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

# CONTEMPORARY AGRICULTURE
# *SAVREMENA POLJOPRIVREDA*

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*Srpski časopis za poljoprivredne nauke*

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# DETERMINING THE PRESENCE AND SPREAD OF PORCINE INFECTION WITH HAEMOTROPHIC MYCOPLASMAS ON OUR FARMS*

BRANISLAV LAKO, ALEKSANDAR POTKONJAK, MLADEN GAGRČIN, BRANISLAVA BELIĆ, OGNJEN STEVANČEVIĆ, IVANA DAVIDOV, BOJAN TOHOLJ[1]

*SUMMARY: Mycoplasma suis is the cause of an immunologically mediated haemolytic anaemia with pigs. This disease is zoonosis and has a big economical impact on pig breeding. Eperythrozoonosis of the pigs is a disease that is spread worldwide and pigs of all ages suffer from it. As a material for this research, porcine peripheral blood smears and full blood with EDTA have been used. The overall number of the units involved in the research was 150. Methods of microscopic examination of the peripheral blood stained in Giemsa and with Acridine orange method findings of haemoplasmas in porcine blood have been used. Classical PCR method has been used to confirm the microscopic findings of haemoplasma in pig's blood. The presence of Mycoplasma suis has been determined among pigs on the examined pig farms in the Republic of Serbia. The prevalence of the pigs infected with haemoplasmas, determined through a microscopic examination of the peripheral blood smear according to Giemsa is 39% and with Acridine orange is 47%.*

***Key words:*** *Mycoplasma suis, Eperythrozoon suis, infection, pigs, Giemsa, Acridine orange.*

## INTRODUCTION

Haemotrophic mycoplasmas (HM) are highly specialised bacteria that are localised

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Original scientific paper / *Originalni naučni rad*

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on the surface of erythrocites of various mammals, leading to their damaging. These causes cannot be cultivated in *in vitro* conditions (Messick 2004). Primarily they were classified as two genus, *Eperythrozoon* and *Haemobartonella*, within the order of *Rickettsiales*. The reclassification took place when a close phylogenetic and phenotypic connection between these epicellular bacteria with mycoplasms was noticed, especially with the *pneumoniae* group of mycoplasma (Messick et al., 2002; Neimark et al., 2002; Tasker et al. 2003). Haemotrophic mycoplasms are pleomorphic little bacteria, 380-600 nm in diameter. Due to their epierythrocytic localisation, they are seen on erythrocytes as coca, sticks or rings that form jaggedness (Messick, 2004). In the smears of peripheral blood stained in Giemsa or with Acridine orange. HM is seen on the surface of erythrocytes as separate round forms of around 0.5 μm in diameter. Besides, HM are seen free floating in blood plasma in a smaller or greater numbers depending on the sort (Neimark et al., 2001; Hoelzle, 2008).

*Mycoplasma suis* (previously known as *Eperythrozoon suis*) is the cause of immunologically mediated haemolytic anaemia amongst pigs (porcine eperythrozoonosis). Wu says that the porcine eperythrozoonosis is, in actual fact, a zoonosis, and also that it is of immense economic importance for pig farming (Wu et al., 2006). This disease is spread all over the world and is registered amongst all pig categories (Messick, 2004). Ritzmann says that the prevalence of the infection in Germany, amongst stalled hogs is 13.9%. Chinese authors report an overall morbidity of 30% and mortality of 10-20% (Wu et al., 2006). In natural conditions, the agent is, for the most part simply transferred mechanically. Intrauterine transmission has been described, too, and it is considered that the disease is spread through vectors (pig louse, *Haematopinus suis*) (Hoelzle, 2008). Prullage mentions that the agent of this disease can in natural environment be mechanically transferred by flies (*Stomoxys calcitrans*) and by mosquitoes (*Aedes aegypti*) (Prullage et al., 1993). Recently, Groebel first points out to an intraerythrocytic localisation of *Mycoplasma suis*, which can explain the lack of the immunological response by the agent, inadequate therapeutic response of a unit to antibiotic and establishing an infection that takes place in a chronic flow or latent infection (Groebel et al., 2009). Upon natural infection the incubation period can be very long and depends on whether a unit is prone to infections, to the intensity of the infection and the influence of non-specific factors. It has been noticed that after the natural infection has taken place, the pig does not show clinical signs for a couple of months, and also that certain units do not develop a clinical picture at all, i.e. that only a latent infection takes place. With experimentally infected pigs that have undergone splenectomy, the incubation period varies from 2 to 10 days.

The disease was first recognised by Kinsley in the USA in 1932 and described it as icteroanaemia of pigs with icterus, "diluted" blood, haemorrhages in lungs, heart and kidneys, splenomegaly and yellow colouring of an enlarged liver. The acute flow of the infection is characterised by bacteraemia and haemolytic anaemia which can, sometimes, have lethal outcome with piglets, stalled hogs or gravid sows. From the epidemiological point of view the really important aspects are the chronic flow and subclinical form of infection, considering the resistance and spreading of the infection amongst the pig population. During these flows the disease can be completely asymptomatic or with the occurrence of anaemia, icterus, slow body growth and development and reproductive dysfunctions (Hoelzle, 2008). Pereyra reported co-infection of *Mycoplasma suis* and

PCV-2 which is the cause of the syndrome of multi-systematic weakness of piglets after post weaning (Pereyra et al., 2006).

The laboratory diagnostics of the pigs infected with *Mycoplasma suis* is based on the microscopic examination of the chemically stained smear of porcine peripheral blood or on the application of the methods of molecular biology that are more sensitive and specific (Hoelzle, 2008). However setting the diagnosis is traditionally based on the epidemiological survey, clinical examination and microscopic examination of the stained smear of the pigs' peripheral blood, although tests like PCR have been developed (Wu et al, 2006). Apart from the application of direct techniques that prove the presence of the agent, serological methods are very important for the diagnosing of the disease in pigs due to their low cost and possibilities for mass production and application. To this day, there has been not a single commercial test for serological diagnostics of porcine infection with *Mycoplasma suis*, and the research and development of serological tests have been based on three methods: RVK, ELISA and the test of indirect hemmaglutination. For these serological tests, non-defined antigens of *Mycoplasma suis* have been used, which is an obstacle in establishing a precise diagnosis (Hoelzle, 2008). Zhang reports that ELISA is the most sensitive, most specific and reliable test for diagnosing eperythrozoonosis of pigs compared to the test of indirect hemmaglutination, and that when applying this test there is not cross-reactivity with the serums of pigs suffering from enzootic pneumonia, classical plague, colibacillosis and toxoplasmosis (Zhang et al., 2008).

Since, until today, a commercial serological test has not been developed that would diagnose eperythrozoonosis in pigs, the aim of this paper is to determine the presence and prevalence of the porcine infection with chemotropic mycoplasmas on our farms, so that exception of the cause can take place as well as characterisation of the antigens which would be used for the preparation of the serological tests ELISA and WB.

## MATERIALS AND METHOD

The materials used in this research are peripheral blood smears, and full blood with EDTA, that have been collected from pigs on two farms in the South Bačka Region (Farm A and Farm B), in a farm in a North Bačka Region (Farm C) and on one farm in Braničevo Region (Farm D) in the Republic of Serbia. The overall number of units involved in the research was 150 (144 units in Farm A, 46 on Farm B, 30 units on Farm C and 30 units on Farm D).

Right after the aseptic puncture of veins, two "fresh" smears of peripheral blood on microscope glass slides of standard size were made from each unit involved in the research. After being dried for 24 hours, the smears of the peripheral blood have been fixed in methanol for two minutes. One fixed smear of peripheral blood was stained in Giemsa (G) solution during a ten-minute period and then it was rinsed in water and left to dry. The other fixed smear of peripheral blood was stained with the solution of Acridine orange (AO) in a citrate buffer 1:20000 with pH of 3.5 for the period of 30 minutes. Then the smear of peripheral blood was rinsed with water and prepared for UV microscopy. The peripheral blood smears stained in Giemsa solution were examined using light microscopy, 1000 times enlarged. The smears of peripheral blood stained with Acridine orange solution were examined using UV microscopy, 400 times enlarged.

To confirm the microscopic findings of haemoplasmas in porcine blood, a method of classical PCR has been used, with specific primers for Mycoplasma suis (msg1-Fw 5' ACAACTAATGCACTAGCTCCTATC-3' i msg1-Rw 5'-GCTCCTGTAGTTGTAGGA ATAATTGA-3')

## RESULTS

The microscopic examination of the smear of peripheral porcine blood has confirmed the presence of haemoplasma in the population of the examined pigs in the Republic of Serbia.

The microscopic examination of peripheral porcine blood stained with Gims on Farm A has proven the presence of haemoplasma with 18 units; on Farm B with 10 units; on Farm C with 5 units and on Farm D with 20 units. The same examination has proven the presence of haemoplasma on Farm A with 22 units; on Farm B with 34 units; on Farm C with 22 units and on Farm D with 6 unites (Table 1, Graph 1, Picture 1).

The microscopic examination of peripheral porcine blood stained with Acridine orange has identified the presence of haemoplasma on Farm A with 20 units; on Farm B with 13 units; on Farm C with 10 units and on Farm D with 22 units. The same examination has not proven the presence of haemoplasmas on Farm A with 20 units; on Farm B with 31 units; on Farm C with 17 units and on Farm D with 4 units (Table 1, Graph 2, Picture 2).

The total of 13 smears of peripheral porcine blood was inadequate for the microscopic examination (Farm A – 4; Farm B – 2; Farm C – 3 and Farm D – 4).

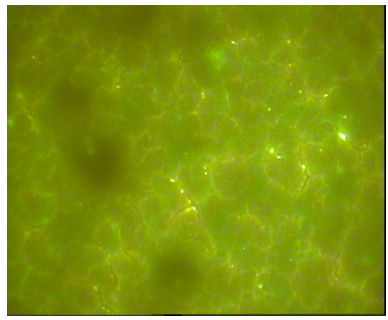
The overall prevalence of the porcine infection with haemoplasmas, determined by the microscopic examination of the peripheral blood smears stained in Giemsa on the examined farms is 39% (Graph 3) and the one stained in Acridine orange is 47% (Graph 4).

The positive microscopic finding of haemoplasmas in peripheral porcine blood smears has been confirmed through the application of the PCR method and the presence of the agent of *Mycoplasma suis* has been proven in the blood of pigs on our farms.

