavko Borojevic has contributed to a significant increase in food production, most notably with the creation of varieties tolrant to photoperiodism, high-yield winter wheat varieties. His contribution has directly or indirectly increased the wheat production from 235 to 300% in the regions of former Yugoslavia and neighboring countries in the period 1968-1990, which finally had a big impact on global production of wheat.

The overall results of the wheat selection in Novi Sad, led by academicianian Borojevic were known throughout the world. Many of his associates considered him the top breeder of winter wheat varieties in the world and the creator of "Yugoslav school of wheat breeding".

The scientific opus created by academician Borojevic has been great contribution at the national and international research level in several research units, among which the most important are: genetic studies of quantitative traits, theory of plant breeding, construction of models of high wheat varieties (which served as a model for other plant species), the creation of high wheat varieties for our agroecological conditions and exploitation of genetic potential for yield production. The result of his work was published in 140 scientific and professional papers, 30 of which in international journals. He also submitted 30 papers (most of which were invited) to the scientific and professional gatherings in the country and abroad. He lived by the words of Gorky: I know what work is: it is the source of all joy, all the most beautiful things in the world, uplifting of human mind and will, that creates and builds.

As a teacher and scholar he was shearing his enormous experience passing it on his protégés, thus leaving his ideas and knowledge to live and after life. His greatness is in developing love for agronomy in his students' minds without imposing - that humane job, providing the necessary food for the welfare and livelihood of the human population. He often addressed them with words: after work every minute of joy seems doubly joyful, soul plays with joy, eyes shine, merry thoughts smile, the man's face becomes more beautiful and younger.

Academician Borojevic, as dedicated scientist, university professor, was a complex personality, intellectual elite, a man full of the highest knowledge and broad education, full of the most valuable human traits and virtues, a man who was next to the knowledge of their profession, successfully followed all the other findings in the social and natural sciences and that was why it all affected the comprehensive development of youth, and through them the whole of the spiritual, economic and social status of society.

There are few professors at the university that poured their philosophical wisdom, scientific intuition, superior know-how and broad education with noble human qualities and virtues in a single unit for which they received numerous awards so appropriate so skillfully. Academician Borojevic was elected a foreign member of Federal Academy of Agricultural Sciences of the former USSR (1979) and the Slovak Academy in 1999. University of Agricultural Sciences in Gedeleu (Hungary) awarded him an honorary doctorate of science in 1977. He was elected regular member of the Vojvodina Academy of Sciences and Arts in 1979, a regular member of the SASA in 1991 and also an honorary member of the American agronomical society. He was an expert of the United Nations for plant breeding n Egypt (1969/70) and invited professor on the University of California (1979).

During his fruitful period, professor Borojevic held a number of scientific, professional and managing functions in this country and international institutions. He was director of the Department for wheat of the Institute for Agricultural Research in Novi Sad (1957-1975), chief editor of Contemporary Agriculture in Novi Sad (1958-1976), Dean of the Faculty of Agriculture in Novi Sad (1960-1962), president of Genetics Society of Yugoslavia (1973-1978), rector of the University of Novi Sad (1974-1976), president of the University Community of Yugoslavia (1974-1976), board member of the European Association of plant breeders (1971-1974), member of the Council of Representatives of the international genetic Congress (1973-1978) and the International Conference on winter wheat (1973-1975), secretary of the Department of Natural Sciences (1980-1984) and President of the Vojvodina Academy of Arts and Sciences (1984-1988), member of the editorial board of international journal Plant breeding, Berlin (1980-1994).

Academician Borojevic has received many military and peacetime medals and awards: Partisan memorials, 1941, Order of Partisan Star III (1945), Medal for bravery (1945), Order and brotherhoods (1946), Order of merit for the people (1948), and the Order of work (1961), the October Prize of the city of Novi Sad (1961 and 1971), award of SR Serbia (1967), Awards of liberation of Vojvodina (1974), Gold Medal Mihajlo Pupin (1974), AVNOJ awards (1975), the Order of the Republic with golden wreath (1982). Academician Slavko Borojevic was proposed by the Serbian Academy of Sciences Award for World Food Prize in 1996, which he unfortunately could not receive because of political samctions imposed on our country and it is not being awarded posthumously.

At The Centemario of Nazareno Staonpelli Green Revolution which was held in Rietiu in Italy from 12 to 14 June 2000, professor Borojevic was awarded the Prime premium, "Nazareno Strampelli 2000" as wheat breeder. The award was handed to prof. dr Katarina Borojevic, academician Borojevic's wife. Slavko Borojevic first, before all the Italians discovered Strampelli's ideas and genes for low plant in Italian varieties (later called the RHT genes which he passed in Novi Sad varieties and spread them all over the world).

Low varieties have enabled high production, and many countries turned from importers to exporters of wheat. Their achievements not only belong to the past and present, because they exist in the bread that feeds millions of people in the world. For such achievements the American Norman Bourlag won the Nobel Prize (1970), although it wahad been done much earlier and with less money in Europe (Bianka, 2000). We must emphasize some experts, among the leading scientists in genetics and selection, and special awards and recognitions received by professor Borojevic.

In his introductory paper titled Agricultural development and selection of wheat in the 21 century, presented at Fifth international wheat conference in Ankara in 1996, Warren Kronstad (USA), stated that Nazareno Strampelli was the first to apply Japanese cultivar Akakomughi for shortening the stem and increasing productivity per unit area.

He was followed by Orville Vogel from Washington State (USA) who created half short wheat cultivar Gaines, which is much used, together with several Japanese varieties of Norman Borlaug at CIMMYT program. This is the famous Green Revolution in the selection half dwarf and dwarf varieties of wheat insensitive to photoperiods. As the third person to contribute, Kronstad points out Slavko Borojevic and his wheat selection school. In this programme some Italian early-stemmed varieties took part, which was a great success in the creation of high species that contributed to the progress of the great selection of winter wheat varieties in the world.

Professor Borojevic is one of top wheat breeders in Europe and the world. He spent many years in production most yielding wheat varieties worldwide (JP Gustafson,

Geneticits Research and Professor of Genetics, University of Missouri, Columbia, MO 65,311, 1994). Professor Borojevic had an international reputation and a special status in the research of improving wheat production. His research has significantly contributed in clarifying the role of genetics and physiology of wheat for the achievement of high yields. (VA Johnson, Professor Emeritus, University of Nebraska, 1994).

Professor Slavko Borojevic is one of the most prominent academicians and research leader in Europe. (Kenneth J. Frey, Cf Curtiss Distinguished Professor of Agriculture, Emeritus, Iowa State University, Ames, Iowa, 1994). Professor Borojevic was one of real giants in the field of wheat selection and genetics. His pioneering contribution to the development of new wheat varieties served as a stable base for modern wheat selection. Slavko Borojevic was so strong and positive in his induvidual influence as he was as scientific worker.

Publishing Council of Biographic lexicon (Belgrade 2001) chose Slavko Borojevic as one of the 100 greatest Serbs in the world.

Professor Borojevic has occupied not only important, but a distinguished place in all domains of his life. His name is a symbol of new principles in agronomic science and education as well as a symbol of timeless values. He was initiator and creator of the preliminary organisation of scientific work on genetics and breeding of wheat in our country, permanent innovation of curricula, the founder and head of graduate studies and specialist studies at the faculty in Novi Sad, the forming of Genetics Society of Yugoslavia, developing and proposing research multidisciplinary projects VANU and another. He possesed leading skills, undisputed authority, pedagogical ability and influence on the environment. Only such charisma and reputation could have directed his vast scientific and pedagogical work into the energy that is not stopped, and his results were extraordinary.

His talent, not only for science but enabling development of others, contributed to all his other work, pedagogical, scientific and professional becoming noticed. The man that was loved and appreciated by all, as it adorned the most beautiful human qualities, thoughtfullness, tolerance, humanity while being the most honest colleague and friend, left a trail forever in the hearts of his numerous students, associates, colleagues, friends and acquaintances and all those whom he benefacted.

Professor Borojevic, a bard amongst world's scientists with his lavish and magnificent and most beautiful human characteristics, will be a model for future generations of scientists, agronomists who will save memory on tireless creator of better future. So, his genetics remain fundamental for life and doctrine closely related to human survival and hope in life.

It was a pleasure learning from him.

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# INFECTIVE AND NON-INFECTIVE ETIOLOGY OF SOW INFERTILITY

## BLAGOJE STANČIĆ<sup>1</sup>, MLADEN GAGRČIN<sup>2</sup>, JELENA STANČIĆ<sup>2</sup>, OGNJEN STEVANČEVIĆ<sup>2</sup>, ALEKSANDAR POTKONJAK<sup>2</sup>

SUMMARY: High reproductive efficiency of sows is the primary factor of successful pig production. 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. In production conditions it is acceptable if the weaning-to-estrus interval is within 7 days, if the farrowing rate is higher than 80% and if the average number of live-born pigs per litter is higher than 10. These values, however, can be frequently reduced, influenced by a number of factors of infective and non-infective etiology. This phenomenon is defined as infertility in sows. This paper presents the basic infective and non-infective factors of infertility in sows, their mechanisms, as well as the possibilities of practical solutions of this significant problem in pig reproduction.

Key words: infertility, infective and non-infective factors, sow.

## **INTRODUCTION**

The reproductive efficiency of breeding herds in industrial pig production is directly dependent on sow fertility parameters. The most significant parameters 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). The value of the above stated parameters of sow fertility can be substantially reduced under the influence of a number of factors of infective and non-infective etiology (Vanroose et al., 2000). Significant reduction of fertility is evident during the warm summer months. This phenom-

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enon is known as "seasonal infertility in pigs".

Infective agents (bacterial and viral infections), almost always, result in substantial reduction of reproductive efficiency of breeding herds, as they cause serious reproductive disorders, which are especially reflected in the increased level of intrauterine mortality, a higher number of stillborn and mummified pigs, the increased number of abortions, as well irregular rebreeding (Hogg and Levis, 1989; Floss and Tubbs, 1993). The disorders that are especially significant are caused by intrauterine infections, caused by porcine parvovirus (PPV), porcine **reproductive** and **respiratory syndrome virus (PRRS)**, and Aujeszky's disease virus (ADV) (Straub, 1994; Gagrčin, 2003), as well as bacterial infections, such as brucellosis, leptospirosis, actinobacillosis, infections caused by E. colli, and so on (Yeske, 2007).

Non-infectious causes of reduced fertility are numerous while the basic causes are nutrition, housing, insemination technologies, stamina, and general health status of breeding sows (Stančić, 2005). 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 present some of the recent findings regarding the phenomenon of seasonal infertility in sows.

## INFECTIVE FACTORS AND SOW FERTILITY

Infective factors (bacterial and viral etologies) cause serious reproductive disorders, which are especially reflected in the increase of the level of intrauterine mortality, a higher number of stillborn and mummified pigs, the increase of the number of abortions, as well as the number of irregular rebreeding (Hogg and Levis, 1989; Floss and Tubbs, 1993). Some of the infections are systemic, while others intrauterine, affecting the very embryos and/or fetus, i.e. placenta and endometrium (Christianson, 1992).

The extreme damages in reproduction are causes by intrauterine infections of porcine parvovirus (PPV), porcine **reproductive** and **respiratory syndrome virus** (**PRRS**), Aujeszky's disease virus (ADV), and Classical swine fever virus (Christianson et al., 1993; Goyal, 1993; Straub, 1994; Vannier et al., 1997; Gagrčin, 2003, Moenning et al., 2003). Regarding the infections of bacterial etiology, significant reproductive disorders are caused by brucellosis, leptospirosis, actinobacillosis, infections caused by E. colli, Chlamydia, Mycoplasma and so on (Mauch and Bilkei, 2004; Laureckiene et al., 2006; Yeske, 2007; De Busser et al., 2008).