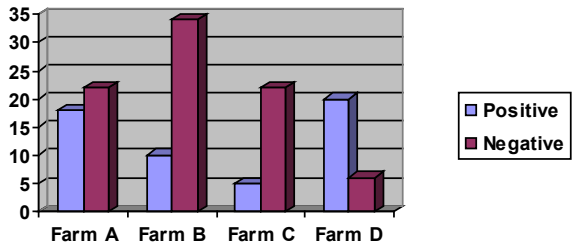
Table 1. Results of the microscopic examination of the smear of peripheral blood smears stained in Giemsa and Acridine orange for the presence of haemoplasmas.

| | Total samples | Total samples | G | | AO | | Prevalence % | |
|---|---|---|---|---|---|---|---|---|
| | | | + | - | + | - | G | AO |
| A | 44 | 4 | 18 | 22 | 20 | 20 | 45.0 | 50.0 |
| B | 46 | 2 | 10 | 34 | 13 | 31 | 23.8 | 30.9 |
| C | 30 | 3 | 5 | 22 | 10 | 17 | 18.5 | 37.0 |
| D | 30 | 4 | 20 | 6 | 22 | 4 | 76.9 | 84.6 |
| Total | 150 | 13 | 53 | 84 | 65 | 72 | 39.0 | 47.0 |

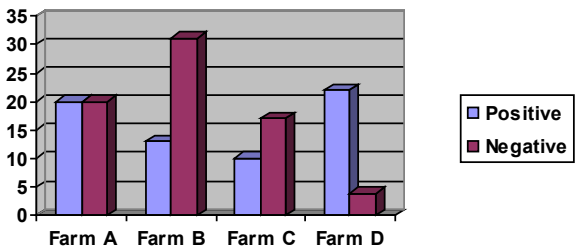
Picture 1. Mycoplasma suis on the surface of an erythrocyte in the peripheral porcine



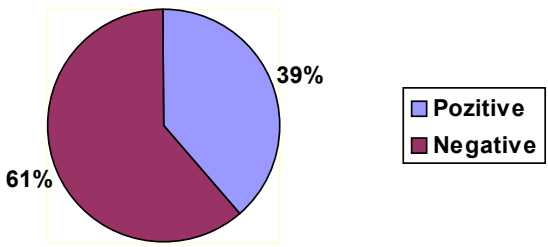
Picture 2. Mycoplasma suis on the surface of an erythrocyte in the peripheral porcine



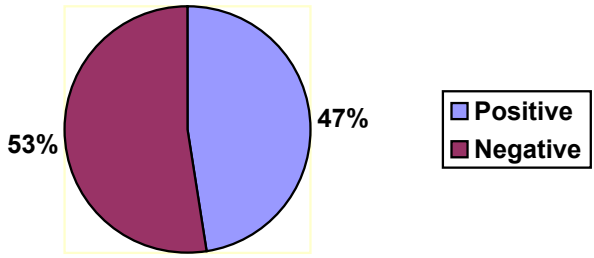

Graph 1. Results of the microscopic examination of the peripheral porcine blood smear stained in Giemsa for the presence of haemoplasmas.




Graph 2. Results of the microscopic examination of the peripheral porcine blood smear stained in Acridine orange for the presence of haemoplasmas.




Graph 3. Results of the overall prevalence of porcine infection with haemoplasmas, determined in a microscopic examination of the peripheral porcine blood smear stained in Giemsa.




Graph 4. Results of the overall prevalence of porcine infection with haemoplasmas, determined in a microscopic examination of the peripheral porcine blood smear stained in Acridine orange.

## DISCUSSION

*Mycoplasma suis* is the cause of an immunologically induced haemolytic anaemia with pigs. This disease is a zoonosis and has a vast economic impact on pig breeding. Eperythrozoonosis of pigs is a worldwide spread disease and is seen among all age categories of pigs (Messick, 2004). The presence of the disease has so far been registerd in America, Europe, Africa, Russia and China. It is considered that the disease is present in other countries and also that it is not directly connected to the climatic conditions (Li et al. 2008). Ritzmann reports that the prevalence of the infection in Germany, in the population of the stalled hogs, is 13.9%, and that the infection is present on 40.3% of pig farms (Ritzmann et al., 2009). Guimaraes has shown that the prevalence of the of the porcine infection with M. suis in Brasil is from 13% to 24% (Guimaraes et al., 2007).

Using a microscopic examination of the peripheral porcine blood smear we have confirmed the presence of *Mycoplasma suis* among the populations of examined pigs in the Republic of Serbia.

We have confirmed an overall prevalence of the infection of pigs with haemoplasmas with a microscopic examination of the peripheral blood stained in Giemsa on the examined farms that is 39%, and 47% in Acridine orange which corresponds to the results of other authors.

## CONCLUSION

Based on the results acquired in this research the following can be concluded:

1. *Mycoplasma suis* infection is present among the population of the examined pigs on the examined pig farms in the Republic of Serbia.
2. The prevalence of the infection with hemoplasmas, determined upon a microscopic examination of the peripheral blood smear stained in Giemsi on the examined farms is 39% and in Acridine orange it is 47%.
3. A laboratory diagnostic protocol has been established for this porcine infection.

Further research work is necessary into the haemotophic mycoplasm with regards to the: possibility of the participation of haemoplasmas as co-factors in the progression of retroviral, neoplastic and immunologically mediated diseases; not knowing the factors

of virulence and pathogen mechanisms in the development of these infections; as well as the need to understand the function of the immunological system which is, in this case, responsible for the occurrence of the opportunistic infections.

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# UTVRĐIVANJE PRISUSTVA I RAŠIRENOSTI INFEKCIJE SVINJA SA HEMOTROPNIM MIKOPLAZMAMA NA NAŠIM FARMAMA

BRANISLAV LAKO, ALEKSANDAR POTKONJAK, MLADEN GAGRČIN, BRANISLAVA BELIĆ, OGNJEN STEVANČEVIĆ, IVANA DAVIDOV, BOJAN TOHOLJ

**Izvod**

Mycoplasma suis je uzročnik imnunološki posredovane hemolitičke anemije kod svinja. Ovo oboljenje je zoonoza i ima veliki ekonomski značaj za svinjarstvo. Eperitrozoonoza svinja je oboljenje raširenо širom sveta i pojavljuje se u svim starosnim kategorijama svinja. Kao materijal za ovo istraživanje korišćeni su razmazi periferne krvi svinja i puna krv sa EDTA. Ukupan broj jedinki uključenih u istraživanje je bio 150. Korišćene su metode mikroskopskog pregleda razmaza periferne krvi obojenih po Giemsa i sa Acridine orange za utvrđivanje prisustva hemoplazmi u krvi svinja. Za potvrdu mikroskopskog nalaza hemoplazmi u krvi svinja, korišćena je metoda klasične PCR. Utvrđeno je prisustvo Mycoplasma suis u populacijama svinja na ispitivanim farmama svinja u Republici Srbiji. Prevalencija infekcije svinja sa hemoplazmama, utvrđena mikroskopskim pregledom razmaza periferne krvi obojenih po Giemsa na ispitivanim farmama isnosi 39 %, a sa Acridine orange iznosi 47 %.

**Ključne reči**: Mycoplasma suis, Eperythrozoon suis, infekcija, svinje, Gimsa, Acridine orange

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# ASSOCIATION PANICO-PORTULACETUM OLERACEAE LOZ. 1962 IN ORGANIC PRODUCTION OF CRUCIFERS*

NIKOLIĆ LJILJANA, KNEŽEVIĆ ALEKSA, STOJANOVIĆ SLOBODANKA, DŽIGURSKI DEJANA, LJEVNAIĆ-MAŠIĆ BRANKA[1]

*SUMMARY: The floristic-phytocoenological study of weed in organic production of crucifers, the Brassicaceae family, was performed during the 2008 growing season in the village of Kisač, the Vojvodina Province. Stands of the association Panico-Portulacetum oleraceae Loz. 1962, the alliance Polygono-Chenopodion Koch 1926. em, Sissing 1946, the order Chenopodietalia albi Tx., Lohm. et Prsg. 1950, the class Stellarietea medie Tx., Lohm. et Prsg. 1950 were registered in the studied site. Twenty-one plant species make the floristic structure of the community. The maximum cover value was found for Portulaca oleracea L. (1130), the characteristic and dominant species of the association. Ambrosia artemisiifolia L. (370) was distinguished among the associated species. Species of wide distribution predominated in the chorological spectrum. The analysis of the represented life forms indicated a terophytic character of the described community.*

***Key words:*** *ass. Panico-Portulacetum oleraceae Loz. 1962, organic production, crucifers.*

## INTRODUCTION

In recent years, organic agriculture has come in the focus of attention because its goal is the production of safe food in addition to biodiversity preservation in agro-ecosystems. Organic agriculture excludes the use of pesticides, growth regulators and mineral fertilizers. On the other hand, it favors the use of organic and microbial fertilizers, taking into account mutual interactions between soil, plants, animals and humans (Manojlović *et al*., 2007; Kovačević, 2008;). Because of the importance of this type of agricultural production, the conditions and principles of organic production are

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Original scientific paper / *Originalni naučni rad*

[1] Dr Ljiljana Nikolić, doc., dr Aleksa Knežević, red. prof., dr Slobodanka Stojanović, red. prof., dr Dejana Džigurski, doc., mr Branka Ljevnaić-Mašić, asis., Faculty of Agriculture Novi Sad.
Corresponding author: dr Ljiljana Nikolić, e-mail: ljnik@polj.uns.ac.rs

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regulated by the Law on Organic Production and Organic Products (Official Gazette of the Republic of Serbia, No. 62/2006).

Vegetable production is an important segment of organic agriculture, which provides a healthy and varied diet for the population. In addition to reduced yields, the organic vegetable production (outdoor and indoor) is associated with almost complete absence of chemical measures of plant protection, increased dependence on the climate, increased input of manual labor, therefore increased job opportunities, increased weed presence and more frequent plant diseases and pests (Lazić, 1998; Kovačević, 2008; Knežević *et al*., 2008a; Knežević *et al.*, 2008b).

The increased presence of weeds in crop production brings negative effects due to a high competition for space, light, water and mineral substances between the cultivated plants – dominant members of agro-phytocoenoses - and weeds (Šinžar and Mišović, 1978).

Starting from the fact that floristic-phytocoenological studies of weed vegetation present in the organic production of vegetables have started recently and that the intensity of weed damage is relatively high, the goal of this paper was to analyze the weed flora in the crucifer crops and establish the vegetation cover status, so that integrated protection of cultivated plants could be applied, which implies the application of agrotechnical measures, crop rotation and biological measures of crop protection.

## MATERIAL AND METHODS

Floristic-phytocoenological study of weeds in the organic production of crucifers (the Brassicaceae or Cruciferae family) was performed during the 2008 growing season according to the methodology of the phytocoenological school Zürich-Montpellier (Braun-Blanquet, 1964) at the Vozars farm located in the vicinity of the village of Kisač (the Vojvodina Province).

The observed taxons were determined according to the publications 'Flora of SR Serbia' (Josifović, 1970-1977) and 'Flora Europaea' (Tutin *et al.*, 1964-1980). Floral elements were presented according ot the publication 'Review of Floristic Species of SR Serbia with Plant-Geographic Designations' (Gajić, 1980), the life forms according to the publication 'Gymnövények' (Ujvárosi, 1973).

The soil type at the studied site was the calcareous black meadow soil on the loess terrace (Živković *et al.*, 1972).

The previous crops to the brassicas had been the table beet (*Beta vulgaris* L. subsp. *esculenta* Salisb.) in 2006 and the lettuce (*Lactuca sativa* L. subsp. *capitata* L.) in 2007.

The following brassicas were grown in the course of the 2008 growing season: the heading cabbage (*Brassica oleracea* L. var. *capitata*) including cultivars with white, red and cone-shaped heads, the kale (*Brassica oleracea* L. var. *sabauda* L.), the Chinese cabbage (*Brassica oleracea* L. v *broccoli* ar. *chinensis*), the cauliflower *(Brassica oleracea* L. var. *botrytis* L.), the romanesco broccoli (*Brassica oleracea* L. var. *botrytis* L. type *romanesco*), the broccoli (*Brassica oleracea* L. var. *botrytis* subvar. *cymosa* / var. *italica*) and the collard (*Brassica oleracea* L. var. *acephala* DC) – Knežević *et al.* 2008b.