Infective agents considerably decrease the fertility in sows, since they influence the disorders of almost all reproductive functions, especially re-establishment of estrus after weaning (extended post-lactation anestrus), the normal course of gestation (rebreeding, abortions, pseudopregnancy), as well as stillborn, mummified and avital pigs. Therefore, Gagrčin et al. (2005) claim that viral (PPV, PRRS, PCV-2) and bacterial (leptospirosis) infections are the reason for the significant increase of mortality in all pigs categories, for the decrease of live-born pigs, increase of stillborn pigs, as well as lowering of the farrowing index. The significant infective causes of infertility and basic clinic symptoms are presented in Table 1.

Table 1. Infective causes of sows infertility Tabela 1. Infektivni uzročnici infertiliteta krmača

| Cause / Uzročnik<br>(Varoose et al., 2000)   | Clinical symptoms / Klinički sipmtomi   |  |  |  |
|--|---|--|--|--|
| Bacterialcauses/Bakterijskiuzročnici   |   |  |  |  |
| Brucella suis  | Abortion in any phase of gestation / <i>Abortus u bilo kojoj fazi gestacije</i> .   |  |  |  |
| Erysipelotrix rhusiopethiae  | Abortion, rebreeding / Abortus, povađanje.  |  |  |  |
| Leptospira Pomona  | Abortion or avital and/or stillborn piglets / Abortus, rađanje avitaln<br>i/ili mrtve prasadi.                                      |  |  |  |
| Streptococcus suis   | Rebreeding, purulent vaginal discharge / Povađanje, gnojni vagina iscedak.  |  |  |  |
| Chlamydia sp.  | Endomatristis, rebreeding, purulent vaginal discharge / Endometritis, povađanje, gnojni iscedak.                                    |  |  |  |
| Actinobacillus   | Vaginitis, endometritis, rebreeding / Vaginitis, endometritis, povađanje.   |  |  |  |
| Mycoplasma suis  | Anemia, piglets mortality, rebreeding / Anemija, mortalitet prasadi, povađanje.   |  |  |  |
| V  | iralcauses/Virusniuzročnici   |  |  |  |
| Svinjski parvovirus (PPV) Mummyficed and/or stillborn piglets / Mumifikovana i/ili mrtva prasad, infertilitet. |   |  |  |  |
| Virus Aujeszky-eve bolesti<br>(ADV)  | Neural disorders, high piglet mortality, abortus / Nervni poremećaji, visok mortalitet prasadi, abortus.                            |  |  |  |
| Svinjski resp. i reprod.<br>sindrom (PRRS)   | Abortion, mummys, stillborn piglets, infertility, pneumonia /<br>Abortus, mumije, mrtvo rođena prasad, infertilitet, pneumonija.    |  |  |  |
| Klasična svinjska kuga   | Stillborn and/or mummyficed piglets, piglets tremor, abortus / Mrt<br>rođena i/ili mumifikovana prasad, tremorom prasadi, abortusi. |  |  |  |
| Afrička svinjska kuga  | Stillborn and avital piglets, abortion / Mrtvo rođena i avitalna prasad, abortus.   |  |  |  |
| Svinjski cirkovirus  | Low fertility / Nizak fertilitet.   |  |  |  |

## NON-INFECTIVE FACTORS AND FERTILITY IN SOWS

Non-infective factors which affect the values of sow fertility parameters can be genetic and paragenetic. The most significant genetic factors are breed, line, crossbreeding, and the level of inbreeding, while paragenetic factors are nutrition, housing, parity structure of breeding herds, climatic conditions (ambient temperature and daily pwarmoperiod duration), application of hormonal preparations and general health (Tomes et al. 1982; Stančić, 2005). Therefore, the reproductive performance of breeding herds is significantly dependent on all of these factors separately, as well as their interactions. Thus, it is important to find out the basic parameters for measuring reproductive performance of breeding herds, as well as technological values of these parameters which provide acceptable reproductive efficiency of breeding herds both from zoo-technological and economic point of view.

## **REPRODUCTIVE PERFORMANCE OF BREEDING HERDS**

Reproductive efficiency of breeding herds can be measured by natural and economic indicators. The basic natural indicator of pig reproductive efficiency is the number of weaned pigs per sow on a yearly basis. This parameter depends on a number of genetic and paragenetic factors, and it varies within a wide range, from 10 to 22 (Stančić, 2005). However, the fertility of sows in a breeding herd is a complex parameter, including the values of numerous reproductive indicators, as presented in Table 2. Namely, in contemporary intensive agriculture, there are technologically desirable values of certain reproductive parameters, which indicate the level of natural and economic reproductive efficiency. In case the values of all or individual reproduction parameters are not within the borderline values, it can be concluded that there is a serious disorder in reproduction in a breeding herd (Wrathall, 1982).

| Parametar  | Standardne<br>vrednosti /<br>Standard<br>values | Granične<br>vrednosti /<br>Borderline<br>values |
|--|---|---|
| Gilts age at fertile insemination / Starost nazimica kod fertilnog osemenjavanja   | 220-240 dana                                    | > 240 dana                                      |
| Weaning-to-estrus interval / Interval zalučenje-estrus                             | ≤ 7 dana  | $\geq 10$ dana                                  |
| Regularan return / Regularno povađanje (18 – 24 i 36 - 48 days/dana)               | 10%   | > 20%   |
| Iregular return / <i>Neregularna povađanja</i> (25 – 35 i ≥ 49 days/ <i>dana</i> ) | 3%  | > 6%  |
| Abortions / Abortusi   | 1%  | > 2,5%  |
| Pseudopregnancy / Paragravidnost   | 1%  | > 2%  |
| Farrowing rate / Vrednost prašenja   | 85%   | ≤ 80%   |
| Live born piglets per litter (gilts) Živorođene prasadi po leglu (nazimice)        | 9.5 - 10.5                                      | < 9.5   |
| Live born piglets per litter (sows) Živorođene prasadi po leglu (krmače)           | 10.5 - 12.0                                     | < 10.5  |
| Stillborn piglets per litter / Mrtvorođene prasadi po leglu                        | 5%  | > 7.5%  |
| Mummyficed piglets per litter / Mumifikovane prasadi po leglu                      | 1.5%  | > 3.0%  |
| Litters with $\leq 8$ piglets / Legala sa $\leq 8$ prasadi                         | 12%   | > 25%   |
| Weaned piglets per litter (gilts) / Zalučeno prasadi po leglu (nazimice)           | 8.5 - 9.5                                       | < 8.5   |
| Weaned piglets per litter (sows) / Zalučeno prasadi po leglu (krmače)              | 9.5 - 11.0                                      | < 9.0   |
| Farrowing index / Indeks prašenja  | 2.0 - 2.4                                       | < 2.0   |

Table 2. Parameters of normal herd reproductive performance (Wrathall, 1982)Tabela 2. Parametri normalne reproduktivne performanse zapata (Wrathall, 1982)

## 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 (Almond and Bilkei, 2005) on a great number of breeding herds during a number of years also indicate significant decrease of fertility parameters in the warm season (Table 3).

| Fertility parameters / Parametri fertiliteta                           | Season / Sezona |              |  |
|--|-----------------|--------------|--|
| Fertifity parameters / Parameter jertifield                            | Cold / ladna    | Warm / Topla |  |
| Weaning-to-estrus interval (days) / Interval zalučenje – estrus (dani) | 5.9             | 7.8          |  |
| Farrowing rate (%) / Vrednost prašenja (%)                             | 91.1            | 78.1         |  |
| Regular returns (%) / Regularna povađanja (%)1                         | 3.1             | 4.5          |  |
| Iregular returns (%) / Neregularna povađanja (%)2                      | 21.1            | 42.2         |  |
| Live born piglets per litter (n) / Živo rođene prasadi po leglu (n)    | 9.9             | 7.8          |  |

 Table 3. Sows fertility parameters in cold and warm season

 Tabela 3. Parametri fertiliteta krmača u hladnoj i toploj sezoni

<sup>1</sup> In intervals / *u* intervalima:  $\leq$  17, 18-24 i 36-48 days after insemination / *dana posle osemenjavanja*.

<sup>2</sup> In intervals / u intervalima: 25-35  $i \ge 48$  days after insemination / dana posle osemenjavanja.

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 stated authors, as well as the results of certain other authors, analysed by Stančić et al. (2002) in a review paper, it can be concluded

that the seasonal infertility is manifested most in considerably extended weaning-toestrus interval, decrease of farrowing rate (%), i.e. achieving high conception rate, as well as lowering of the number of vital live-born pigs per litter.

**Extended weaning-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 farrowing rate of sows (% of farrowed sows out of the number of inseminated ones), 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 yellow body of pregnancy (Wrathall et al., 1986). Namely, recent research indicates that high ambient 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 (LTH) from hypophysis, which is necessary for enhancement of secretory activity of yellow body of pregnancy after 16<sup>th</sup> 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 very yellow body 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 in farrowing are rather contradictory. This can be attributed to the fact that, on one hand, litter size is influenced by the interaction of a number of 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 pigs 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 mortality of pigs during lactation period is increased, as well, which also significantly decreases the total number of produced pigs 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 in 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 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 contact with a boar.
- Control the quality of all ejaculated semen of boars, and make a smaller number of inseminating doses, with the increasing number of spermatozoids with progressive motility.
- Apply the third insemination with 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 value.

## DISCUSSION

Infertility or lowered fertility in pigs can, basically, be of infective or non-infective etiology. Infectious factors, bacterial or viral etiology, most frequently affect uterus or conceptus (fetus and /or fetal membrane), which results in embryo or fetus mortality, and consequently, abortion, irregular rebreeding, dead-born, avital or mummified pigs (Christianson, 1992; Hogg and Levis, 1989; Floss and Tubbs, 1993; Varoose et al., 2000; Gagrčin, 2003).

Non-infectious factors which cause pig infertility are numerous and can be genetic or paragenetic. The most important paragenetic factors are nutrition, housing, parity structure of breeding herds, climatic conditions (ambient temperature and daily photoperiod duration), application of hormonal preparations and general health (Tomes et al., 1982; Stančić, 2005). Unfavourable effects of these factors are reflected in significant reduction of sow fertility during the warm period of the year. This phenomenon is known as "summer or seasonal infertility syndrome" (Love, 1978; Rozeboom et al., 2000).

The lowered fertility during summer casues considerable zoo-technological and sanitary-veterinary problems, as well as economic losses in the intensive pig production. Therefore, the aim of this paper is to: (a) present in more detail the findings pertinent to the phenomenon of summer infertility in sows, on the basis of the results of other authors, and (b) according to the results of own research, indicate the effect of warm and cold seasons of the year on the basic parameters of sow fertility in our conditions of intensive pig production.

Although the domestic pig breeds are reproductively active during the whole year (manifesting ovulation 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; Stan-čić et al., 2002). Namely, substantially lower values of the stated 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) i 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 (so-called irregular rebreeding). Our research 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 yellow body of pregnancy (Wrathall et al., 1986). Namely, recent research indicate that high ambient 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 (LTH) from hypophysis, which is necessary for enhancement of secretory activity of yellow body of pregnancy after 16th 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 bigger decrease in gilts than in sows.

The phenomenon of the seasonal infertility in sows 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 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 cycle manifestation (Mauget, 1982).

Although all the factors of seasonal infertility, as well as the mechanisms of their physiological effects are known, 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) The infective agents, especially of bacterial and viral etiology, pathologically affect uterus and/or conceptus, which results in embryo and fetus mortality, rebreeding, abortion, stillborn, avital and mummified pigs.
- 2) 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 farrow-

ing rate (%), significantly increased number of sows with irregular rebreeding (embryo mortality), and abortion, substantially higher number of stillborn avital and mummified pigs per litter.

- 3) Negative effects of the warm period of the year are more evident in gilts than in sows.
- 4) 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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# INFEKTIVNA I NEINFEKTIVNA ETIOLOGIJA INFETILITETA KRMAČA

## BLAGOJE STANČIĆ, MLADEN GAGRČIN, JELENA STANČIĆ, OGNJEN STEVANČEVIĆ, ALEKSANDAR POTKONJAK

## Izvod

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Ključne reči: infertilitet, infektivni, neinfektivni, faktori, krmača.