## RESULTS AND DISCUSSION

A detailed floristic-phytocoenological analysis of the weed vegetation growing in the crucifer crops indicated the presence of stands of the association *Panico-Portulacetum oleraceae* Loz. 1962 whose syntaxonomic position according to Kojić *et al.* (1998) is as follows:

Class: *Stellarietea medie* Tx., Lohm. et Prsg. 1950
Order: *Chenopodietalia albi* Tx., Lohm. et Prsg. 1950
Alliance: *Polygono-Chenopodion* Koch 1926. em Sissing 1946
***Ass. Panico-Portulacetum oleraceae* Loz. 1962**

According to the studies conducted so far, stands of the *Panico-Portulacetum oleraceae* association are fairly widespread, especially in some parts of the Vojvodina Province and Serbia (the Mačva region, locations in the vicinity of Belgrade). They grow in various row crops (corn, sunflower, sugarbeet, carrots, etc.), as well as in orchards and vineyards across the Vojvodina Province (Kojić and Pejčinović, 1982; Kojić *et al.*, 1998).

Main features of the stands of the examined association are given by the characteristic species of the association, *Panicum crus-galli* L. and *Portulaca oleracea* L. Of the characteristic species of the order *Chenopodietalia* and the class *Stelarietea*, the following species were registered: *Amaranthus retroflexus* L., *Polygonum lapathifolium* L., *Datura stramonium* L., *Solanum nigrum* L., *Chenopodium album* L. *and Sinapis arvensis* L. (Phytocoenological Table 1).

Phytocenological table 1. The plant community Panico-Portulacetum oleraceae Lozanovski 1962
*Fitocenološka tabela 1. Asocijacija Panico-Portulacetum oleraceae Lozanovski 1962*

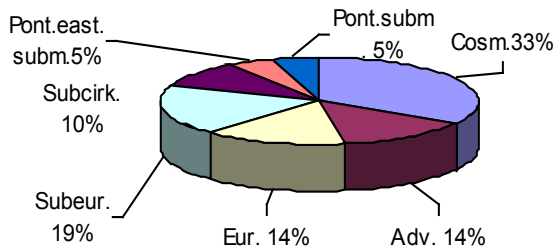
| Floral elements | Life form | | 1 | 2 | 3 | 4 | 5 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Characteristic species of the community** *Panico-Portulacetum oleraceae* | | | | | | Degree of presence | Covering values |
| Cosm. | $T_4$ | *Portulaca oleracea* L. | 3.3 | 2.2 | +.1 | +.1 | +.1 | V | 1130 |
| Cosm. | $T_4$ | *Panicum crus-galli* L. | +.2 | +.1 | +.1 | | +.1 | IV | 40 |
| | | **Characteristic species of the order** *Chenopodietalia* **and class** *Stellarietea* | | | | | | | |
| Adv. | $T_4$ | *Amaranthus retroflexus* L. | 1.1 | +.1 | +.1 | +.1 | 1.1 | V | 230 |
| Subcirk. | $T_4$ | *Polygonum lapathifolium* L. | 1.1 | 1.1 | +.1 | +.1 | +.1 | V | 230 |
| Cosm. | $T_4$ | *Datura stramonium* L. | +.1 | +.1 | +.1 | +.1 | | IV | 40 |
| Cosm. | $T_4$ | *Solanum nigrum* L. | +.1 | +.1 | | +.1 | +.1 | IV | 40 |
| Cosm. | $T_4$ | *Chenopodium album* L. | +.1 | +.1 | | | +.1 | III | 30 |
| Subeur. | $T_3$ | *Sinapis arvensis* L. | +.1 | +.1 | | +.1 | | III | 30 |
| | | **Companions** | | | | | | | |
| Subeur. | $T_4$ | *Sonchus oleraceus* L. | 1.1 | +.1 | | +.1 | +.1 | IV | 130 |
| Eur. | $T_1$ | *Senecio vulgaris* L. | +.1 | +.1 | | +.1 | 1.1 | IV | 130 |
| Subeur. | $G_3$ | *Cirsium arvense* (L.) Scop. | +.2 | +.1 | +.1 | | +.1 | IV | 40 |
| Adv. | $T_4$ | *Ambrosia artemisifolia* L. | | +.1 | | 2.1 | +.1 | III | 370 |
| Cosm. | $G_3$ | *Convolvolus arvensis* L. | +.1 | | +.1 | +.1 | | III | 30 |
| Subevr. | $T_4$ | *Bilderdykia convolvulus* ( L.) Dum. | | +.1 | | +.1 | +.1 | III | 30 |
| Cosm. | $G_1$ | *Sorghum halepense* (L.) Pers. | +.1 | +.1 | | | +.2 | III | 30 |
| Subcirk. | $T_4$ | *Chenopodium hybridum* L. | +.1 | +.1 | | +.1 | | III | 30 |
| Pont.east.subm. | $T_4$ | *Hibiscus trionum* L. | +.1 | | | +.1 | | II | 20 |
| Eur. | $G_1$ | *Agropyrum repens* (L.) P.B. | | | | +.2 | | I | 20 |
| Adv. | $T_4$ | *Amaranthus blitoides* S. Watson | | | | | +.1 | I | 10 |
| Eur. | $T_4$ | *Matricharia inodora* L. | | | | +.1 | | I | 10 |
| Pont.-subm. | $T_4$ | *Heliotropium europaeum* L. | +.1 | | | | | I | 10 |

Legend: Cosm. - Cosmopolitian; Adv. - Adventive; Subcirk. - Subcirkumpolar; Subeur. - Subeurasian; Eur. – Eurasian; Pont.east.subm. - Pontic East Submediterranean; Pont.-subm.- Pontic Submediterranean; T – Terophyta, G – Geophyta.

The association was found to be composed of 21 plant species. High degrees of presence, V and VI (characteristic group), were observed for the following weed species: *Amaranthus retroflexus* L., *Polygonum lapathifolium* L., *Datura stramonium* L., *Solanum nigrum* L., *Sonchus oleraceus* L., *Senecio vulgaris* L., *and Cirsium arvense* (L.) Scop. The highest cover percentage was determined for *Portulaca oleracea* L. (1130), the characteristic species of the association, followed by *Ambrosia artemisiifolia* L. (370), *Amaranthus retroflexus* L. (230), *Polygonum lapathifolium* L. (230), *Sonchus oleraceus* L. (130) and *Senecio vulgaris* L. (130). The other species were present in low numbers.

Plant distribution according to height was evident in the analyzed stands. Three strata could be observed. The lowest stratum (to the height of some 20 centimeters) was composed of: *Portulaca oleracea* L., *Convolvulus arvensis* L. *and Hibiscus trionum* L.. The second, intermediate stratum (to the height of 50 cm) was dominated by *Polygonum lapathifolium* L., *Cirsium arvense* (L.) Scop., *Ambrosia artemisiifolia* L. *and Chenopodium album* L.. *The third stratum,* above 60 cm in height, included the species *Panicum crus-galli* L.,, *Sonchus oleraceus* L., *Sorghum halepense* (L.) Pers., *Amaranthus retroflexus* L. , etc.

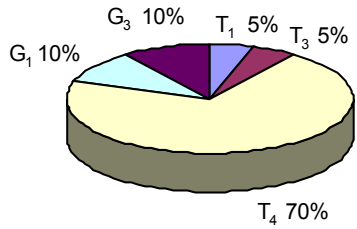
A general characteristic regarding the floristic composition of the association could be seen from the chorological spectrum (Figure 1). The predominant species in the spectrum were those of wide distribution (90%). Cosmopolitan elements were dominant among these species with 33% (7 species). Sub-Eurasian floristic elements were present with 19% (4 species), Eurasian with 14% (3 species), adventive also with 14% (3 species) and sub-circumpolar with 10% (2 species). Only two species belonged to floral elements of narrow distribution (Pontic−sub-Mediterranean and Pontic−east-sub-Mediterranean). Therefore, we may conclude that the described stands of the Panico-Portulacetum oleraceae association were predominated by species of wide distribution, which is one of the basic characteristics of weed communities (Kojić et al., 1998).




Graph. 1. Chorological spectrum of the association Panico-Portulacetum oleraceae Loz. 1962
*Graf. 1. Spektar areal tipova asocijacije Panico-Portulacetum oleraceae Loz. 1962*

The biological spectrum of the association provides a comprehensive picture of the environmental conditions and characteristics of the analyzed sites (Figure 2). Dominance of therophytes was evident in the biological spectrum (80%; 17 plant species). Most frequent among these were annual weeds that germinate in the spring and produce seed at the end of summer - T4 therophytes (70%; 15 plant species). The characteristic species of the association belong to this group. Geophytes (G1 and G3) were represented by 4

plant species (20%). The obvious therophytic character of the analyzed association is a typical characteristic of communities of segetal weeds (Kojić et al., 1988; Knežević and Baketa, 1990).




Graph. 2. Biological spectrum of the association Panico-Portulacetum oleraceae Loz. 1962
*Graf. 2. Biološki spektar asocijacije Panico-Portulacetum oleraceae Loz. 1962*

The stands of the association found in the organic production of crucifers were relatively poor in terms of flora. They were characterized by low frequencies of the species characteristic for the order and class, and a complete absence of the characteristic species of the alliance, which is definitely a consequence of intensive anthropogenic influence and which is in accordance with literature data (Knežević and Baketa, 1990).

Crucifers are grown in thin stands, which encourage the development of stands of the described association. Therefore, it is recommended to monitor the development of stands of this association and to control them, especially during early stages of development, while the cultivated plants are too small to successfully compete against weeds for basic environmental factors (light, water, minerals).

## CONCLUSION

Stands of the association *Panico-Portulacetum oleraceae* Loz. 1962., the alliance *Polygono-Chenopodion* Koch 1926. em, Sissing 1946, the order *Chenopodietalia albi* Tx., Lohm. et Prsg. 1950, the class Stellarietea medie Tx., Lohm. et Prsg. 1950 were found in a plot dedicated to the organic production of crucifers located in the vicinity of the village of Kisač, the Vojvodina Province.

Twenty-one plant species comprise a total floristic structure of the community. Distinguished for high degrees of presence, V and IV, were the species *Amaranthus retroflexus* L., *Datura stramonium* L., *Solanum nigrum* L., *Polygonum lapathifolium* L., *Sonchus oleraceus* L., *Senecio vulgaris* L., *and Cirsium arvense* (L.) Scop. The highest cover percentage was found for *Portulaca oleracea* L., the characteristic and dominant species of the association (1130). *Ambrosia artemisiifolia* L., an invasive and highly competitive species, stood out among the accompanying species for high numbers (370). The stands of the analyzed association grew on the calcareous black meadow soil on the loess terrace.

Species of wide distribution predominated in the chorological spectrum of the described association (90%).

A major feature of the community was its therophytic character (80%), with the predominance of T4 therophytes (70%).

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# ASOCIJACIJA *PANICO-PORTULACETUM OLERACEAE* LOZ. 1962 U ORGANSKOJ PROIZVODNJI KUPUSNJAČA

NIKOLIĆ LJILJANA, KNEŽEVIĆ ALEKSA, STOJANOVIĆ SLOBODANKA, DŽIGURSKI DEJANA, LJEVNAIĆ-MAŠIĆ BRANKA

**Izvod**

Floristično-fitocenološka proučavanja korova u organskoj proizvodnji kupusnjača, familija Brassicaceae, obavljena su tokom vegetacionog perioda 2008. godine (Kisač, Vojvodina). Na proučavanom staništu konstatovane su sastojine asocijacije *Panico-Portulacetum oleraceae* Loz. 1962, sveze *Polygono-Chenopodion* Koch 1926. em, Sissing 1946, reda *Chenopodietalia albi* Tx., Lohm. et Prsg. 1950, klase *Stellarietea medie* Tx., Lohm. et Prsg. 1950. Floristički strukturu zajednice čini 21 biljna vrsta. Najveću pokrovnu vrednost ima karakteristična vrsta i edifikator asocijacije *Portulaca oleracea* L. (1130). Među pratilicama ističe se *Ambrosia artemisifolia* L. (370). U spektru areal-tipova dominiraju vrste širokog rasprostranjenja. Analiza zastupljenih životnih formi ukazuje na terofitski karakter opisane zajednice.