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# THE IMPORTANCE OF SOIL ORGANIC CARBON FOR SOIL, AGRICULTURE AND GLOBAL CLIMATE CHANGE

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SUMMARY: Due to the facts that soil is the largest terrestrial carbon pool (2,500 Gt) and that soil organic carbon (SOC) has a strong impact on climate change, an increasing interest and new approaches to SOC pools and dynamics have been noted in recent years. Many new SOC-related investigations and analytical methods have appeared. These investigations are predominantly focused on carbon sequestration aspects, climate change mitigation, effects of land use change and SOC pools analyzing. Current practices which lead to SOC concentration and SOC stock decrease are unpopular because of their negative effect on global warming and soil degradation. On the other hand, carbon sequestration options cited in this paper are good practical, environmentally-friendly experiences which help to conserve the present and increase the future SOC levels.

*Key words:* Soil organic carbon, SOC, SOC pools, carbon sequestration, land use, soil type.

## **INTRODUCTION**

According to Volk (2008), the largest carbon (C) pools on the Earth are the oceans holding approximately 43,000 gigatons (Gt) of C, while the atmosphere holds 800 Gt and land plants 600 Gt. Excluding carbonate rocks, soils represent the largest terrestrial pool of carbon. Soil organic carbon (SOC) plays a key role in terrestrial eco-systems (Zhang et al., 2008). The global soil carbon pool of 2,500 Gt includes about 1,550 Gt of SOC and 950 Gt of soil inorganic carbon (SIC) (Lal, 2004). In the last two decades one of the central issues of soil science investigations is SOC, carbon associated with soil

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organic matter. There are a few reasons for this fact: firstly, global warming is predominantly induced by  $CO_2$  emission, which is rising with SOC decomposition process; secondly, SOC improves the physical, chemical and biological properties of the soil, helps stabilize soil particles, thus decreasing erosion, improves soil structure and workability, enhances aeration and water infiltration, increases water-holding capacity, and stores and supplies nutrients for growth of both plants and soil micro-organisms; and finally, SOC has an important role in increasingly spreading sustainable agricultural systems, such as organic farming.

#### SOC POOLS AND ANALYTICAL APPROACHES

Soil organic C is difficult to study because it is a complex mixture of substances (multiple C pools) having turnover rates that range from days to thousands of years. Soil organic C is the single largest component of soil organic matter, usually about 58%.

We can divide SOC at least in three pools: labile, intermediate and stable.

Labile (active, light) pool is a more recent product of dead plant and animal organisms, which is used as food for microorganisms. This is easily decomposable a small SOC pool (5%). Light fraction is commonly refers to a plant-like and less stable fraction with high C concentration (Golchin et al., 1994).

Intermediate (slow) pool with turnover times that range from less than 10 to more than 100 years is a large SOC pool (60–85%), made up of fractions with different turnover times.

Stable (passive, heavy) pool occurs as a product of long-term chemical transformations and represents chemically very complex matter, persistent to microbial decomposition. It is a stabilized or physically protected material that has a mean residence time of thousands of years, participating in SOC with 10–40%. Heavy fraction is a more stable and high-density organo-mineral fraction having lower C concentrations (Golchin et al., 1995). One of the most important functions of a stable fraction is the contribution in cation exchange capacity, enhancement of soil aggregation and the prevention of soil leaching.

An important prerequisite for analyzing SOC is removing inorganic carbon (IC) if it is present. The most common method for its removal is sample treatment with HCl or  $H_2SO_4$ . Using HCl can cause organic C compounds loss thus a better alternative is to remove IC using  $H_2SO_4$  in combination with FeSO<sub>4</sub>. According to Schumacher (2002), estimating the total organic carbon (TOC) content can be performed by semi-quantitative (loss-on-ignition (LOI),  $H_2O_2$  digestion), or quantitative methods (wet oxidation followed by titration with Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> 6H<sub>2</sub>O or photometric determination of Cr<sup>3+</sup> wet oxidation followed by the collection and measurement of evolved CO<sub>2</sub>; and dry combustion at high temperatures in a furnace with the collection and detection of evolved CO<sub>2</sub>). *Wet chemistry* methods (determination of SOC by oxidation with a mixture of  $\kappa_2 cr_2 o$ , and  $H_2SO_4$ ) are widely used, particularly in Russia and East European countries (Slepetiene et al., 2008).

Qualitative analysis of SOC is more difficult to perform. The well-known classic methods of SOC fractionating into humic and fulvic acid, and humin are not meaningful for the description of soil organic matter. Much more rational methods, which have been devised in last decades, separate SOC fractions by their resistance to chemical decomposition. These methods are more commonly used then the old ones and they divide SOC into the pools (e.g. labile, intermediate and stable). But no standard method has been established to physically separate SOC pools. That is the reason why SOC is still poorly understood. For the structural characterization of SOC fractions we can find methods in literature based on nuclear magnetic resonance (NMR) spectroscopy and methods based on diffuse reflectance infrared fourier transform (DRIFT) spectroscopy (Schumacher, 2002). Oxidizable SOC fraction predicted by mid and near DRIFT spectroscopy are determined in combination with partial least squares (PLS) regression method.

Many alternative methods for SOC fractionating have also appeared recently. One of the most interesting methods for fractionating SOC is the modified Walkley-Black method described by Chan et al. (2001). This method separates different C fractions by their oxidizability using varying concentrations of  $H_2SO_4$ . Blair et al. (1995) describe a method for determining labile SOC fraction based on susceptibility to neutral 333 mM KMnO<sub>4</sub> oxidation. Non-labile SOC is taken to be the difference between total and labile SOC. This method was simplified by Weil et al. (2003). Sohi et al. (2001) cite alternative physical fractionation procedures for separating SOC into free, intra-aggregate and organomineral fraction.

## THE AMOUNT OF ORGANIC CARBON IN SOILS

The amount of SOC is naturally balanced and determined by pedogenetic factors: climate, parent material, topography and vegetation. Different soils contain wide ranges of SOC levels. For instance, SOC ranges from about 10% in humid regions to less than 0.5% in arid climates. Peatlands can contain up to 95% of SOC. Soil organic C stocks vary naturally among different soil types (Table 1). In soils of Vojvodina (Serbia), in the 0-100 cm soil layer, the largest SOC stocks were found in regosols (204 t C ha-1), vertisols (171 t C ha<sup>-1</sup>), gleysols (165 t C ha<sup>-1</sup>), and chernozems (165 t C ha<sup>-1</sup>) and the lowest in solonchaks (87 t C ha<sup>-1</sup>), fluvisols (93 t C ha<sup>-1</sup>), and arenosols (99 t C ha<sup>-1</sup>) (Belić et al., unpublished). Hagedorn et al. (2001) reported that in forest soils, C dynamics were most affected by soil type. According to Robert et al. (2001), there is a lot of SOC variation depending on soil type. Parent material mostly affects SOC content by their texture. Clay offers chemical protection to organic matter through adsorption onto clay surfaces, which again prevents organic matter from being decomposed by bacteria. Soils with high clay content therefore tend to have higher SOC than sandy soils. Total SOC content increased with precipitation and clay content and decreased with temperature and the importance of these controls varied with depth, climate dominating in shallow layers and clay content dominating in deeper layers, possibly due to increasing percentages of slowly cycling SOC fractions at depth (Jobbágy and Jackson, 2000).

The naturally balanced harmony of soil was disturbed when anthropogenic factor started changing land appearing as a new pedogenetic factor which can rapidly affect soil properties. Human activities as land use, changes of farming systems, land management have a great impact on SOC decline and can sometimes result in soil degradation. Most of degraded soils have lost a significant part of the original SOC reserves (Manojlović and Aćin, 2007). Blum (2008) cites SOC losses as one of the eight major threats to the soil according to the *Thematic Strategy for Soil Protection*. There are many factors responsible for the decline in SOC. Jones et al. (2004) indicated some of them: conversion of grassland, forests and natural vegetation to arable land, deep

ploughing, overgrazing with high stocking rates, soil erosion by water and wind, leaching and forest fires. Changes in total SOC with change in land use and management can partly be explained by the way C is allocated in different fractions of soil organic matter (SOM). Mass distributions of different SOC fractions are influenced by land use and management (Tan et al., 2007). The investigation of SOC values in soils of the Golija mountain under different land uses demonstrates that land use system and altitude are important factors affecting SOC. The results show the highest SOC stock under forest, lowest under grass; decreasing trend in SOC from higher to lower altitudes; and the lowest cumulative soil respiration under forest and highest under grass (Manojlović et al., unpublished).

However, SOC concentration and SOC stock can be preserved and/or increased by proper practices (conservation tillage, application of farmyard manure, ploughing down harvest residues with mineral fertilizers and in some cases crop rotation) (Manojlović et al., 2008). Bot and Benites (2005) say that traditional mould-board plough and disc-tillage cropping systems tend to cause rapid decomposition of soil organic matter. Cuvardic et al. (2004), indicate that for Nordic conditions crop rotations and different fer-tilization systems have small impact on SOC changes (from 3.8 to 3.7%), although the tendency towards declining SOC content over years has been pronounced with cereal rotations. Belić (1999) stated that ameliorative practice mainly improves qualitative and quantitative SOC characteristics of non-productive soils such as solonetz. The addition of organic waste material (sugar beet leaves, tops and taproots) increased the average organic matter content of the solonetz topsoil significantly from 2.95% in the control treatment to 7.19% (Gajić et al., 2009).

Although there is a limit on the amount of organic C that can be stored in soils, large losses in the past mean that many agricultural soils have the potential for large increases. Based on their potential for increasing SOC stocks, we can divide soils into three categories; those with a high, medium or low potential. Soils formed mostly on loess, such as chernozems, which are characterized by a powerful solum and loamy mechanical composition, have a high potential for an increase of SOC content through a change of land use and tillage. According to local authors (Miljković, 1996, cit. Nejgebauer), the SOC content of South Pannonian chernozem is 2.5-3.5%, while the same content of the rare bands of unploughed chernozems under steppe vegetation is 5%, which indicates that the original SOC content of this soil may have been twice as high (4.5-7%). According to Ćirić (2008), Vojvodinian chernozem on loess terrace contains 1.5-1.9% of SOC in the surface layer exposed to continuous ploughing. Škorić (1986) noted that the natural grassy steppe vegetation had been ploughed up for the past 150 years and that there had been no biological accumulation as in the years before. Soils formed on alluvial sediment have a medium potential for increasing their SOC reserves, and this potential varies greatly according to the nature of the sediment, i.e. the clay content. Soils formed on eolian sand have a low potential for SOC reserves increase because of their sandy mechanical composition, good aeration, and a humus-accumulating layer that is maintained in the initial stages of formation under the influence of eolian erosion. Approximately 85% of agricultural soils in Vojvodina is affected by wind erosion with an annual loss of over 0.9 ton material per hectare (Vidojević and Manojlović, 2007). Land use is a good predictor of SOC content, but it usually masks the effect of soil type on SOC because soil type mostly determines land use.

Monitoring SOC change over time is fundamental in efforts to increase SOC lev-

els and provides a good measure of the impact of land management on soil health. It is very important to establish baseline levels of SOC and observe trends in these in response to changes in land management practices and provide data for C accounting. At a regional level SOC monitoring has to be performed over a minimum period of 5-10 years, because SOC cannot be rapidly changed in few years. Nesić et al. (2008) recorded a 0.68% decrease in humus content in soils of Srem in the 1992-2006 monitoring period. In the same monitoring period a 0.24% increase in humus content of in South Bačka region (Nešić et al., 2009) was observed.

If we look at the vertical distribution of SOC, we can conclude that the surface layer usually has the highest level of SOC which decreases with the depth of the soil profile (Table 1). In Maryland and Iowa fields Ritchie et al. (2007) found highest SOC concentrations in the surface layers which decreased slowly through the tilled layer with greater decreases with depth below this tilled layer.

Table 1. Soil organic carbon stocks in the most common WRB soil types in Vojvodina (Source: Belić et al., unpublished)

Tabela 1. Rezerve organskog ugljenika zemljišta u najzastupljenijim WRB tipovima zemljišta u Vojvodini

| WRB soil type<br>Tip zemljišta po WRB | No. of<br>samples <i>Broj</i><br>uzoraka | Area<br>Površina<br>(ha) | SOC stocks ± standard deviation<br>Rezerve SOC ± standardna devijacija<br>(t C ha-1) |         |          |
|---------------------------------------|--|--------------------------|--|---------|----------|
|                                       |  |                          | 0-30 cm  | 0-60 cm | 0-100 cm |
| Arenosols / Arenosoli                 | 92                                       | 10 510                   | 41±18  | 70±17   | 99±21    |
| Chernozems / Černozemi                | 191                                      | 1 304 416                | 72±19  | 126±21  | 165±24   |
| Cambisols / Gajnjače                  | 3  | 56 164                   | 52±36  | 93±14   | 132±5    |
| Fluvisols / Fluvisoli                 | 17                                       | 194 522                  | 46±14  | 71±10   | 93±19    |
| Gleysols / Ritske crnice              | 28                                       | 364 115                  | 75±26  | 124±25  | 165±39   |
| Planosols / Pseudogleji               | 12                                       | 116 424                  | 59±17  | 91±10   | 118±10   |
| Regosols / Regosoli                   | 5  | 3 806                    | 71±49  | 129±63  | 204±82   |
| Solonchaks / Solončaci                | 9  | 19 865                   | 42±17  | 66±11   | 87±10    |
| Solonetzs / Solonjeci                 | 29                                       | 80 333                   | 61±18  | 91±10   | 113±12   |
| Vertisols / Smonice                   | 14                                       | 36 139                   | 74±28  | 129±22  | 171±20   |

### IMPORTANCE OF SOC FOR SOIL AND AGRICULTURE

Even in small amounts SOC is very important for soil fertility which is determined by chemical, physical and biological properties. Higher levels of SOC reduce bulk density and nutrient leaching, increase aggregate stability, cation exchange capacity, pH buffering, water-holding capacity, infiltration, resistance to soil compaction, resistance to soil erosion and enhance soil fertility. Wildlife habitat, biodiversity and biological activity are greater in soils rich in SOC, which can increase crop health and vigour. With proper level of SOC crops are more able to withstand drought and soil is a more effective water filter, which makes ground and surface water quality better. Soil organic C can also influence plants' health by reducing dust, allergens and pathogens in the air and by binding pesticides and thus decreasing their activity. Soil organic C can directly help plants by providing air pockets which allow microorganisms to turn the nitrogen from the air into nitrate and ammonia and by providing  $CO_2$  contained in these air pockets, which increases plant growth. SOC also enhances plant and microbial growth through growth stimulating compounds and root growth, by making it easy for roots to penetrate through the soil.

Organic farming practices are regarded as being beneficial for the environment by promoting soil quality and sequestering SOC (Leifeld et al., 2009). One of the basic characteristics of organic farming is an increased input of organic fertilizers (manure, compost, peat, guano). A key prerequisite for the realization of the basic principle of organic farming, *fertilize the soil to feed plant*, is increase in SOC content (Čuvardić, 2006). The application of manure increases C biomass, total number of bacteria, the number of azotobacter and aminoheterotrophs and activity of enzymes dehydrogenase and urease (Đurić et al, 2008). However, the studies on the effects of organic agriculture on soil fertility have shown that in the first few years of organic farming implementation in Vojvodina (Serbia) there is no difference in SOC between conventional systems and those employing farmyard manure (Čuvardić et al., 2005; 2006).

## CARBON SEQUESTRATION AND IMPACT OF SOC ON GLOBAL CLIMATE CHANGE

Greenhouse gases (GHGs), carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), concentrations in the atmosphere can vary as a result of some natural process, such as volcanic activity or temperature changes. But since the Industrial Revolution certain human activities (burning of oil, coal and gas, land use, and agriculture) have increased concentrations of GHGs in the atmosphere. According to IPCC (2007), since pre-industrial times, increasing emissions of GHGs due to human activities have led to a marked increase in atmospheric concentrations of the long-lived GHGs (atmospheric CO<sub>2</sub> concentrations have increased by almost 100 ppm in comparison to its preindustrial levels, reaching 379 ppm in 2005).

In order to stop global warming, scientists have deeply started to analyze both quality and quantity of SOC. Soil organic C is a relatively dynamic, continuously exposed to synthesis and decomposition processes in soil. During photosynthesis, C is assimilated in  $CO_2$  state from atmosphere and integrated into plant bodies, which are later exposed to microbiological decomposition processes. Soil organic C is created in the synthesis process from residues of dead plants and soil-related animals. At the same time SOC decomposition process takes place and  $CO_2$  is created as a result.

Soils annually emit between 6.8 and 7.9 Gt  $CO_2$  equivalents, mainly as  $CH_4$  from intact peatlands and from rice agriculture; as N<sub>2</sub>O from unmanaged and managed soils; and as  $CO_2$  from land-use change (Leifeld, 2006). Along the decreasing emission of GHGs into the atmosphere, sequestering  $CO_2$  in the soil is one of the leading methods of mitigating global warming. Carbon sequestration implies transferri ng atmospheric  $CO_2$  into long-lived pools and storing it securely so it is not immediately reemitted (Lal, 2004). Simpler, SOC sequestration is a positive flux between terrestrial C and atmosphere C, while the emission in  $CO_2$  state is negative. It is natural, cost-effective and environmentally-helpful process. Some of the ways to enhance SOC sequestration are no-tillage or reduced tillage practice, organic production, cover crops, crops rotation, fertility management, forest regeneration and fertilization, conversion of cropland to grassland and forest, etc. Manojlović et al. (2008) found significantly higher SOC stock in continuous corn rotation in 0-40 cm depth with no tillage and disc tillage compared to chisel plow tillage and moldboard plow tillage, while SOC stock was not significantly affected by tillage methods at 0-40 cm soil depth with corn/soybean rotation. The results of a long-term field trial established at Chernozem showed that in corn monoculture (CC), fertilizer application compared to no fertilization in a 32-year period, did not increase SOC stock in the 0-30 cm soil layer (Table 2) (Manojlović et al., 2008). However, fertilization treatments with harvest residues (F+HR) or FYM (F+FYM) increased SOC concentration and SOC stock compared to control. Soil organic C stock was increased to 6.81 Mg ha<sup>-1</sup> with F+HR treatment and to 15.2 Mg ha<sup>-1</sup> with F+FYM. The highest sequestration was noticed with F+FYM in corn/spring barley rotation (CB). Using no-tillage, reducing summer fallow, including hay in rotation with wheat (Triticum aestivum L.), plowing green manures into the soil, and applying N and organic fertilizers were the practices that tended to show the most consistent increases in SOC storage (Vanden Bygaart et al., 2003). Lal et al. (2007) reported that the rate of SOC sequestration ranges from 100 to 1000 kg ha<sup>-1</sup> a year depending on climate, soil type, and local land management system. Management systems have a major influence on the total SOC as well as the relative distribution of the various C pools that reside within it (Sherrod et al., 2005). Smith (2004) claims that there is a significant potential in Europe to decrease the flux of C to the atmosphere from cropland, and for cropland management to sequester soil C, relative to the amount of C stored in cropland soils at present. Lal (2004), indicates that the C sink capacity of the world's agricultural and degraded soils is 50 to 66% of the historic C loss of 42 to 78 Gt of C and emphasizes that the rate of SOC sequestration with the adoption of recommended technologies depends on soil texture and structure, rainfall, temperature, farming system, and soil management. Freibauer et al. (2004) state that a substantial spatial component in the net sequestration potential may be expected, because of regional differences in soil, site, and climatic conditions. Oren et al. (2001) indicate that the assessment of future C sequestration should consider the limitations imposed by soil fertility, as well as interactions with nitrogen deposition. Smith et al. (2008) emphasize that there are significant opportunities for GHG mitigation in agriculture but for the potential to be realized numerous barriers like barriers to implementation, including climate and non-climate policy, and institutional, social, educational and economic constraints need to be overcome. The same author indicates that the total biophysical potential of approximately 5.5-6 Gt CO<sub>2</sub>-eq yr<sup>1</sup> would never be realized due to these constraints, but with appropriate policies, education and incentives, it may be possible for agriculture to make a significant contribution to climate change mitigation by 2030. A good example of the incentive to encourage SOC sequestering is Australian Soil Carbon Accreditation Scheme (ASCAS). Due to the fact that Australia has the highest per capita rate of greenhouse gas emissions in the world (Jones, 2008), Australian farmers will be paid \$90/tonne annually and retrospectively for the increase in their SOC.

Table 2. Impact of crop rotation and fertilization methods on SOC concentration and SOC stock in chernozem (Source: Manojlović et al., 2008)

| Treatment<br>Tretman | Duration<br>(years)<br><i>Trajanje</i> | SOC<br>SOC<br>(g kg-1) | SOC stock<br>SOC rezerve<br>(Mg ha-1) | U         | elated to control<br>odnosu na kontrolu |
|----------------------|--|------------------------|---------------------------------------|-----------|---|
|                      | (godine)                               |                        |                                       | (Mg ha-1) | (kg ha-1 yr -1)                         |
| Control, CC          | 32                                     | $1.40\pm0.01\ d$       | $61.0\pm0.47\ d$                      |           |   |
| F, CC                | 32                                     | $1.42\pm0.02\ d$       | $61.7\pm0.83~d$                       | 0.76      | 24                                      |
| F + HR, CC           | 35                                     | $1.56\pm0.02\ c$       | $67.8\pm0.73~c$                       | 6.81      | 195                                     |
| F + FYM, CC          | 35                                     | $1.75\pm0.01\ b$       | $76.2\pm0.44\ b$                      | 15.2      | 435                                     |
| FYM, CB              | 30                                     | $1.78\pm0.04\ b$       | $77.3\pm1.73\ b$                      | 16.4      | 547                                     |
| F+FYM, CB            | 30                                     | $2.08\pm0.05\;a$       | $90.3\pm2.00\ a$                      | 29.3      | 978                                     |

Tabela 2. Uticaj plodoreda i načina đubrenja na koncentraciju i rezerve SOC u černozemu

Notes: Control, without fertilization; F, mineral fertilizers; F + HR, mineral fertilizers + harvest residues; F+ FYM, mineral fertilizers + farmyard manure; CC, continuous corn; CB, corn/ spring barley rotation (corn in sampling year). Mean values and standard error followed by the different letters in the columns or rows are significantly different at p < 0.05.

Napomene: Kontrola, bez đubriva; F, mineralna đubriva; F + HR, mineralna đubriva + žetveni ostaci; F + FYM, mineralna đubriva + stajnjak, CC, monokultura kukuruza, CB, rotacija kukuruz / jari ječam (uzimanje uzoraka u godini pod usevom kukuruza). Srednje vrednosti i standardne greške obeležene različitim sovima u kolonama i redovima su statistički značajne za prag značajnosti od p < 0.05.

### CONCLUSIONS

As the largest terrestrial pool of C, the soil has an important role in global C cycle and global warming. Soil organic C is a key factor of soil C dynamics as it can strongly affect GHGs concentrations in the atmosphere and improve soil fertility.

The role of the soil in global warming mitigation should be very important when it comes to C sequestration – transferring atmospheric  $CO_2$  into long-lived pools and storing it securely. Carbon sequestration can be achieved by no-tillage or reduced tillage practice, organic production, cover crops, crops rotation, fertility management, forest regeneration and fertilization, conversion of cropland to grassland and forest, etc.

Different soil types have a naturally wide range of SOC concentrations, which are decreased in recent period by land use change (conversion of grassland, forests and natural vegetation to arable land), deep ploughing; overgrazing with high stocking rates; soil erosion, by water and wind; leaching and forest fires. In order to achieve our goals we have to perform detailed analyses and monitor SOC concentrations and pools as a baseline for the mitigation of global warming and soil degradation, as well as the improvement of soil quality.