**Ključne reči:** ass. Panico-Portulacetum oleraceae Loz. 1962, organska proizvodnja, kupusnjače.

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# EFFECT OF DIFFERENT LEVELS OF NITROGEN FROM PLOWED UNDER HARVEST RESIDUES ON GRAIN YIELD OF CORN

DRAGANA LATKOVIĆ, BRANKO MARINKOVIĆ, MIROSLAV MALEŠEVIĆ, GORAN JAĆIMOVIĆ, DANIJEL JUG[1]

*SUMMARY: Results from an international stationary field trial (ISDV) have been analyzed for effects of increasing nitrogen doses on yield of corn. The trial has been established on a calcareous chernozem soil, at Rimski Šančevi experiment field of the Institute of Field and Vegetable Crops, Novi Sad. The average yield of grain obtained in the variants with plowed under harvest residues was 12.22 t ha. It was higher by 1.02 t ha$^{-1}$ or 9.1% than the average yield obtained in the fertilized variants that received no harvest residues. On average for both analyzed hybrids, the highest average yield (12.76 t ha$^{-1}$) was achieved in the fertilization variant with 150 kg ha$^{-1}$ of nitrogen combined with plowing under of harvest residues. On average, the yield of the hybrid NS 6010 was highly significant as compared with that produced by the hybrid NS 510*

***Key words**: corn, nitrogen, fertilization, plowing under of harvest residues*

## INTRODUCTION

Corn (Zea mays, L.) is a major field crop on the global scale. Yielding potential of corn hybrids was significantly increased during last 50 years, particularly in regions favorable for corn growing (Russel, 1991; Tollenar *et al*., 1994). Yield increases have been achieved thanks to the development of genetically superior hybrids, increased stand density, intensified application of mineral fertilizers, decreased row distance and improved crop protection, in the first place against weeds and pests (Carlone & Russel, 1987., Dwyer *et al*. 1991., Tollenar, 1991).

Nitrogen is a constitutive element of various compounds present in plants. Most of these compounds play important physiological roles – those are proteins, chlorophyll,

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enzymes, chromosomes, nucleic acids (Hageman and Below, 1984). Earl & Tollenar (1997) and Tollenar *et al*. (1994) maintained that nitrogen is essential for leaf area duration in corn, as well as that it tends to increase all yield components of corn.

Harvest residues of field crops comprise voluminous biomass which is important for matter cycling in the agroecosystem, especially when organic fertilizers are not applied in adequate amounts. Regular removal of harvest residues from fields or their burning in combination with irregular application of organic fertilizers may be quite harmful in the long run (Kastori and Tešić, 2006).

Analyzing a period of 18 years, Starčević *et al*. (1999) concluded that a significant increase of corn grain yield was achieved with 110 kg $Nha^{-1}$, was maximum yield was obtained with 195 kg $Nha^{-1}$. Taking into account production economy and nitrogen balance, these authors found that optimum nitrogen amounts ranged from 110 to 150 kg $ha^{-1}$, when harvest residues were plowed under.

## MATERIAL AND METHODS

A stationary field trial was established at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops, Novi Sad, in 1971/72. The trial was established on the calcareous chernozem soil. Effects of increasing nitrogen doses and harvest residues on corn yield were assessed in a three-crop rotation of corn, soybean and wheat. The trial had nine variants, six with and three without plowing under of harvest residues. The experiment included the following variants of nitrogen fertilization (factor A):

a) treatments with plowed under wheat straw: 0, 60, 90, 120, 150 and 180 kg N $ha^{-1}$ (every third year 5 $tha^{-1}$ of absolutely dry wheat straw are plowed under before corn planting; 50 kg N $ha^{-1}$ of fertilizer are added (10 kg of mineral N per 1t of straw) for more efficient decomposition),

b) treatments without plowed under wheat straw: 0, 90 and 150 kg N $ha^{-1}$.

The trial included 2 corn hybrids annually (factor B). This paper reviews 6-year average yields (two rotations) of two currently tested corn hybrids, NS 510 and NS 6010.

Nitrogen was applied two times, one half before basic tillage, another before planting. All variants invariable received equal amounts of phosphorus and potassium, 80 kg of $P_2O_5$ and $K_2O$ $ha^{-1}$. These fertilizers were added before basic tillage.

The data obtained for corn yield (adjusted to 14% moisture) were statistically processed by the analysis of variance for a two-factor split-plot experiment (the statistical software GenStat v.9), testing the significance of differences between treatment means by the LSD test. The effect of increasing nitrogen doses on yield was assessed by the regression analysis and it was shown graphically.

## RESULTS AND DISCUSSION

The F-test showed that fertilization system and hybrid had highly significant effects on grain yield in the experiment ($F_{pr}$<0.001, Table 1). Their interaction (N dose x hybrid) showed no statistically significant effect ($F_{pr}$=0.30), i.e., both hybrids reacted similarly to the increasing N doses. On average for the entire 6-year trial, the yield of grain was 11.88

t ha$^{-1}$. However, the yield of grain in the variants with plowed under harvest residues was 12.22 t ha$^{-1}$ (an average for all variants that received N fertilizer), which was 1.02 t ha$^{-1}$ or 9.1% higher than the average for the variants without harvest residues.

Table 1. Grain yields (t ha$^{-1}$) with different N doses in variants with and without harvest residues
*Tabela 1. Prinos zrna (t ha$^{-1}$) pri različitim dozama N na varijantama sa i bez zaoravanja žetvenihostataka*

| Fertilization variant (A) *Varijanta đubrenja (A)* | | | Hybrid (B) *Hibrid (B)* | | Average(A) *Prosek(A)* |
|---|---|---|---|---|---|
| | No. R. br. | N dose (kg ha$^{-1}$) Količina N (kg ha$^{-1}$) | NS-510 | NS-6010 | |
| With HR Sa zaoravanjem žetvenih ostataka | 1 | 0 | 10.56 | 10.97 | 10.77 |
| | 2 | 60 | 11.64 | 12.35 | 12.00 |
| | 3 | 90 | 12.34 | 12.65 | 12.50 |
| | 4 | 120 | 12.37 | 12.88 | 12.63 |
| | 5 | 150 | 12.22 | 13.30 | 12.76 |
| | 6 | **180** | 12.08 | 13.29 | 12.68 |
| | **Average /*Prosek*** | | **11.87** | **12.57** | 12.22 |
| Without HR Bez žetvenih ostataka | 7 | 0 | 9.58 | 10.61 | 10.09 |
| | 8 | 90 | 11.03 | 12.15 | 11.59 |
| | 9 | 150 | 11.15 | 12.72 | 11.93 |
| | **Average/ *Prosek*** | | **10.59** | **11.83** | **11.20** |
| **Average (B)/*Prosek (B)*** | | | **11.44** | **12.32** | **11.88** |

| | | A | B | BxA | AxB |
|---|---|---|---|---|---|
| **LSD** | **5%** | 0.55 | 0.26 | 0.77 | 0.76 |
| | **1%** | 0.75 | 0.35 | 1.04 | 1.01 |

The highest yield on average for the two hybrids (12.76 t ha$^{-1}$) was obtained in the variant with 150 kg N ha$^{-1}$ combined with plowed under harvest residues. This yield was highly significant in relation to the control variant and the variant with 60 kg N ha$^{-1}$ and plowed under harvest residues, while it was not statistically significant in relation to the variants with 90 and 120 kg N ha$^{-1}$. These results indicate that it is not economically justifiable to apply more than 120 kg N ha$^{-1}$, as had been reported earlier by Starčević *et al*. (1999).

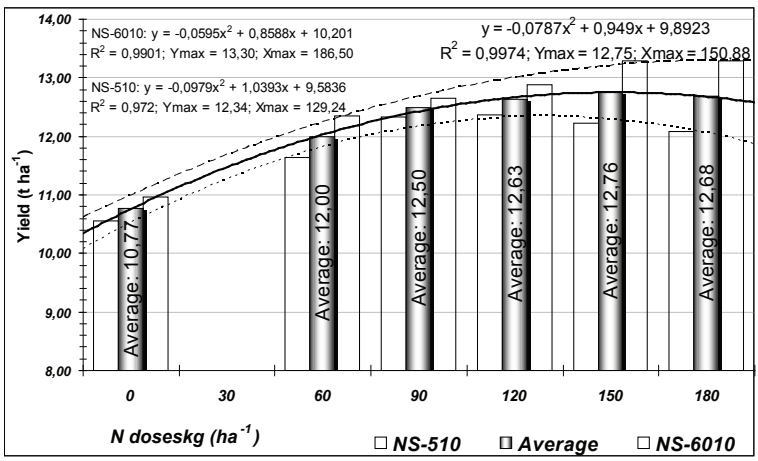
The hybrid NS 6010 had a statistically highly significant yield in relation to the other hybrid, NS 510. In the variant with and without harvest residues, the hybrid NS 6010 out yielded NS 510 by 0.7 tha$^{-1}$ and 1.24 tha$^{-1}$, respectively.

In the variants with plowed under harvest residues, with both hybrids and on their average, the curve for grain yield increase followed the quadratic regression pattern (Graph 1). Based on the calculated regression equations, the theoretically averaged maximum grain yield (12.75 t ha$^{-1}$ on average for the studied hybrids) is obtained with 150.88 kg N ha$^{-1}$ (r2=0.9974). The theoretically maximum yields of the hybrids NS 6010 and NS 510 (13.30 and 12.34 t ha$^{-1}$, respectively) are obtained with 186.50 kg N ha$^{-1}$ and 129.24 kg N ha$^{-1}$, respectively.

The highest grain yield (11.93 t ha$^{-1}$) in the variants without plowed under harvest residues (Table 1) was obtained with 150 kg N ha$^{-1}$. However, this yield was significant

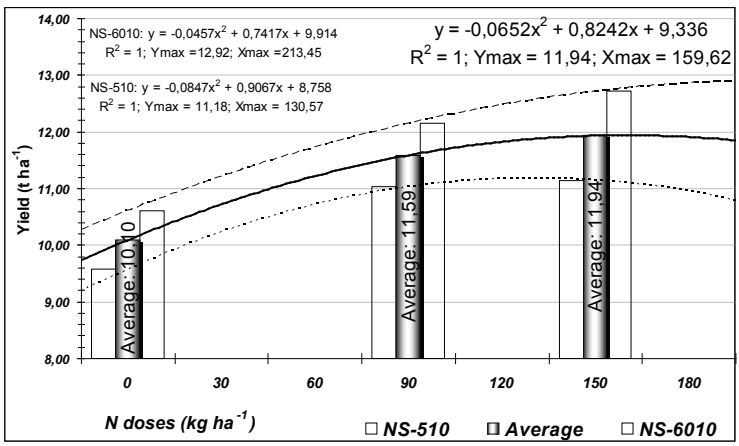
only in relation to the yield in the control variant (10.09 t ha$^{-1}$), and it was not significant in relation to the yield in the variant with 90 kg ha$^{-1}$ (11.59 t ha$^{-1}$).

In the case of the variants without plowed under harvest residues, the curve for the grain yield increase also followed the quadratic regression pattern. On average for both hybrids, the theoretically maximum yield of 11.94 t ha$^{-1}$ would be obtained with 159.62 kg N ha$^{-1}$ (Graph 2). Similar effects of increasing N doses on grain yield of corn were reported by Starčević *et al.* (1999) and Latković & Starčević (2006).