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# ZNAČAJ ORGANSKOG UGLJENIKA ZEMLJIŠTA U POLJOPRIVREDI I GLOBALNIM KLIMATSKIM PROMENAMA

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#### Izvod

Imajući u vidu činjenice da su u zemljištu najveće terestrijalne rezerve ugljenika (2500 Gt) i da organski ugljenik zemljišta (SOC) ima jak uticaj na klimatske promene, poslednjih godina su evidentna sve veća interesovanja za SOC. Nova istraživanja su uglavnom orijentisana na aspekte vezivanja C, ublažavanja klimatskih promena, efekte promene načina korišćenja zemljišta i analiziranje frakcija SOC. Aktivnosti čoveka, koje utiču na smanjenje sadržaja SOC su nepopularne zbog negativnog uticaja na globalno zagrevanje i degradaciju zemljišta. S druge strane, praktična rešenja navedena u ovom radu, kojima se ostvaruje vezivanje ugljenika u zemljištu su ekološki održiva iskustva koja pomažu očuvanju sadašnjeg i povećanju budućeg nivoa SOC.

**Ključne reči**: Organski ugljenik zemljišta, SOC, frakcije SOC, vezivanje ugljenika, način korišćenja zemljišta, tip zemljišta.

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# AGRICULTURAL BUDGET AS A FORM OF FINANCING AGRICULTURE IN THE REPUBLIC OF SERBIA

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SUMMARY: Authors are investigating the support of the state with regard to financing the agricultural sector over the past period. In January 1994, the credit placement from the central bank primary issuing, which had long been reliable and standing source of funding agriculture, was terminated by the Programme of the monetary system reconstruction. That led to the agrarian budget as a pooled and consolidated form of the state support to agriculture, which is implemented by subsidizing the agricultural production. Agrarian budget funds from 2004 to 2006 were exploited for the purpose of financing agricultural production, which represents a very specific form of the state support and which used to be realized through commercial banks.

*Key words:* agriculture, state, credit, agricultural budget, Republic of Serbia.

## **INTRODUCTION**

Agricultural budget is an integral part of the central, federal or regional budget which determines the scope of necessary funds (premiums, recourse, subsidies, grants, loans)<sup>2</sup>, as well as instruments of state interventionism (agricultural policy) and protec-

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<sup>&</sup>lt;sup>2</sup> **Premium** - Additional Incentive payments (to producers or exporters or importers). **Regress** - a form of economic transfers that states stimulate sales and consumption of certain products. It creates stable economic conditions for the development of certain activities and areas. With regress governent usually wants to achieve the following objectives: increase agricultural production and on that basis, the growth and stabilization of the aggregate supply of agricultural products, faster introduction of the results of the modern development of science, technology and techniques in agricultural practices, increasing and stabilizing agricultural income. Regress can be given to farmers for purchase of certain reproduction materials, such as mineral fertilizers,

tion of pricing policies of agricultural products.

## **RESEARCH RESULTS**

Reconstruction programs of credit-monetary system, whose implementation started on 24th January 1994 abolished the agricultural finance from the primary emission of the central bank on the grounds that these investments affect the growth of domicile inflation. In the given situation it was necessary to find a new way of financing that would recognize specific of agriculture. At the same time, due to tight supply of money, we have the growth of interest rates on loans that were approved by commercial bank, which significantly increased costs of agricultural production and led companies in a difficult financial situation (Pejanović et al, 2008).

For these reasons, it was realized that it is necessary to establish safe and permanent source of financing for agriculture, and therefore agricultural budget was established, as part of the budget of the Republic of Serbia. Decision on the establishment of the agricultural budget was adopted in late 1995 and in 1996 he officially became part of the state budget. Agricultural budget was designed as an integrated source of state support for agriculture in maintaining current production, and development dimensions of this economic activity. According to some authors, this is the way of regressing, not financing agriculture, while in support of the state agricultural budget exists in all developed countries.

At first the agricultural budget was relatively modest and it was one billion dinars. The initial intention was that the agricultural budget provides funds for stimulating priority needs of agriculture, extension services and funds for the revitalization of the village. It was clear that the funds allocated for this purpose is insufficient and that it is necessary to increase it if it is meant to seriously develop agricultural production, because its progress is not possible without strong economic assistance of the State. Only in this way the modernization of agricultural production can be achieved, the optimal supply of markets with food and it can ensure quality life to farmers. For example, in the European Union, that counted 25 countries at the end of 2006th, and had 480 million inhabitants, the average subsidy per capita, in the above year, was 127 euros. In our country, in the same period, subsidies to agricultural producers goes to an average amount of 17 to 20 euros, 70 euros in Hungary, and even 130 euros per capita in Slovenia. In the European Union in 2006 subsidiris were amounted to 360 euros per hectare, and in our country 32 euros per hectare. We can conclude that our agriculture has almost ten times

means of plant protection, quality seed, brood stock and for the purchase of machinery (tractors, harvesters), and spare parts. **Subsidy** (Latin subventio - help) is the stimulation, the amount by which the state covers the difference between the price paid by the consumer and producer cost (payments to farners). The aim of the subsidies is to encourage production of certain products, promoting and exports. Downside is the reduction of efforts to increase productivity and profit-ability of production. **Grants** (Latin dotare - richly equipped) is the amount that is awarded when revenues are not sufficient to cover expenses, or a way of helping producers in solving problems. Usually it is assigned with a purpose, and so the recipient agrees to spend the earmarked. **Credit** (Latin credere - delegate) the debtor - trust relationship in which the debtor gives the creditor the right to dispose of a certain amount of money to a specific time and under certain conditions, which are more favorable than the market.

less support in relation to developed countries (Pejanović and Milić, 2008).

Long term objectives of the agricultural budget should be related to<sup>3</sup>:

- 1) increase production, productivity, export and monitoring of technological innovations in agricultural production;
- 2) improving the quality of production and increase competitiveness of agriculture;
- 3) the growth of life standard of the population and ensuring adequate income to agriculture;
- 4) environmental protection and production of healthy food;
- 5) development of rural areas and keeping people on them.

According to the share of agriculture, in gross domestic product and expenditure budget of the Republic of Serbia (Table No. 1), in the period from 1996 to 2006, it can be concluded that the importance of agriculture for the economy as a whole did not had the adequate support of the state for this very important activity. For example, in the period from 1996 to 2002 the share of agriculture in gross domestic product was relatively stable and amounted to about 20%, while the share of agricultural budget in state cash register reduced every year and from the initial 8.3%, in 2002 came to only 4.3%. The importance of agriculture in the economy and its participation in the state budget illustratively is shown in the fact that during 2001 production recorded a growth of 20%, which led to GDP growth of 5%<sup>4</sup>. That same year, the participation of agriculture in the total state budget was only 3.1%.

Table 1. Comparative review of the participation of agriculture in GDP and expenditure budget of the Republic of Serbia  $(%)^{5}$ 

| Year  | Percent of agrar in GDP | Percent of agricultural budget in total budget<br>of the Republic of Serbia |
|-------|-------------------------|---|
| 1996. | 19,50                   | 8,30  |
| 1997. | 19,30                   | 6,00  |
| 1998. | 18,30                   | 5,80  |
| 1999. | 21,40                   | 5,00  |
| 2000. | 23,20                   | 5,60  |
| 2001. | 24,00                   | 3,10  |
| 2002. | 20,00                   | 4,30  |
| 2003. | 18,46                   | 4,04  |
| 2004. | 15,00                   | 6,12  |
| 2005. | 13,50                   | 4,38  |
| 2006. | 12,90                   | 5,28  |

Tabela 1. Uporedni prikaz učešća poljoprivrede u domaćem bruto proizvodu i budžetskim izdacima Republike Srbije (%)

<sup>&</sup>lt;sup>3</sup> Sinđić, V.: "Dinar za razvoj, poslovanje, konkurentnost,",magazin "Poljoprivreda", februar 2007.godine, str.21.

<sup>&</sup>lt;sup>4</sup> Magazin" Ekonomist", Beograd, broj 86 od 14.01.2002.godine, str.20.

<sup>&</sup>lt;sup>5</sup> Bogdanov Natalija: "Poljoprivreda u međunarodnim integracijama i položaj Srbije", Monografija, Društvo agrarnih ekonomista Jugoslavije,Beograd, 2004. godina, str.102 i str.125 (podaci za period od 1996-2001.god.); Sinđić, V.: " Dinar za razvoj, poslovanje, konkurentnost", Magazin "Poljoprivreda", februar 2007.godine, str.21 i str.27(podaci za 2002. i 2003. godinu); Paraušić Vesna, Cvijanović, D.: "Poljoprivreda Srbije – programi kreditne podrške države i komercijalnih banaka u periodu od 2004-2007. godine", str.188 (podaci za period od 2004. do 2006. godine).

Based on the data shown in Table No. 2 it can be concluded, that in the entire period, the largest single appropriation of funds from the agricultural budget was for premium for milk and they went up to about 1/3 of total budgetary resources. Note that this measure had mainly social dimension because premiums were paid to producers, regardless of the amount of milk delivered, so that a large number of farms in the Republic was eligible for this support. After that we have premiums for plant production, which primarily were intended to change sowing structure in favor of industrial plants. According to data presented in Table No. 2 it can be concluded that the funds in the period observed were continuously separated for breeding cattle, while (since 2001.) incentives for revitalization of villages have been abolished. In the next year, the budget will start to pay incentives for raising plantations of fruit and especially vines and plums, because their production in the previous period was significantly reduced. Also, subsidies for the purchase of wheat were introduced and since 2003 subsidies for export of agricultural and food products, and subsidies for expanditure and improvement of the quality of land area, which is in accordance with the requirements of modern agricultural production in the European Union.

| No  | Purpose  | 1996. | 1997. | 1998. | 1999. | 2000. | 2001. | 2002. | 2003. |
|-----|--|-------|-------|-------|-------|-------|-------|-------|-------|
| 1.  | Premium for milk                               |       | 28.0  | 34.7  | 34.6  | 47.4  | 45.4  | 33.7  | 27.5  |
| 2.  | Premium for plant production                   |       | 17.2  | 5.8   | 7.7   | -     | 16.7  | 15.0  | 23.8  |
| 3.  | Recourse for breeding cattle                   |       | 6.3   | 8.6   | 4.8   | 4.0   | 2.7   | 2.8   | 2.5   |
| 4.  | Revitalization of village                      |       | 10.0  | 7.4   | 6.7   | 5.1   | -     | -     | -     |
| 5.  | Agricultural services                          | -     | 2.0   | 2.4   | 2.2   | 2.2   | 1.2   | 1.4   | 1.1   |
| 6.  | Measures for improvement of livestock          | -     | 4.0   | 6.8   | 6.2   | 7.3   | 2.9   | 1.9   | 1.4   |
| 7.  | Animal healthcare                              | -     | 8.0   | 8.9   | 8.1   | 8.4   | 15.7  | 11.9  | 9.9   |
| 8.  | Improvement of agricultural land               | -     | 3.7   | 3.6   | 2.5   | 1.1   | 0.8   | 0.8   | 0.9   |
| 9.  | Stimulating of plantation growth               | -     | -     | -     | -     | -     | -     | 1.8   | 1.3   |
| 10. | Subvencion for weath puchase                   | -     | -     | -     | -     | -     | -     | 5.6   | 2.0   |
| 11. | Expansion and maintenance of land tenure       | -     | -     | -     | -     | -     | -     | -     | 5.9   |
| 12. | Stimulating of export of agricultural products | -     | -     | -     | -     | -     | -     | -     | 4.3   |
| 13. | Forestry and hunting                           | -     | 3.0   | 3.4   | 8.6   | 8.4   | 2.5   | 1.8   | -     |
| 14. | Multipurpose usage of water                    |       | 14.0  | 14.7  | 14.7  | 12.4  | 7.9   | 18.6  | 18.0  |
| 15. | Other obligations stipulated by the law        | 38.5  | 3.8   | 3.6   | 3.8   | 3.6   | 4.1   | 4.8   | 1.3   |

Table 2. Structure of agricultural budget of the Republic of Serbia for 1996-2003 (in %)<sup>6</sup> Tabela 2. Struktura agrarnog budžeta Republike Srbije u periodu 1996.-2003. godine (u %).