Graph. 1. Effect of nitrogen dose on grain yield of corn in variants with plowed under harvest residues

*Graf. 1. Uticaj količine azota na prinos zrna kukuruza na varijantama sa zaoravanjem žetvenih ostataka*




Graph. 2: Effect of nitrogen dose on grain yield of corn in variants without plowed under harvest residues

*Graf. 2. Uticaj količine azota na prinos zrna kukuruza na varijantama bez zaoravanja žetvenih ostataka*

As shown in Table 2, when comparable variants were considered, the effect of

long-term plowing under of harvest residues on grain yield of corn ranged in dependence of the tested hybrids and nitrogen doses from mere 360 kg (in the case of the hybrid NS 6010 in the control variant) to a significant amount of 1310 kg ha$^{-1}$ (in the case of the hybrid NS 510 in the variant with 90 kg N ha$^{-1}$). On average for the two hybrids, plowed under harvest residues increased the yield of corn grain in the control variant (without N application) and the variant with 90 kg of N by 680 kg ha$^{-1}$ and 910 kg ha$^{-1}$, respectively. In the variant with the most intensive fertilization (150 kgN ha$^{-1}$), the plowed under harvest residues increased the yield of grain by 830 kg ha$^{-1}$.

Table 2. Effect of plowed under harvest residues on grain yield of corn (t ha$^{-1}$)
*Tabela 2. Efekat zaoravanja žetvenih ostataka na prinos zrna kukuruza (t ha$^{-1}$)*

| N dose *Doza azota* | Harvest residues (HR) *Žetveni ostaci* | Hybrid (B) *Hibrid (B)* | | Average *Prosek* |
|---|---|---|---|---|
| | | NS-510 | NS-6010 | |
| 0 kg N ha$^{-1}$ | With HR *Sa zaoravanjem ŽO* | 10.56 | 10.97 | 10.77 |
| | Without HR *Bez zaoravanja ŽO* | 9.58 | 10.61 | 10.09 |
| | **Difference /*Razlika*** | **0.98** | **0.36** | **0.68** |
| 90 kg N ha$^{-1}$ | With HR *Sa zaoravanjem ŽO* | 12.34 | 12.65 | 12.50 |
| | Without HR *Bez zaoravanja ŽO* | 11.03 | 12.15 | 11.59 |
| | **Difference /*Razlika*** | **1.31** | **0.50** | **0.91** |
| 150 kg N ha$^{-1}$ | With HR *Sa zaoravanjem ŽO* | 12.22 | 13.30 | 12.76 |
| | Without HR *Bez zaoravanja ŽO* | 11.15 | 12.72 | 11.93 |
| | **Difference /*Razlika*** | **1.07** | **0.58** | **0.83** |
| Average foe all 3 N doses Prosečno za sve 3 doze N | With HR *Sa zaoravanjem ŽO* | 11.71 | 12.31 | 12.01 |
| | Without HR *Bez zaoravanja ŽO* | 10.59 | 11.83 | 11.20 |
| | **Difference /*Razlika*** | **1.12** | **0.48** | **0.81** |

Literature references mention a number of instances where plowing under of harvest residues is as effective as manure application with respect to soil properties and yield performance improvement. Experiments conducted in various countries including ours have indicated that plowing under of harvest residues affected yield performance and quality (Kastori *et al*., 1990), increased total N and C contents, improved soil fertility and reduced nitrogen leaching (Nicholson *et al*., 1997), and increased grain yield (Ortega *et al*., 2000; Pracházková *et al*., 2002; Silgram *et al*., 2002). Application of nitrogen fertilizers significantly affects the amount of harvest residues whose incorporation increases the humus content and efficiency of carbon retention in the soil (Halvorson *et al*., 1999).

## CONCLUSION

Following conclusions can be drawn on the basis of the study of the effect of increasing nitrogen doses on grain yield of corn in variants with and without plowed under harvest residues.

- The average yield obtained in the variants with plowed under harvest residues was 12.22 t $ha^{-1}$ or higher by 1.02 t $ha^{-1}$ (or 9.1%) in relation to the average of the fertilization variants without plowed under harvest residues.
- The highest average yield for the two hybrids (12.76 t $ha^{-1}$) was achieved in the fertilization variant with 150 kg $ha^{-1}$ of nitrogen combined with plowing under of harvest residues.
- The average yield of the hybrid NS 6010 was highly significant as compared with that produced by the hybrid NS 510.
- In the case of individual hybrids and their average, in variants both with and without plowed under harvest residues, the increase of grain yield in response to the increasing nitrogen doses created a quadratic regression curve.
- In the comparable variants, the effect of long-term plowing under of harvest residues on grain yield of corn ranged from only 360 kg of grain (in the case of the hybrid NS 6010 in the control variant) to a significant amount of 1310 kg $ha^{-1}$ (in the case of the hybrid NS 510 fertilized with 90 kg N $ha^{-1}$).

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## EFEKAT ZAORAVANJA ŽETVENIH OSTATAKA PRI RAZLIČITIM KOLIČINAMA AZOTA NA PRINOS ZRNA KUKURUZA

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### Izvod

U radu su analizirani rezultati iz stacionarnog međunarodnog poljskog ogleda (ISDV) sa rastućim dozama azota u đubrenju kukuruza. Prinos zrna dobijen na varijantama sa zaoravanjem žetvenih ostataka iznosio je u proseku 12.22 t $ha^{-1}$ i bio je za 1.02 t $ha^{-1}$ (odnosno za 9.1%) veći u odnosu na prosek varijanti đubrenja bez zaoravanja žetvenih ostataka. Najveći prinos u proseku za oba ispitivana hibrida (12.76 t $ha^{-1}$) dobijen je pri đubrenju sa 150 kg $ha^{-1}$ azota uz zaoravanje žetvenih ostataka. Hibrid NS 6010 ostvario je u proseku statistički vrlo značajno viši prinos u odnosu na drugi ispitivani hibrid NS 510.

**Ključne reči**: kukuruz, azot, đubrenje, zaoravanje žetvenih ostataka

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# INFLUENCE OF VARIETY, SEED TUBER MASS AND NUMBER OF SPROUTS ON POTATO YIELD

MILE DARDIĆ, RATKO DIMITRIĆ[1]

*SUMMARY: This work shows the results of the research of the influence of the variety, seed tuber mass and number of sprouts per seed tuber on the yield of potato under the conditions of Lijevče field. Researches were conducted on two most often cultivated varieties, Desiree and Jaerla, planting tuber mass 30±5 g, 50±7,5 g and 70±10 g. Seed tubers were cut to one, two, and three sprouts. Experimental data were processed as tri-factorial trial by years of research. The research results showed that seed tuber mass, number of sprouts per tuber and variety have the largest impact on potato yield, with significant interaction between the studied factors.*

***Key words**: variety, tuber mass, number of sprouts.*

## INTRODUCTION

It is unnecessary to write about the importance of potato as a cultivated crop. It presents a pillar of nutrition for population in many countries around the world. According to the method of use it is an important product for processing into different products and an important ingredient of cattle food. According to researches of the International Center for Potato (*CIP*) this species is considered the fourth most important cultivated plant in the world.

In the Republic of Srpska and in BiH potato occupies an important place. Plant areas under this crop throughout the country are about 41000 ha, of which in Republic of Srpska are about 18000 ha and about 23000 ha in the Federation of BiH. Potato covers 7,6% and all other vegetables cover 7,8% ( *MSTEO BiH 2007*) of the total arable land in the Republic of Srpska. According to the volume of annual production of approximately 460000 tons potato is the second most important cultivated crop and it comes after the corn and before the wheat.

Traditionally, potato in Lijevče field occupies significant plant area, larger than

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the Republic average (*Dardić, 2008*), with the application of modern technology which includes regular irrigation. Also manufacturers grow potato as early (new potato) or as medium late for consumption during the autumn and winter. Regardless of the production purpose, assortment is representative for this and wider production area. The largest plant area takes *Desiree*, then *Jaerla* and *Kenebek*, and gradually largest plant areas are under the varieties *Marabel, Laura, Aladin, Kondor, Romano, Margarita etc.* The production of seed potato has been redeployed in the area of low infection pressure and slow pace of run up (isolated mountain platform) in which there is no production of comestible potato.

In the production of comestible or seed potato, choice of appropriate tuber mass is of crucial importance for consumption of seed per hectare. Price of healthy potato seedling is high and in structure of production cost presents the largest item. Many researchers agree that the desirable tuber mass for planting is about 50 grams (*Struik et al. 1999., Milošević, 1999., Đorđević et al. 1994., Poštić et al*.) and that yield increases by planting tuber with larger mass. Producers in this area, as well as those that produce for their own needs, each year are buying seminal potato class A (original) or even E (elite) and regularly conducting germination. In order to plant larger production area, numerous manufacturers are buying smaller fraction or cut tuber seed in multiple parts. Some manufacturers even cut tuber in as many parts as there are sprouts on it.

The specificity of potato reproduction by tubers and choice of the appropriate tuber mass for planting are the basic problems of this research. With planting tuber with the mass larger than 50 grams, with desirable structure of each variety, makes this production economically questionable. A manufacturer is left with dilemma whether he, by planting smaller tubers and larger planting area, has accomplished higher yields, and the researchers are obliged to select the optimal mass of seed tuber for planting, with the appreciation of biological specificity of cultivated varieties.

## MATERIAL AND METHOD

Field experiment by random block system in four repetitions has been set on test field of the Agricultural Institute in Banja Luka, in vegetation season in the year 2003 and 2004. The size of the basic parcel was 10,5m2, apropos, row length 5m and width 2,1m, so that inter-row spacing was 0,7m, what is standard spacing for mechanical planting. Planting has been carried out manually, on row spacing between tubers of 0,33m, so the set of 43500 tubers per hectare was made.

In the first year of the research, planting was made at the beginning of the second decade of April and in the second year at the beginning of third decade of April with germinating tubers. In the well prepared land, planting was made at the same depth of 7 cm regardless of the seed tuber mass. Fertilization was carried out only with mineral fertilizers before sowing in the amount of 170 units of pure nutrients N, 200 units of P and 200 units of K, added by using NPK fertilizers and urea. During vegetation necessary measures of care which local producers use in potato production were performed. For protection against weed, herbicide based on the active matter prometrin (*Prometrin S-50*) is applied in the amount of 3 kg/ha after planting. Diseases were under control by conducting two spraying from the beginning of blooming with cooper basis agents

(cooper oksichloride) and metalaksil+mancozeb (*Ridomil MZ*). Pests (marten) were held under the control with alfa-cipermetrin (*Fastak*) and dimetoat (*Sistemin 40*) insecticide.

Researches were conducted on two varieties; Desiree and Jaerla which are produced on significant plant areas. The seminal (seedling) material categories E (elite) have been used in each year of planting. Germination of seminal tubers was carried out in a warehouse and in dutchman with single-layered tubers at temperature 15-18°C with good hotbed luminance. Weight preparing and tubers cutting on 30g±5g, 50g±7,5g and 70g±10 g is carried out in laboratory just before planting. During potato vegetation phenological observations (germination, flowering, and maturation) were performed. Tuber extraction was done by hands and by excavations. The total yield was determined by weighting and tuber summation from every accounting parcel. Only tubers with individual weight larger than 30g are included in the calculation of the total yield of varieties.

The research results, tuber yield by variety, tuber mass and number of sprouts were statistically processed as tri-factorial trial by the years of research.