In this period agrarian policy of Serbia had a duty to help the financially exhausted agriculture. In fact, agriculture was responsible to provide food safety during the period of economic isolation. Also, this economic activity is additional financial exhaustion with perennial economic recession, so it is understandable that the measures of agricultural policy, institutionaly, qualitatively and quantitatively must be very different from those in the European Union (Pejanović, 2009).

<sup>&</sup>lt;sup>6</sup> Bogdanov Natalija: "Poljoprivreda u međunarodnim integracijama i položaj Srbije", Monografija, Društvo agrarnih ekonomista Jugoslavije, Beograd, 2004. godina, str.126.

| No  | Purpose   | 2004  | 2005  | 2006  |
|-----|---|-------|-------|-------|
| 01. | Support to plant production                       | 12.9  | 16.2  | 11.1  |
| 02. | Support to livestock breeding                     | 19.0  | 18.5  | 13.1  |
| 03. | Market support measures for agricultural products | 3.0   | 2.6   | 2.2   |
| 04. | Measures promoting the production                 | 5.5   | 6.4   | 8.7   |
| 05. | Measures and actions in agriculture               | 0.2   | 4.9   | 2.3   |
| 06. | MEASURES TO SUPPORT RURAL DEVELOPMNET             | 20.1  | 6.4   | 5.1** |
| 07. | CREDIT POLICY MEASURES                            | -     | 13.6  | 20.7  |
| 08. | Other measures                                    | 14.4* | 1.4   | 0.7   |
| 09. | TOTAL SUPPORT TO PRODUCTION                       | 75.1  | 70.0  | 63.9  |
| 10. | Advisory services                                 | 1.0   | 1.2   | 0.8   |
| 11. | Veterinary management                             | 8.8   | 5.2   | 7.5   |
| 12. | Plant protection management                       | 0.6   | 0.6   | 0.7   |
| 13. | Republic Directorate for Water                    | 12.3  | 16.9  | 17.4  |
| 14. | Forest management                                 | 1.8   | 2.5   | 1.9   |
| 15. | Ministry expenditure                              | 2.4   | 3.6   | 3.2   |
| 16. | Other costs                                       | -     | -     | 4.6** |
| 17. | PROFESSIONAL AND PUBLIC SERVICE                   | 24.9  | 30.0  | 36.1  |
|     | AGRICULTURAL BUDGET                               | 100.0 | 100.0 | 100.0 |

Table 3. Structure of agricultural budget for 2004 - 2006 (in %)<sup>7</sup> *Tabela 3. Struktura agrarnog budžeta u periodu 2004.-2006. godina (u%)* 

\* Weath purchase \*\* Means of National Investement plan (NIP)

In domestic agricultural policy, during 2004, there is a turning point, which is reflected in the effort to adapt agriculture to a market economy as in developed countries. For this purpose, new items were introduced to the agricultural budget, which is shown in Table No. 3 First of all, those are incentives for developing the market of agricultural and food products, incentives for rural development, and incentives for the development of agricultural lending. Priority development projects are funded from grants, support is given to long-term lease of agricultural land (in order to increase the property). Premiums for milk remain as an important social dimension of our agriculture. The novelty is that all premiums are paid directly to farmers and not to food processors as in the previous period. For that purpose, there will be a mandatory registration of farms, because only these farms are entitled to get the state support.

Based on the data presented in Table number 3 it can be concluded that the dominant share of the agricultural budget in 2004 goes to measures that will support production and market, even 55%. Measures encouraging rural development have constituted 20.1% of the agricultural budget in the specified year and the cost of professional and public services 24.9%. In the following, 2005 new measures were introduced, encouraging credit policy which participated in the agricultural budget with 13.6%, while the financing of measures to enhance the production and the market, spent half of the agricultural budget for the year. Costs of professional and public services were in 2005

<sup>&</sup>lt;sup>7</sup> Sinđić, V.: "Dinar za razvoj, poslovanje, konkurentnost", magazin "Poljoprivreda", februar 2007.godine, str.21.

30% of budget funds assigned to agriculture and rural development incentives 6.4%<sup>8</sup>. In 2006 credit policy measures increased their participation in the agricultural budget and went up to 20.7%, rural development measures are constituted at 5.1% (financed from the funds of the National Investment plan), and measures to support production and market of agro-food products have reduced their participation in the agricultural budget to 38.1%, while the share of the costs of professional and public services increased to 36.1%. The growth of these costs was conditioned by the need to build local agricultural institutions in order to support the implementation of the model applied by agriculture in the European Unio (Pejanović, 2009).

It can be concluded that the observed ten-year period of means of the agricultural budget was insufficient to meet the current needs of domestic agriculture, let alone the development and the need for structural adjustment of production in accordance with the requirements of the European Union. Also, funds taken from the state budget were not equivalent to the importance of agriculture for the national economy. Specifically, in the period from 1994 to 2006, the share of agriculture in the creation of gross domestic product goes to an average of 19%, and if the consideration of textiles this share amounted to even more than 40%. At the same time, the share of agricultural products in total exports went to 25%, while the separated funds from the state budget for agriculture went to an average of only 5%. For example, in surrounding countries, on average, the state annually sorts out for agriculture from 10% to 14%.

However, not all funds in the agricultural budget in the period of last two years were intended for agricultural production incentives, but were placed in the form of the loan, the return funds, which are significantly diminished funds for subsidies. There is an opinion that the credit function is not in accordance with pre-set function of the agricultural budget, because from it, it can only grant Incentive Grant funds and not revolving funds.

In order to display integrity it is necessary to point out that in this period from the budget of the Autonomous Province of Vojvodina a part of incentives for the development of agricultural production has been sorted out.

| Year | Total budget of APV |                  | Involvement of agricultural budget in total budget of APV |
|------|---------------------|------------------|---|
| 2004 | 14.993.076.565,39   | 365.548.571,85   | 2,4 %   |
| 2005 | 20.433.898.970,97   | 573.511.830,84   | 2,8 %   |
| 2006 | 25.825.845.496,85   | 1.014.001.881,44 | 3,9 %   |

Table 4. Involvement of agricultural budget in total budget of AP Vojvodine<sup>9</sup> Tabela 4. Učešće agrarnog budžeta u ukupnom budžetu AP Vojvodine

With the analysis of the data shown in Table No. 4 it can be assumed, that the

<sup>&</sup>lt;sup>8</sup> In 2005 premiums for basic farming culture were abolished, and only the production of hops, tobacco, rapeseed and pumpkin is still regresed. Regresing procurement of planting materials, fertilizers and mineral oil, in addition to credit funds and incentives for rural development becomes the main form of state support to agricultural producers.

<sup>&</sup>lt;sup>9</sup> Source: Archive of Secretariat for Agriculture, Forestry and Water Management of the Executive Council of Vojvodina.

funds allocated for agriculture in the provincial budget was insufficient for its funding, because agriculture has not received an adequate level of funds according to its economic importance, given that, it is the primary economic activity in the province. Unfavourable financial situation is somewhat mitigated by the credit funds placed under a relatively favorable terms from specialized funds established by the Assembly of AP Vojvodina.

## CONCLUSION

The problem of domestic agriculture in the reported period was based on the pending issue of financing agriculture, inadequate government support for this important economic activity, inadequate comparison between the contribution of agricultural domicile production of gross domestic products and financial investment in the local agriculture. Also, the financial position of agriculture in the period was further on endangered with the disparity in prices of agricultural and industrial products, inconsistent agricultural policies, and inadequate credit support, both in quantitative and in qualitative terms. With abolition of agricultural credit from the primary emission of the Central bank, this category has remained without beneficiary loans, as well as a secure and permanent funding source, which takes the specificities of its production into account.

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# AGRARNI BUDŽET KAO OBLIK FINANSIRANJA POLJOPRIVREDE REPUBLIKE SRBIJE

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## Izvod

Autori razmatraju podršku države finansiranju poljoprivrednog sektora u proteklom periodu. U januaru 1994. godine, kreditni plasmani iz primarne misije centralne banke, koji su dugo bili pouzdan i trajan izvor finansiranja poljoprivrede, ukinuti su putem Programa rekonstrukcije monetarnog sistema. To je dovelo do agrarnog budžeta, kao zajedničkog i konsolidovanog oblik državne podrške poljoprivredi koja se sprovodi putem subvencionisanja poljoprivredne proizvodnje. Sredstva agramog budžeta su u periodu od 2004. do 2006. godine korišćena za potrebe finansiranja poljoprivredne proizvodnje, što predstavlja specifičan oblik državne podrške koja je realizovana preko komercijalnih banaka.

Ključne reči: poljoprivreda, država, kredit, agrarni budžet, Republika Srbija.

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# GEOGRAPHIC CONDITIONS FOR QUAIL PRESENCE IN VOJVODINA HUNTING GROUNDS

## ZORAN A. RISTIĆ, BRANKO RISTANOVIĆ, MILOSAVA MATEJEVIĆ<sup>1</sup>

SUMMARY: Based on the gathered statistical indicators regarding the bagging turtledoves and qualis in Vojvodina for the period from 1979. to 2008. (total number of bags, total number of bags in hunting tourism) we come to a conclusion that the doves were most important quarry for development of hunting tourism. Until a certain point (the middle 80 s) when the majority of hunting was steadily focusing on quails and the hunt for turtledoves was drastically dropping. Because of these facts (based on the assessment of quantity in our and other European countries) we can say with certainty that these species were not in danger because the number that was hunted was just 2 to 5% of the total number hunted in august and september (when these birds come, because of their removal) in large numbers, to hunting grounds of Vojvodina.

Key words: quail, hunt, game

## **INTRODUCTION**

The quail (*Coturnix coturnix*) is a migratory bird and the smallest member of Gallinae family in our field hunting grounds. It comes to our parts from North Africa at the end of April or in early May and it is nesting here. At the end of August, when chicks are grown up, they flock again and fly over Italy and Sicily or over Bosporus to Africa, where they spend winter. Nevertheless, it often happens that quail is present in our parts in October, November and even December, especially if the fall and winter were mild.

This bird is dispersed in almost whole Europe, except in Iceland, Scandinavia, northern Finland and northern Russia. Their habitat consists of plain terrains with meadows, fallows, wide fields under clover and stubble. The country with far highest number of quail is definitely Spain (about 370,000 pairs) and partly France (100,000).

Review scientific paper / Pregledni naučni rad

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Population of this species in Serbia is not investigated enough, and it is estimated to about 20,000 pairs, uniformly spread in Pannonian plane (Vojvodina) and in hills and mountains parts of Serbia.

## IMPORTANCE OF ECOLOGICAL CONDITIONS IN VOJVODINA FOR QUAIL POPULATION

Vojvodina is situated at the north of Serbia. It borders to Hungary at north, Croatia at west, Bosnia and Herzegovina at southwest and Romania at east. Borderline towards central Serbia is partly on river Sava and partly on Danube.

Generally speaking, Vojvodina is situated in southern part of Pannonian plain and represents a natural bridge connecting Central and West Europe with Balkans and the Middle East. Its area is 21,533 km<sup>2</sup> with about 2 million people. Three main regions are Banat (9,296 km<sup>2</sup>), Bačka (8,729 km<sup>2</sup>) and Srem (4,220 km<sup>2</sup>), separated by rivers Danube and Tisa. Sava River, third largest in Vojvodina, separates Srem from Mačva region in central Serbia.

Although seemingly a monotonous plane, Vojvodina has stair-like relief. The first members of such relief are alluvial river planes. These areas comprise 32% of Vojvodina territory and are often endangered by floods. In Danube's alluvial plane, at Nera river mouth, is situated the lowest point in Vojvodina (65 m).

Diluvial planes or loess terraces were formed by piling up of aeolian material and by deposition of river silt (38% of area). They are 3-5 m higher than previous relief element and wear traces of old river channels filled in by loess. Most prominent fragments of terraces are Bačka, Srem, Pančevo and Zrenjanin terraces.

The next elements of Vojvodina relief are loess plateaus. Most prominent fragment of a loess plateau is Titel hill, with ellipsoid shape, which extends in northwestsoutheast direction for 17 km, and is only 7 km wide. Highest point is at 137 m, at the northeastern periphery. Other loess plateaus (Telečka, Fruška gora, south Banat) are not so prominent and have valleys as a geomorphological shape with distinct cross-section in shape of overturned trapezoid. All plateaus were formed by diluvial piling up of aeolian material.

Highly characteristic elements of Vojvodina relief are most certainly two sandy desserts: Banatska (Deliblatska) in southeastern Banat, between Danube River and western slopes of Carpathian Mountains. It is 60 km long, and about 25 km wide at the widest point, with ellipsoid shape along southeast-northwest axis. By its origins, orography, climate, flora and fauna, Deliblatska peščara is the unique phenomenon in the Europe, and sometimes was called European Sahara. Subotička peščara represents far periphery of spacious Bajska peščara, located in Hungary. Its height is between 100 and 134 m, which is the highest point in Bačka region. Most sand is located between villages Horgoš and Tavankut, while two smaller parts are near Gornji Tavankut and Riđica.

Higher altitudes belong to two mountains: lower Fruška gora (Crveni Čot, 539 m) which is longer (78 km) and wider (25 km) and higher Vršačke planine (highest point in Vojvodina – Gudurički vrh, 641 m).

Surface hydrography is characterized by large rivers – Danube, Tisa, Sava – but also by smaller ones as Begej, Tamiš, Karaš, Nera and Bosut. Small water flows of Vojvodina, but not without importance, are Jegrička, Krivaja, Čik, Plazović and Mostonga in Bačka; Zlatica, Brzava, Rojga and Moravica in Banat; Spačva, Šidina and Studva in

Srem. Danube is not the largest, but certainly is the most important river in Europe. It enters Vojvodina in its middle course at 1433 kilometers, at junction of three borders: Hungary, Croatia and Serbia, and exits at mouth of Nera River. This part of Danube is a typical lowland river. It floods at higher water level (between April and July) and meanders, producing numerous river islands and by-channels. Between by-channels and ponds unique natural oases are formed, inhabited by diverse plant and animal species. All settlements at Vojvodina banks of Danube have rich game resources. Rare bird species are present here, as well as prize specimens of deer and wild boar. By its natural biodiversity, most prominent hunting grounds are Karađorđevo, Kozara, Apatinski rit, Plavna, Deliblatska peščara, Koviljski rit.

Pedological composition of Vojvodina is diverse, due to different relief elements. Loess plateaus and higher parts of diluvial terraces are covered by fertile chernozem (43.4%). Meadow black soil (17.2%) dominates in diluvial terraces, and alluvial-diluvial soils (9.2%) at lowest elements of Vojvodina relief – inundation planes. Swamp black soil, low quality agricultural land, is present in lower parts of diluvial terraces and in tectonic depressions. Fruška gora and southern Srem are full of utisol (2.6%) and are excellent woodland soil. Similar use is frequent for sand soils that are present in 30,429 ha (1.4%). Lowest parts of Vojvodina relief (eastern Banat depressions) are covered by vertisol (6.5%). The rest of Vojvodina space is covered by initial soils, salt marsh (Tisa valley, Banat tectonic depressions) and gley (southwestern Srem).

Natural vegetation of Vojvodina, before human interventions, was comprised mostly of forests. In alluvial planes, dominant species were poplar and willow, as well as oak forests. In Banat depressions, forests of ash and bay oak were present, and loess areas were under ash, linden and oak forests. In Fruška gora oak and hornbeam dominated, and in Vršačke planine wainscot oak. Modern picture is quite different: most of Vojvodina is under agricultural land – fields, meadows, orchards and vineyards (75.5%). The rest is pastures (5.7%) and forests (6.2%). This disposition of agricultural areas is very favorable for quails.

Fauna is comprised of relatively small number of species. Nevertheless, Vojvodina, although lowland area with small forestation and large percent of agricultural fields, still offers almost ideal conditions for breeding, and therefore for hunting of all game species. It is not often to find areas where, at relatively small space, it is possible to breed such large number of small and large game species. Hunting grounds in Vojvodina are among most well-known ones in Europe. Advantages of Vojvodina, as a hunting-touristic destination, are not only in large number of species but also in high general hunting and hotel management culture. Lowland area has long hunting tradition, since Vojvodina has long been most popular hunting destination in Europe. Most popular game species in this area are roe deer, wild boar, fallow deer, moufflon, brown hare, pheasant, partridge and quail. Area of 2.000.000 ha within 89 organized hunting grounds is well known among hunters worldwide.

#### MATERIAL AND METHODS

In order to understand issues affecting hunting in general and therefore in Vojvodina too, especially regarding quail as a part of hunting-touristic offer in Vojvodina, it is important to know historical facts. This problem is not researched enough and apart of several science papers, it is not possible to obtain detailed scientific data regarding hunting tourism in general and total hunting of quail.

Material for this paper was collected from Statistic institute of Serbia, Hunting Association of Vojvodina, Long-term program of hunting development for Vojvodina 2000-2010 and Long-term program of hunting development in Vojvodina from 1984. We also used price lists for foreign hunting tourists which are given every year before start of hunting season by Hunting association of Serbia and Vojvodina as authorized by Law on hunting, and are distributed to hunters' societies. Statistical data were collected regarding general catch of quail in Vojvodina hunting grounds between 1979 and 2008. Besides data about total catch, data about catch in hunting tourism were also collected.

Data ate shown as a diagram in order to extract conclusions regarding realized hunting tourism for quail in 30-year period.

Present hunting season for quail is given, as well as hunting season in effect for previous period. Based on quail biology as given in paper, an optimal hunting season for our hunting grounds conditions was suggested.

From statistical data regarding catch of quail, it is evident that hunting was done within limits for sustainable management of this species for all hunting grounds in Vojvodina.

## LITERATURE OVERVIEW

Number of European quail population, without Russia, is between 640 and 880 thousand pairs. It is evenly dispersed in whole Serbia, from valleys to high mountain meadows, between 1.000 and 1.800 m altitude (Zlatibor, Pešter, Stara Mt., Šara Mt., Prokletije etc.). It is most often in areas with lush pastures and meadows, but it is also nesting at edge of agricultural fields. Its number in Serbia, in reproductive period, is still not investigated enough and general estimates range between 20 and 30 thousand pairs. Universal trend in European population is fluctuation and mild decrease, and it is officially listed as a vulnerable species. Main reasons of decreasing number are plowing of pastures, meadows and fields, pesticide use, excessive hunting using forbidden decovs, and large number of enemies and dangers they encounter during long journey to warmer climate. Our hunting societies would considerably contribute to quail protection if they persist in reduction of predator number (magpies, crows, foxes, cats) and also on preserving natural habitats, lowering use of harmful pesticides and preventing use of decoys during hunting, as well as forbidding setting stubble to fire. Present hunting season for quail in Serbia (August 01 – September 30) is determined well. Although quail is rare in Serbia during November and December, it is nevertheless good to limit hunting during those months. Plans for sustainable annual hunting use should not exceed forty to sixty thousand individuals, certainly without using decoys during hunting.

Quail is the bird that travels different routes for spring and autumn migration. It appears in autumn en masse in Egypt, while in spring months it appears in Tunisia, where is almost absent in fall. It looks it arrives to our parts over Tunisia and Italy, and leaves over Bosporus (Ristić, 2004). It is interesting that Ristić (2004) notes, after Professor A. Toši in article "Quail hunting in Italian sea coast area" published in "Lovac" magazine in 1951, that in this area quail was hunted not only in summer and fall, but also in spring. In those days, Italian Ministry of agriculture allowed quail hunting until May 21, 1950. After that, a wide polemics ensued, and due to permanent Commission for migratory birds of International Council for Game and Wildlife Conservation (CIC), after several years this hunting was banned.

According to Ristić (2004), author Č. Đorđević (1956: "Lovac" No.4) in article "Some notes about a quail", wrote: "Some say that during a migration males fly separate from females. Allegedly, large flocks of males come first, and after two weeks females follow. Whether this is true or not, one is certain – when migration starts, quails go from our fields individually or in a small flocks, and only near Mediterranean Sea they aggregate in large flocks. In such way they fly to Africa continent, and some reach even the Cape of Good Hope".

In their monograph "Prepelice" (Quail), Divjak and Ćeranić wrote: "Quails always return using different route, than in opposite direction. Most of our quails during migration flies over Greece, Dardanelles, Palestine and Suez and arrive in Egypt in large groups, and in Tunisia they are absent at the time. But in spring, Tunisian coast is full of quails, since they return over Mediterranean Sea, Apennine peninsula (Italy) and Balkan peninsula towards northern and central Europe."

In Europe, three main migration routes were noted:

- eastern coast route, from north along North Sea coast, Atlantic coast, along France and Spain towards Morocco;
- 2) Italian-Mediterranean route to Tunisia
- 3) Balkans route over Bosporus, Palestine and Suez to Egypt.

Russian ornithologists add even fourth route, along large river of Volga and Caspian Sea, towards Turkmenistan and north-west Russia. First route is mainly used by birds from Scandinavia and northwest Russia, second by birds from central Europe, third by birds from south Europe and fourth by all kinds of migratory birds from northern Russia.

As for Vojvodina, migratory corridors may not be fully established directly, but only as an increased hunting pressure in certain regions. Highest catch by far in Vojvodina during period observed (1978-1989) was noted in hunters' society in Bačka Topola, with 64,446 quails for 12 years, which is in average 5,370 birds per year. At the second place is Sombor (14,804), and then follow Žitište (14,623), Senta (12,405), Apatin (10,048) and so on.

Due to large number of predators and other dangers during long migration to southern parts, number of quail is notably decreasing every year. This represents a subject of much discussion at international bird-protection conventions, and International league for bird protection has been sent a petition to all southern Europe countries, demanding every government to limit destruction of quail by laws and regulations. Many countries have signed International convention on protection of migratory birds. Nevertheless, this appeal is poorly met, and hunters still widely hunt for this bird because of its tasty meat and attractive ways of hunting.

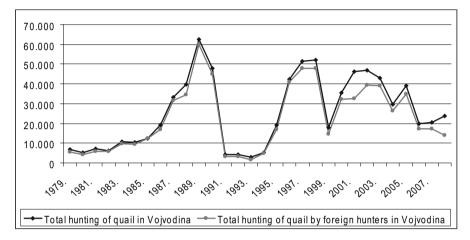
#### RESULTS

For 30-year period observed, between 1979 and 2008, total of 745,498 quails was caught in Vojvodina, or in average 25,552 quails per year. Highest catch was noted in 1989 and lowest in 1993 – 62,425 and 2,837 quails, respectively.

Foreign tourist hunters, in the same period, have caught 675,472 quails or in average 22,516 per year. Based on catch in foreign hunting tourism, it is evident that a number of quails caught was rising between 1979 and 1989, when a peak was noted in foreign hunting

tourism – 59,999 quails. Lowest catch was in 1993 with only 1,758 quails caught. In 1979-1990 period, total of 241,261 quails was caught by foreign tourist hunters, which is in average 20,105 quails per year. Since 1991, catch was oscillating up to year 2008. In this period total of 434,211 quails was caught, or in average 24,123 quails per year.

Average percent of quails caught in hunting tourism, compared to total number caught in Vojvodina was 88.24% for given period. This leads to conclusion that foreign tourist hunters are the most interested in hunting quail, while domestic hunters are not so much interested in hunting this attractive species. Domestic hunters caught 90,026 these birds in a 30-year period, or in average only 3000 per year.



Graph 1. Total number of bagged quails in Vojvodina throught hunting -tourism for the period from 1979. to the year 2008.