Trial is set on alluvial, carbonate-sandy land eutric fluvisol type.The results of chemical analysis show that the land on which the trial is placed is weak acid to neutral soil reaction, medium to good provided with easy accessible phosphorus and potassium (Table 1).

Table 1. Chemical characteristic of soil
*Tabela 1. Hemijske osobine zemljišta*

| Year of research<br>*God. istraživanja* | Ph | | Humus (%)<br>*Humus (%)* | $P_2O_5$<br>(mg/100g) | $K_2O$<br>(mg/100g) |
|---|---|---|---|---|---|
| | $H_2O$ | KCl | | | |
| 2003 | 7.03 | 6.43 | 1.87 | 17.7 | 25.1 |
| 2004 | 7.17 | 6.67 | 1.92 | 19.6 | 27.3 |

According to the classification by Gračanin, this soil belongs to low humus provided group of soil.

Climatic conditions in years of research were different. Average annual air temperature in the year 2003 was higher for 0,4°C of the same in the year 2004 and even higher for 0,9°C from multi-annual series for years 1961-2004 (Table 2).

Table 2. Average monthly air temperature, rainfall and multi-annual average for Banja Luka
*Tabela 2. Srednje mjesečne temperature vazduha, padavine i višegodišnji prosjek za Banja Luku*

| Month<br>*Mjesec* | Air temperature(°C)<br>*Temperature vazduha (°C)* | | | Rainfall ($l/m^2$)<br>*Padavine ($l/m^2$)* | | |
|---|---|---|---|---|---|---|
| | 2003 | 2004 | 1961-2004 | 2003 | 2004 | 1961-2004 |
| I | -0.3 | 0.1 | -0.2 | 123.3 | 105.1 | 71.3 |
| II | -1.4 | 2.7 | 2.0 | 22.5 | 76.3 | 62.9 |
| III | 6.2 | 6.0 | 6.5 | 23.5 | 70.4 | 77.5 |
| IV | 11.0 | 11.9 | 10.9 | 56.7 | 166.4 | 90.9 |
| V | 18.9 | 14.8 | 15.9 | 75.0 | 86.1 | 95.4 |
| VI | 24.2 | 19.6 | 19.4 | 35.9 | 104.3 | 111.6 |
| VII | 23.1 | 21.6 | 20.9 | 50.5 | 129.6 | 94.5 |
| VIII | 24.5 | 21.4 | 20.6 | 48.6 | 45.0 | 82.8 |
| IX | 15.5 | 16.0 | 16.1 | 93.4 | 63.0 | 94.3 |
| X | 9.9 | 14.2 | 11.1 | 149.1 | 50.0 | 80.5 |
| XI | 8.2 | 6.3 | 6.1 | 41.8 | 116.6 | 98.2 |
| XII | 1.4 | 2.6 | 1.2 | 54.4 | 107.3 | 89.3 |
| Average<br>*Prosjek* | 11.8 | 11.4 | 10.9 | | | |
| Annual amount<br>*Godišnja suma* | | | | 774.9 | 1120.1 | 1047.0 |

The total annual sum of rainfall in the year 2003 is significantly less than multi-annual average (Table 2) and during the entire period of potato vegetation lack of rainfall was expressed. In the year 2004 amounts of rainfall were sufficient and had favorable distribution for potato production.

## RESULTS AND DISCUSSION

High yield of comestible tuber potato is the goal of every manufacturer. As numerous factors are affecting the yield, to determine those which are crucial isa starting base for achieving the production goal. The mass of seed tuber, number of sprouts per tuber and variety (Table 3) had the biggest influence on the yield of comestible tubers per hectare in the year 2003. However, the differences in yield between varieties were significant and variety Jaerla had much significantly larger yield than variety *Desiree*.

Table 3. Influence of the tuber seed mass and number of tuber sprouts on potato yield in year 2003.
*Tabela 3. Uticaj mase sjemenske krtole i broja klica po krtoli na prinos krompira u 2003. godini (t/ha)*

| *Variety (A)* Sorta (A) | *Mass 30±5 g* Masa 30±5 g | | Masa 50±7,5 g *Mass 50±7,5 g* | | Masa 70±10 g *Mass 70± 10 g* | | Prosjek za sortu *Variety average* |
|---|---|---|---|---|---|---|---|
| | 1 kl. *1spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | |
| Desiree | 19.83 | 20.40 | 23.10 | 25.13 | 22.25 | 23.88 | 22.43 |
| Jaerla | 16.83 | 18.83 | 25.78 | 26.30 | 26.53 | 27.45 | 23.62 |
| broj klica (C) *number of sprouts (C)* | 18.33 | 19.62 | 24.44 | 25.72 | 24.39 | 25.67 | 23.03 |
| masa (B) *Mass (B)* | 18.98 | | 25.08 | | 25,03 | | |

LSD sorta/*variety* (A) 0.05=0.69 0.01=0.92
Masa/*mass* (B) 0.05=0.85 0.01=1.12
broj klica (C) 0.05=0.69
*sprouts number* 0.01=0.92
int. (AB) 0.05=1.20 0.01=1.59
AC 0.05=0.98 0.01=1.30
BC 0.05=1.20 0.01=1.59
ABC 0.05=1.70 0.01=2.24

Regardless of the variety and seed tuber mass, the lowest yield was achieved with the smallest number of sprouts per tuber seed. Statistically significant differences in yield are determined between tubers with one and with more sprouts. By increasing number of sprouts per seed tuber the yield is growing regardless of the tuber seed mass. The results of the research conducted by *Lešić et al. 2002*., stipulate that the increase in planted tuber mass reduce the number of tuber seed and increase number of main stems and yield.

The mass of planted tuber has got an extremely significant influence on potato yield in this climatic adverse year. The minimum tuber yield is achieved with the minimum mass (18,98 t/ha) and significantly lower than the yield resulting from the tuber seed

mass of 50g and 70g. Differences in yield resulting from planting tubers with mass of 50 and 70g are random and not statistically significant.

Accomplished yield of potato in the year 2004 was significantly higher compared to the first year of research (Table 4). The analysis of variance has determined that the mass of tuber seed has got the crucial influence on the yield of the comestible potato tubers, whereas the number of sprouts per tuber seed has got smaller influence, and the variety has got the smallest influence. The yield of the variety Desiree in climatic more favorable year is significantly higher compared to the variety *Jaerla*.

Table 4. Influence of the tuber seed mass and number of tuber sprouts on potato yield in year 2004

*Tabela 4. Uticaj mase sjemenske krtole i broja klica po krtoli na prinos krompira u 2004. godini (t/ha)*

| Sorta (A) *Variety (A)* | Masa 30±5 gr *Mass 30±5 gr* | | Masa 50±7,5 gr *Mass 50±7,5 gr* | | Masa 70±10 gr *Mass 70±10 gr* | | Prosjek za sortu *Variety average* |
|---|---|---|---|---|---|---|---|
| | 1 kl. *1spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | |
| Desiree | 25.55 | 27.73 | 28.55 | 31.95 | 29.70 | 32.35 | 29.31 |
| Jaerla | 24.75 | 26.13 | 28.68 | 30.05 | 29.40 | 30.30 | 28.22 |
| broj klica (C) *number of sprouts (C)* | 25.15 | 26.93 | 28.62 | 31.00 | 29.55 | 31.33 | 28.76 |
| masa (B) *Mass (B)* | 26.04 | | 29.81 | | 30.44 | | |

LSD sorta/*variety* (A) 0.05=0.59 0.01=0.73
masa/*mass* (B) 0.05=0.72 0.01=0.95
broj klica (C) 0.05=0.59
*sprouts number 0.01=0.73*
int (AB) 0.05=1.02 0.01=1.35
AC 0.05=0.84 0.01=1.10
BC 0.05=1.02 0.01=1.35
ABC 0.05=1.45 0.01=1.91

By changing or increasing the number of sprouts per tuber seed, the yield is increasing and the differences are statistically significant. And with increasing of tuber seed mass that differences are larger.

The smallest yield of comestible potato tubers is accomplished with the smallest tuber seed mass (26,04 t/ha) and by increasing mass of seed tuber the yield is increasing and differences are statistically significant only for the mass between 30 and 50g. By increasing mass of seed tuber above 50g the yield increases but the resulting differences are incidental.

## CONCLUSION

Based on the researches conducted on the influence of tuber seed mass and the number of sprouts per tuber seed on potato yield we can make the following conclusions.

Results of the research are showing that the yield of comestible potato tubers is under the influence of the numerous factors, and that choice of tuber seed mass and number of sprouts per tuber seed play important role in formation of total yield. Researches conducted show that in climatic different years, potato yield significantly depends on tuber seed mass. Planting tubers with mass of 30g provides the smallest yield. By increasing the mass of tuber seed to 50g, the yield increases by about 15%, but that upgrowth is smaller than increased yield which is obtained from larger planting area achieved by planting tubers of 30g. The number of sprouts on tuber seed had an impact on potato yield. With greater number of planting sprouts is accomplished a greater number of main stems and higher yield, but with smaller tubers on average. Planting tubers with one sprout in conditions of Lijevče field provides a smaller yield which is partially compensated by even and higher tuber mass so for early production it can be recommended to plant smaller mass tuber.

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# UTICAJ SORTE, MASE SJEMENSKE KRTOLE I BROJA KLICA NA PRINOS KROMPIRA

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**Izvod**

U radu su predstavljeni rezultati istraživanja uticaja sorte, mase sadne krtole i broja klica po sadnoj krtoli na prinos konzumnog krompira u uslovima Lijevče polja. Istraživanja su provedena na dvije najčešće gajene sorte, Desiree i Jaerla, sadnjom mase krtole 30±5g, 50±7,5g i 70±10g. Sadne krtole su sječene na jednu i dvije do tri klice. Eksperimentalni podaci obrađeni su kao trofaktorijalni ogled po godinama istraživanja. Rezultati istraživanja su pokazali da na prinos krompira najveći uticaj ima masa sadne krtole, broj klica po krtoli i sorta sa značajnom interakcijom između istraživanih faktora.

**Ključne riječi:** sorta, masa krtole, broj klica.

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# THE ROLE OF SEED TUBER MASS AND THE NUMBER OF SPROUTS IN THE DEVELOPMENT OF GENERIC POTATO STALKS

MILE DARDIĆ, RATKO DIMITRIĆ[1]

*SUMMARY: In order to research the influence of variety, seed tuber mass and the number of seed tuber sprouts on the development of generic stalks per tuber, two most cultivated varieties of potato, Desiree and Jaerla, were selected. The research was conducted in the north-western part of Republic of Srpska (Banja Luka) planting tubers weight 30±5gr, 50±7,5gr, and 70±10gr planst with one sprout per tuber and 2-3 sprouts per seed tuber. The results were statistically processed and they have shown that the number of developed generic stalks per tuber depends the most on the sprouts number, less on tuber mass and the least on variety. The largest number of generic stalks per tuber or thickest texture is achieved by planting tubers weighing 70g with two to three sprouts per tuber.*

***Key words:*** *variety, tuber mass, generic stalks.*

## INTRODUCTION

According to the planted areas and total volume of the production, the potato has important place in the diet of world population. It presents the base in the diet of the population in poor countries, an important foodstuff in developed countries and important raw material for processing and food industries. The potato in the Republic of Srpska, regardless of the length of the period when it became cultivated crop, the method of production, tradition, addiction in consumption and consumption *“per capita”*, today represents an extremely important crop (*Dardić, 2008*).