Grafikon 1. Ukupan odstrel prepelica i odstrel u lovnom turizmu u Vojvodini za period od 1979. do 2008. godine.

#### DISCUSSION

General wealth of bird species in Serbia is considerable. Up to now a total of about 360 species was noted, from which 333 species are permanently present. In Vojvodina, there are 320 species, from which 188 are nesting, or 131 without water birds (Vasić, 1995). From these, 260 species or 72% are reproducing in Vojvodina, while in winter period 220 species are present. Since even 274 bird species in hunting grounds of Serbia are listed as rare in nature, hunters are responsible to obey laws and regulations and protect endangered and rare species. Special attention must be paid to identification of permanently protected game species and to obeying regulated hunting seasons. Hunters' societies must organize actions to reduce number of game predators without jeopardizing permanently protected game species.

Main ways of bird diversity protection and management in Serbia and Vojvodina are to identify priority activities/projects, to coordinate methods of collecting and organizing data, to develop central databases, to promote sustainable biodiversity management (hunting tourism), to apply global conventions for bird and nature preservation, to enhance international cooperation etc. Special attention must be paid to rare, endangered and disperse species, as well to most valuable international bird habitats in Serbia (IBA areas). There are 35 such areas with 7.670 km<sup>2</sup> area.

Importance of bird game in Europe in general, and therefore in Serbia, has been rising during last decades. This may be connected to depleting number of attractive mammals and decreasing number of some, previously numerous game bird species. Game birds present in Serbia and Vojvodina during migration or wintering mostly belong to ordo Anseriformes, and then come bald coot (*Fulica atra*), quis (*Scolopax rusticola*), ringdove (*Columba palumbus*), turtledove (*Streptopelia turtur*) and **quail (***Coturnix coturnix***).** In Serbia territory, there are over 100 bird species that may be listed as a water habitat birds, and among them most important are from family Anatidae (27 species) and Scolopacidae (22 species).

Table 1. Review of most important game bird species in Serbia and Vojvodina (Puzović S, 2001) Tabela 1. Pregled posebno značajnih vrsta pernate divljači u Srbiji i Vojvodini (Puzović S, 2001)

| Species                           | Estimated<br>number<br>of specimens<br>wintering in<br>Serbia | Estimated number of nesting pairs in Serbia (1996-2000)    |                         | Annual catch in<br>Serbia<br>(1996 - 2000)            | Plan of sustainable<br>annual hunting<br>management<br>in Serbia        |  |
|-----------------------------------|---|--|-------------------------|---|---|--|
|                                   | (1996-2000)<br>January  | Serbia<br>88.361 km2                                       | Vojvodina<br>21.526 km2 | (specimens)   | (2001 - 2010)<br>(specimens)  |  |
| Vrsta                             | Procena broja<br>jedinki na<br>zimovanju u<br>Srbiji          | Procena broja gnezdećih parova<br>u Srbiji<br>(1996-2000.) |                         | Okvirni godišnji<br>odstrel u Srbiji<br>(1996 - 2000) | Plan održivog godišnjeg<br>lovnog korišćenja u Srbiji<br>(2001 - 2010.) |  |
|                                   | (1996-2000.)<br>januar  | Srbija<br>88.361 km2                                       | Vojvodina<br>21.526 km2 | (1990 - 2000)<br>(jedinki)                            | (2001 - 2010.)<br>(jedinki)   |  |
| Quail<br>Coturnix<br>coturnix     | 0   | 20.000-<br>30.000  | 10.000-15.000           | 20.000-30.000   | 40.000-60.000<br>*further restrictions?                                 |  |
| Prepelica<br>Coturnix<br>coturnix | 0   | 20.000-30.000  | 10.000-15.000           | 20.000-30.000   | 40.000-60.000<br>*dalja ograničenja?                                    |  |

General factors causing population decrease, i.e. depleting number of birds, are mostly changes due to agriculture, changes in ways of soil cultivation and usage, application of mechanical and chemical measures, land consolidation, choice of cultivars, leaving land untilled, changing ways of cattle breeding etc. More than 50% of bird species is tied to habitats that are subject to these changes. Intensive agriculture influences about 40% of bird species, and their populations are shrinking. These changes may be listed as:

- 1) Losing habitats depletion, shrinking, degradation and losing basic characteristics of a habitat, especially depletion and degradation of water habitats.
- 2) Pollution indirect or direct introduction of harmful substances into ecosystem.
- 3) Hunting pressure and harrying cause is primarily in poor application of predator-erasing measures, or deficient law regulation and misinformation, including authorized and unauthorized predator-erasing which influences negatively about a third of bird species in our country. Hunting pressure especially affects migratory species. There is also considerable inadvertent harrying and disturbing of animals, even in good intentions to observe and take pictures.

- 4) Unbalanced use overexploitation of all game birds populations, in order to sell or to use as a food.
- 5) Climate changes long-term changes of temperature and humidity are also potentially negative factors for all bird game species.

## CONCLUSION

Strict definition of parameters for sustainable hunting management is of high importance for population management of bird game that is present in Serbia during migration and wintering. Those parameters should address following problems: 1. Present and desired state of population; 2. Possibilities to increase population; 3. Habitat vitality; 4. Estimating population percentage that may be used in long-term sustainable way; 5. Possibility to increase habitat capacity; 6. Decreasing negative factors effect; 7. Possibility of substitution and relieving hunting pressure; 8. Sustainable level of usage and hunting methods; 9. Areas with allowed hunting; 10. Periods of free hunting; 11. Ways and methods to monitor effects of measures undertaken; 12. Plan changes and corrections; 13. Species status according to law regulations (hunting/environment protection).

Priority is to define new status of bird game, with strict grouping according to possibilities and needs of their hunting usage and protection. Therefore, special attention will be paid to: 1. Present game species and their present usage and protection; 2. Promotion of new, potentially important game species; 3. Promotion of present, currently under-used game species.

Within basic aims, important segment of future efforts must be pointed to defining following population, ecological and hunting issues: 1. Investigation of local populations and trends; 2. Inventory of significant habitats (with classification, determination of spatial dispersion and area, importance and productivity for each species, present hunting usage, negative factors, necessary control and protection measures); 3. Investigation of spring/fall migration and wintering (determination of composition and number of bird game species at every significant habitat, for every season); 4. Investigation of hunting pressure (known, real, potential, possible/optimal); 5. Preparation of management program with sustainable hunting usage.

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# GEOGRAFSKI USLOVI PRISUTNOSTI PREPELICA U LOVIŠTIMA VOJVODINE

## RISTIĆ A. ZORAN, BRANKO RISTANOVIĆ, MILOSAVA MATEJEVIĆ

## Izvod

Cilj ovog rada je da prikaže kakvo je stanje prepelica u Evropi i kod nas, da iz postojeće literature i dugoročnih programa se vidi pregled o planskom i racionalnom odstrelu, odnosno lov koji se zasniva na održivom korišćenju. U radu su izneti počeci njenog lova, sa najvećom ekspanzijom, kakvo je trenutno stanje, kao i perspektiva šta činiti u narednom periodu. Da bi se sačuvala ova veoma lepa i korisna poljska koka potrebno je da joj se obezbedi stanište (što više lucerišta, ostavljanje u letnjem periodu strnjišta i sl.), kao i smanjivanje svih vrsta predatora na podnošljiv broj, a lov samo u dozvoljenim kvotama, na osnovu usvojenih planskih dokumenata (Dugoročnih programa, lovnih osnova i godišnjih planova gazdovanja lovištima). Zabrana bilo kakve upotrebe vabilica u lovištima.

U sklopu osnovnih ciljeva, značajan segment rada u narednom periodu treba da bude usmeren ka definisanju sledećih značajnih populacionih i lovnih pitanja: 1. Istraživanje zavičajne populacije i trendova; 2. Inventarizacija značajnih staništa; 3. Istraživanje prolećne - jesenje seobe (utvrđivanje sastava i brojnosti prepelica na svakom značajnom staništu, po sezonama); 4. Istraživanje veličine lovnog pritiska prepelica, što je dato i u ovom radu za staništa u Vojvodini. Za period od 1979 do 2008. godine, odnosno za period od 30 godina, u Vojvodini je ukupno odstreljeno 765.498 prepelica ili prosečno godišnje 25.552 prepelice. Najveći odstrel u odnosu na posmatrani period zabeležen je 1989. godine, 62.425 preopelica i najmanji 1993. godine 2.837 prepelica. 5. Izrada programa upravljanja i održivog korišćenja.

Ključne reči: prepelica, lov, divljač.

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# INTRODUCTIONS TO AUTHORS ON WRITING PAPERS FOR THE JOURNAL "CONTEMPORARY AGRICULTURE"

The journal "Contemporary Agriculture" publishes original scientific papers, surveys and former reports.

A paper is written in English. It should comprise a short summary in English. The whole script of the paper, including tables, graphs, schemes, drawings and photographs, can have 6 typed pages at the maximum, Portrait, in single spacing. Margins:Top 2.0cm,Left 4.2 cm, Bottom 8.7 cm, Right 4.2 cm. For typing the paper the Times New Roman font, 10 pt, should be used. Justify with the indent of the first line 0.6 cm. (Portrait - Paragraph – Indents and Spacing – Special – First Line 0.6. No pagination.

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*Key words* : minimum 3 and maximum 6 words. Below the summary, Font Size 10.

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**DISCUSSION** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the title.

**CONCLUSION** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the title.

LITERATURE (bold), centred, Font Size 10.

STANCIC, B., GRAFENAU, P., PIVKO, J., OBERFRANC, M., BUDINCEVIC, A., SAHINOVIC, R. : The ovulation and fertility in suckling pigs at the synchronization of estrus with Regumate, Biotechnology in livestock breeding , 16(3-4)49-54,2000.

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After the literature a short table of contents should be written in English as follows:

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Graph. 1. Sperm concentration in ejaculates according to seasons of year (Font Size 9, italic).

Citing the authors in the paper: Stančić et al., 2005) – if there are more than two authors. If there are only two authors, then - (Stančić and Šahinović, 1995). Or - Stančić et al. (2005).

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The papers should be sent to the following address : The editor's office of the journal "Contemporary Agriculture" The Faculty of Agriculture 8 D. Obradovića 8 Square 21 000 Novi Sad *Phone:* ++ 021/450-355

We are grateful to all the authors for their cooperation.

Editor - in - chief Prof.dr Milan Krajinović

# UPUTSTVO AUTORIMA ZA PISANJE RADOVA U ČASOPISU »SVREMENA POLJOPRIVREDA«

U časopisu »Savremena poljorivreda«, objavljuju se originalni naučni radovi, pregledni radovi i prethodna saopštenja.

Rad se, u celosti, piše na engleskom jeziku. Treba da sadrži i kratak izvod na srpskom jeziku (Izvod). Celokupan tekst rada, uključijući tabele, grafikone, sheme, crteže i fotografije, može da ima maksimalno 6 kucanih stranica, A4 formata (Portrait), normalnog proreda (Single Space). Margine: Top 2,0cm, Left 4,2cm, Bottom 8,7cm, Right 4,2cm. Za kucanje rada koristiti font Times New Roman, 10pt. Justify poravnanje sa uvlakom prvog reda 0,6cm (Format  $\rightarrow$  Paragraph  $\rightarrow$  Indents and Spacing  $\rightarrow$  Special  $\rightarrow$ First Line 0,6). Bez paginacije (numerisanja stranica rada).

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Key words: minimalno 3, a maksimalno 6 reči. Ispod izvoda, Font Size 10.

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STANČIĆ, B., GRAFENAU, P., PIVKO, J., OBERFRANC, M., BUDINČEVIĆ, A., ŠAHINOVIĆ, R.: Ovulacija i fertilitet nazimica kod sinhronizacije estrusa preparatom Regumate. Biotehnologija u stočarstvu, 16(3-4)49-54, 2000.

Redosled radova je po abecednom redu početnog slova prezimena prvog autora, bez numeracije! Tekst literature Font Size 9.

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