The edible reproductive part of potato is tuber which contains approximately 25% of dry matter. The content of dry matter varies significantly and is mostly depended on the variety and growing conditions. The tuber is a modified underground part of the stem and primal plant organ for wintering and reproduction in our climatic conditions. Technologically, we perceive the tubers as „seed“, but not real botanical seed, rich soft

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Original scientific paper / *Originalni naučni rad*

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roe where there is up to 200 seeds. Because the basic way of reproduction, by seed tubers, emphasized the meaning of tuber as a reproductive organ that has got a decisive part in potato production.

The stalk as an aerial vegetative part develops from the tuber sprout and number of stalks developed at the stem tuber depends on several factors; sprout number, tuber mass, variety, tuber physiological age and growing conditions (*Struik, 1999; Lešić, 2002*). The primary, main and secondary side stalks develop on the stem tuber. If the secondary stalks are developed from primary, but under the ground, they form a stole the same as the primary so we call them together gender stalks. Therefore, the development of gender stalks presents a basic indicator of the assembly. For most cultivated potato varieties the optimal assembly is estimated to be 150000 gender stalks per hectare (*Lešić, 2002*).

Many manufacturers in this area grow early potatoes for consumption (new (baby) potatoes) as the main crop and after that they use land for later planting of various cabbages or kornison cucumbers. In the production of early potato, producers use tuber seeds class A (original) or even class E (elite) knowing that only with healthy seedlings they can achieve early production and high yield. In order to plant larger production area, many manufacturers cut seed tuber in multiple parts. Many manufacturers, for the production of early potato, cut tubers in as many parts as there are sprouts on it. And the results of the research (*Đorđević et al. 1994; Arends et al. 1999; Lešić et al. 2002*) show that planting smaller tubers or the tuber part developing primary (main) stalks and slow the development of secondary stalks. The planting tuber or tuber part with one sprout is assumed not to have achieved the optimal number of generic stalks per hectare necessary for achieving high yield of young potato.

## MATERIAL AND METHOD

The field experiment by random block system in four repetitions has been set on the test field of the Agricultural Institute in Banja Luka, in vegetation season in year 2003 and 2004. The size of the basic parcel was 10,5 $m^2$, *apropos*, row length 5m and width 2,1m, so that inter-row spacing was 0,7m, what is a standard spacing for mechanical planting. The planting has been carried out manually, on row spacing between tubers of 0,33m, so the set of 43500 tubers per hectare was made.

In first year of the research, the planting was made at the beginning of the second decade of April, and in the second year at the beginning of third decade of April with germinating tubers. In the well prepared land, planting was made at the same depth of 7 cm regardless of the seed tuber mass. The fertilization was carried out only with mineral fertilizers before sowing in the amount of 170 units of pure nutrients N, 200 units of P and 200 units of K, added by using NPK fertilizers and urea. During vegetation necessary measures of care which local producers use in potato production were performed. For protection against weed, herbicide based on the active matter prometrin (*Prometrin S-50*) is applied in the amount of 3 kg/ha after planting. Diseases were under control by conducting two spraying from the beginning of blooming with cooper basis agents (cooper oksichloride) and metalaksil+mancozeb (*Ridomil MZ*). Pests (marten) were held under the control with alfa-cipermetrin (*Fastak*) and dimetoat (*Sistemin 40*) insecticide.

The research was conducted on two varieties; Desiree and Jaerla, which are

produced on significant plant areas. For research is used seminal (seedling) material categories E (elite) in each year of planting. The germination of seminal tubers was carried out in warehouse and in dutchman with single-layered tubers at the temperature of 15-18°C with good hotbed luminance. The weight preparing and tuber cutting on 30g ± 5g, 50g ± 7,5g and 70g ± 10g were carried out in laboratory just before planting. During potato vegetation phenological observations (germination, flowering, maturation) and counting number of gender stalks per tuber seed in row for each variety and repetition were performed.

The research results, the number of the gender stalks per variety, mass of the tuber seed and number of sprouts were statistically processed as tri-factorial trial by the years of research and mean value was tested by LSD test.

The trial is set on alluvial, carbonate-sandy land eutric fluvisol type. The results of the chemical analysis show that the land on which the trial is placed is week acid to neutral soil reaction, medium to good provided with easy accessible phosphorus and potassium (Table 1).

Table 1. Chemical characteristic of soil
*Tabela 1. Hemijske osobine zemljišta*

| God. istraživanja *Year of research* | Ph | | Humus (%) *Humus (%)* | $P_2O_5$ (mg/100g) | $K_2O$ (mg/100g) |
|---|---|---|---|---|---|
| | $H_2O$ | KCl | | | |
| 2003 | 7,03 | 6,43 | 1,87 | 17,7 | 25,1 |
| 2004 | 7,17 | 6,67 | 1,92 | 19,6 | 27,3 |

According to the classification by Gračanin, this soil belongs to low humus provided group of soil.

The climatic conditions in years of research were different. The average annual air temperature in year 2003 was higher by 0,4°C of the same in year 2004 and even higher by 0,9° C from multi-annual series for years 1961-2004 (Table 2).

Table 2. Average monthly air temperature, rainfall and multi-annual average for Banja Luka
*Tabela 2. Srednje mjesečne temperature vazduha, padavine i višegodišnji prosjek za Banja Luku*

| Mjesec *Month* | Temperature vazduha (°C) *Air temperature(°C)* | | | Padavine (l/m$^2$) *Rainfall (l/m$^2$)* | | |
|---|---|---|---|---|---|---|
| | 2003 | 2004 | 1961-2004 | 2003 | 2004 | 1961-2004 |
| I | -0,3 | 0,1 | -0,2 | 123,3 | 105,1 | 71,3 |
| II | -1,4 | 2,7 | 2,0 | 22,5 | 76,3 | 62,9 |
| III | 6,2 | 6,0 | 6,5 | 23,5 | 70,4 | 77,5 |
| IV | 11,0 | 11,9 | 10,9 | 56,7 | 166,4 | 90,9 |
| V | 18,9 | 14,8 | 15,9 | 75,0 | 86,1 | 95,4 |
| VI | 24,2 | 19,6 | 19,4 | 35,9 | 104,3 | 111,6 |
| VII | 23,1 | 21,6 | 20,9 | 50,5 | 129,6 | 94,5 |
| VIII | 24,5 | 21,4 | 20,6 | 48,6 | 45,0 | 82,8 |
| IX | 15,5 | 16,0 | 16,1 | 93,4 | 63,0 | 94,3 |
| X | 9,9 | 14,2 | 11,1 | 149,1 | 50,0 | 80,5 |
| XI | 8,2 | 6,3 | 6,1 | 41,8 | 116,6 | 98,2 |
| XII | 1,4 | 2,6 | 1,2 | 54,4 | 107,3 | 89,3 |
| Prosjek *Average* | 11,8 | 11,4 | 10,9 | | | |
| Godišnja suma *Annual amount* | | | | 774,9 | 1120,1 | 1047,0 |

The total annual sum of rainfall in year 2003 is significantly less than multi-annual average (Table 2) and during the entire period of potato vegetation lack of rainfall was expressed. In the year of 2004, the amounts of rainfall were sufficient and had favorable distribution for potato production.

## RESULTS AND DISCUSSION

Manufacturers are often planning assembly and yield of potato according to number of planted stem tubers per hectare. The stem or planted tuber may develop different number of main (generic) stalks that are better indicator of assembly than number of planted tubers. The number of main stalks on stem tuber depends on several factors of which the most important are variety, growing conditions and tuber physiological age (*Lešić et al. 2002; Dardić, 2004.*). Main stalks grow directly from the stem tuber and on them are formed stoles with tubers. Under favorable conditions of production, from the underground part of the main stalk emerges secondary stalks that can be considered as main, forming stoles as well as main, but they end with smaller tubers. Secondary stalks developed from the main stalk above ground are not forming stoles. Increasing number of stalks per stem tuber usually does not result in higher yield than the small number of large tubers (*Arends, 1999; Lešić et al. 2002*).

The number of main stalks developed from stem tuber varies according to the year of the research and usually is around three to five. During the year of 2003, the development of a small number of main stalks per tuber was influenced by less favorable climatic conditions (drought) during the entire period of potatoes upgrowth. Differences between the tested varieties in the development of main stalks per tuber were not significant (Tab.3).

Table 3. Influence of the tuber seed mass and number of tuber sprouts on the number of main stalks in year 2003

*Tabela 3. Uticaj mase sjemenske krtole i broja klica po krtoli na broj glavnih stabljika u 2003. godini*

| Sorta (A) *Variety (A)* | Masa 30±5 gr *Mass 30 5 gr* | | Masa 50±7,5 gr *Mass 50±7,5 gr* | | Masa 70±10 gr *Mass 70±10 gr* | | Prosjek za sortu *Variety average* |
|---|---|---|---|---|---|---|---|
| | 1 kl. *1spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | |
| Desiree | 1,24 | 3,53 | 1,41 | 3,70 | 1,49 | 3,83 | 2,53 |
| Jaerla | 1,14 | 3,03 | 1,35 | 3,37 | 1,55 | 3,75 | 2,37 |
| broj klica (C) *number of sprouts (C)* | 1,19 | 3,28 | 1,38 | 3,54 | 1,52 | 3,79 | 2,45 |
| masa (B) *Mass (B)* | 2,24 | | 2,46 | | 2,66 | | |

LSD sorta/*variety* (A) 0,05=0,40 0,01=0,53
masa/*mass* (B) 0,05=0,49 0,01=0,65
broj klica (C) 0,05=0,40 *sprouts number* 0,01=0,53
int. (AB) 0,05=0,70 0,01=0,93
AC 0,05=0,57 0,01=0,76
BC 0,05=0,70 0,01=0,93
ABC 0,05=0,93 0,01=1,31

Regardless of other studied factors, with the increase of the planted tuber number of the main stalks per stem tuber is increasing but these differences in this year were not statistically significant. The dominant influence on development of a number of main stalks per stem tuber had number of sprouts at the time of planting tuber (Table 3). The minimum number of main stalks per stem tuber developed *Jaerla* variety with the lowest mass of seed tuber (1,14), and the greatest *Desiree* variety with the largest seed tuber mass (3,83). With increasing planted tuber mass and with the increase in the number of the sprouts per planted tuber number of developed main stalks is growing with highly significant difference between the tested factors. The largest number of main stalks per planted tuber is achieved with the largest mass of seed tuber and with the largest number of sprouts per tuber (3,79).

Development of main stalks per planted tuber in year 2004 depended on studied variety, planted tuber mass and number of sprouts per tuber (Tab.4). Significantly greater number of main stalks per tuber developed *Desiree* variety in relation to *Jaerla* variety. The influence of the planted tuber mass on the development of main stalks is significant. The lowest number of the main stalks per tuber is achieved with the smallest tuber mass and the differences are highly significant between tested masses (Table 4).

Table 4. Influence of the tuber seed mass and number of tuber sprouts on the number of main stalks in year 2004

*Tabela 4. Uticaj mase sjemenske krtole i broja klica po krtoli na broj glavnih stabljika u 2004. godini.*

| Sorta (A) *Variety (A)* | Masa 30±5 gr *Mass 30±5 gr* | | Masa 50±7,5 gr *Mass 50±7,5 gr* | | Masa 70±10 gr *Mass 70±10 gr* | | Prosjek za sortu *Variety average* |
|---|---|---|---|---|---|---|---|
| | 1 kl. *1spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | 1 kl. *1 spr.* | 2-3 kl. *2-3 spr.* | |
| Desiree | 1,62 | 3,49 | 1,54 | 3,72 | 1,64 | 4,05 | 2,68 |
| Jaerla | 1,27 | 3,08 | 1,33 | 3,32 | 1,44 | 3,38 | 2,30 |
| broj klica (C) *number of sprouts (C)* | 1,45 | 3,29 | 1,43 | 3,52 | 1,54 | 3,72 | 2,49 |
| masa (B) *Mass (B)* | 2,37 | | 2,48 | | 2,63 | | |

LSD sorta/*variety* (A) 0,05=0,06 0,01=0,08
masa/*mass* (B) 0,05=0,07 0,01=0,10
broj klica (C) 0,05=0,06
*sprouts number* 0,01=0,08
int.(AB) 0,05=0,10 0,01=0,13
AC 0,05=0,08 0,01=0,11
BC 0,05=0,10 0,01=0,13
ABC 0,05=0,14 0,01=0,19

Decisive influence on what will be the number of the main stalks developed by planted tuber again had a number of the sprouts. Planted tubers with one sprout have developed even in the favorable year the smallest number of the main stalks, and significant differences were only between the least and the greatest tuber mass. Conducted tests show that tubers with largest mass develop significantly greater number of main

stalks in relation to planted tubers with smaller mass. Also, the results of research by *Đokić, 1975., Arends, 1999., Lešić et al. 2002*., show that with the increase of tuber mass increases the number of main stalks developed from the stem tuber. In addition, conducted researches show that there is significant interaction between variety, planted tuber mass and number of sprouts per tuber. Therefore, the choice of the optimal mass of planted tuber and number of sprouts per tuber for each variety is presented by the most desirable relation between these two tested factors. Planting largest mass tubers with the largest number of sprouts per tuber provides the largest number of main stalks which means the largest assembly.

## CONCLUSION

Based on the researches conducted on influence of the variety, seed tuber mass and number of sprouts per planted tuber on the number of the main (generic) stalks per tuber we can make the following conclusions;

Main stalks developed from the stem tuber (primary and secondary) are a better indicator of the assembly then the number of planted tubers. The number of developed main stalks varies according to the year of research, variety, seed tuber mass and number of sprouts per seed tuber. The number of sprouts and seed tuber mass have a decisive influence on the number of the developed main stalks. Generally, by increasing the number of sprouts per planted tuber and increasing the mass of tuber seed, number of main stalks is growing with tendency of smaller influence between massive planting fractions. In all the years of research and especially in the more favorable climatic year *Desiree* variety develop a greater number of main stalks in relation to *Jaerla* variety. The interaction of varieties, tuber mass and number of sprouts per tuber is significant and shows that for any variety choice of the optimum mass and number of sprouts per tuber is the base for obtaining optimal number of main stalks.

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## ULOGA MASE SADNE KRTOLE I BROJA KLICA U RAZVOJU RODNIH STABLJIKA KROMPIRA

MILE DARDIĆ, RATKO DIMITRIĆ

### Izvod

Za istraživanja uticaja sorte, mase sjemenske krtole i broja klica na sadnoj krtoli na razvoj rodnih stabljika po krtoli odabrane su dvije najviše gajene sorte krompira, Desiree i Jaerla. Istraživanja su provedena u području sjeverozapadnog dijela Republike Srpske (Banja Luka) sadnjom krtola mase 30±5g, 50±7,5g i 70±10g sađenih sa jednom klicom po krtoli i 2-3 klice po sadnoj krtoli. Dobijeni rezultati su statistički obrađeni i pokazuju da broj razvijenih rodnih stabljika po matičnoj krtoli najviše zavisi od broja klica, manje od mase krtole, a najmanje od sorte. Najveći broj rodnih stabljika po krtoli, odnosno najgušći sklop ostvaren je sadnjom krtola mase 70g sa dvije do tri klice po krtoli.

**Ključne riječi:** sorta, masa krtole, rodne stabljike

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# THE FUNCTIONAL STATE OF LIVER CELLS IN DAIRY COWS IN POSTPARTAL PEROID AND DURING LACTATION

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*SUMMARY: The objective of this study was to determine characteristic blood parameters, i.e. indicators of the functional state of liver in the puerperal cows (n=15) and in those (n=15) from day 90 to day 100 of lactation. Blood glucose levels were statistically significantly lower (P<0.01) in the puerperal cows than in the cows examined during the maximum lactation period, which suggested a decreased gluconeogenesis in the liver. Significantly lower blood levels of total protein(P<0.01), albumin(P<0.01), urea (P<0.01)and triglyceride(P< 0.05) were recorded in the puerperal cows, which suggested the reduced synthetic capacity of liver cells in the early lactation cows. Blood bilirubin levels in the puerperal cows were significantly higher (P<0.05), which clearly indicated the reduced excretory capacity of the liver. Significantly increased (P<0.01) AST, GGT and LDH activities in the blood in the puerperal cows clearly evidenced the disturbed morphological and functional integrity of liver cells and the release of these intracellular enzymes into the blood. The obtained results suggested that fatty infiltration and different degrees of liver cell degeneration were recorded in the puerperal cows, as opposed to the cows during maximum lactation showing preserved morphological and functional capacities of hepatocytes.*

***Key words:*** *cows, protein, lipids, bilirubin, enzymes.*

## INTRODUCTION

During the transition period, from immediately before to after parturition, and with the establishment of lactation, the organism in high-yielding dairy cows is pushed to its physiological limits, reaching maximum until day 120 of lactation, resulting in a substantial load on the organism, specifically on the digestive organs, liver, udder and the

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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; Djoković et al. 1998, 2007).

Moderate fatty infiltration of liver cells in dairy cows during transition and maximum lactation 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, Sevinc et al. 2003, Lubojacka et al. 2005; Djoković et al. 1998, 2007). Severe fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (AST, GGT, LDH), the activity thereof in the blood being considerably elevated ( Pechova 1997; Lubojacka et al. 2005).

Considering the increased metabolic loads on the liver in lactating dairy cows, the objective of the present 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 activities of liver-specific enzymes (AST, GGT, LDH) in th dairy cows in postpartal period and during maximum lactation.

## MATERIAL AND METHOD

Puerperal cows and those from day 90 to day 100 of lactation (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 1 to day 15 after parturition (n=15). The second group (group B) comprised clinically healthy cows from day 90 to day 100 of lactation (n=15). The examined cows were four to six years old and average weight was 650 kg. 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.

Blood was sampled from all examined cows by punction of vena jugularis. Two test tubes of blood (approximately 20 ml) were taken per punction. Serum separation after spontaneous coagulation at room temperature was performed by centrifugation at 3000 revolutions/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) and urea (cat. No. 11536). The levels of total

cholesterol (cat. No. 11828), total protein (ct. No. 11500), albumin (cat. No. 11547) and bilirubin (cat. No. 11515) and the activities of the liver-specific enzymes: AST- aspartate aminotransferase (cat. No. 11830), GGT – gama-glutamyl transferase (cat. No. 11584), LDH- lacto-dehydrogenase (cat. No. 11552), were determined spectrophotometrically. All biochemical blood parameters were assayed using a Cobas Mira device at the biochemical laboratory Medicus 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 those on the activities of AST, GGT and LDH in dairy cows in puerperal period and maximum lactation.

Table 1. Blood levels of glucose, triglyceride, total cholesterol, total protein, albumin, urea and bilirubin and the activity of AST, GGT and LDH in the puerperal cows (group A), and in those during maximum lactation (group B) and the statistical significance of the obtained means

| Gruop | A | B | P<0,05 | P<0,01 |
|---|---|---|---|---|
| n | 15 | 15 | | |
| Glucose (mmol/l) | 2.21± 0.48 | 3.11 ± 0.46 | | A:B |
| Triglycerides (mmol/l) | 0.18 ± 0.08 | 0.27 ± 0.10 | A:B | |
| Total cholesterol (mmol/l) | 2.26 ± 0.59 | 2.75 ± 0.84 | | |
| Total protein (g/l) | 63.51 ± 7.70 | 72.71 ± 6.65 | | A:B |
| Albumin (g/l) | 29.54 ± 3.89 | 35.43 ± 3.83 | | A:B |
| Urea (mmol/l) | 3.85± 0.98 | 5.50 ± 1.30 | | A:B |
| Bilirubin (µmol/l) | 4.44 ± 1.11 | 2.73 ± 0.95 | | A:B |
| AST (IJ/l) | 64.41± 18.08 | 39.47 ± 7.36 | | A:B |
| GGT (IJ/l) | 14.65 ± 4.25 | 8.45 ± 1.86 | | A:B |
| LDH (IJ/l) | 1805 ± 386.8 | 1167 ± 336.1 | | A:B |

Table 1 shows that blood levels of glucose, urea, total protein and albumin were statistically significantly lower in the puerperal cows than in those during maximum lactation ($P<0.01$). Statistically significantly lower blood levels of triglyceride ($P<0.05$) were found in the puerperal cows than in the maximum lactation cows. Blood bilirubin values and the activity of the AST, GGT and LDH enzymes were statistically significantly higher ($P<0.01$) in the puerperal cows than in the cows during maximum lactation.

## DISCUSSION

Glucose is a blood parameter defining the energy metabolism in 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<0.01) blood glucose values as compared to the lactating cows. The above results are in agreement with the literature data (Veenhuizen et al. 1991; Grummer 1995; Reist et al. 2002; Djoković et al. 1998, 2007) 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 early lactation cows. The data are in agreement with the results obtained by other authors (Pechova et al. 1997; Veenhuizen et al.1991; Vazquez-Anon 1994; Sevinc et al. 2003; Djoković et al. 1998, 2007). 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 (Lubojacka et al. 2005). 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< 0.01) in the early lactation cows than in the other group of cows, suggesting the reduced synthetic capacity of the liver cells in the puerperal cows. The reduced synthesis of total protein, albumin and urea at the beginning of lactation in dairy 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). Blood bilirubin value is a highly sensitive indicator of the functional capacity of liver cells. The blood bilirubin values recorded in the present study were within physiological limits (0.85- 5.60 μmol/l) in all examined groups of cows (Jovanović,1984). Significantly higher (P<0.01) values were determined 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 activities of enzymes are highly important blood parameters used in evaluating the degree of hepatocyte damage. The study focused on determining the blood activities of AST, GGT and LDH enzymes. AST is located in the cytoplasm and mitochondria of different tissues and organs, its highest activities being detected in the heart and skeletal musculature as well as in the liver. The AST activity is found to be the most sensitive indicator in diagnosing fatty liver in cows (Pechova et al. 1997; Lubojacka et al. 2005). Blood AST activites in this study were found to be statisticallysignificantly higher (P<0.01) in the puerperal cows than in the lactating ones, which suggested the development of fatty infiltration of hepatocytes, cell membrane damages and the release of the intracellular enzyme into the blood. Blood GGT activity was above physiological limits in the postparturient cows 10-20 IJ/l (Jovanović,1984), being statistically significantly higher (p<0.01) as compared to the other group of cows, indicating the increased activity of the enzyme in the puerperal cows. GGT is a microsomal and membrane-bound enzyme found mostly in the liver, kidneys and small intenstines. The increase in the activity of this enzyme results from

damages of the cellular structure of hepatocytes (Jovanović et al. 1993; Kupczynski et al. 2002; Lubojacka et al. 2005). LDH is not an organ-specific enzyme, being found at high concentrations in the muscles, heart, kidneys and the liver. It is released into the blood in cases of acute cell damage of the above organs. The study revealed a statistically significantly higher ($P<0.01$) activity of LDH in the puerperal cows, clearly indicating hepatocyte damage and the enzyme release into the blood. According to Pechova et al. (2007), the blood activity of LDH is correlated with the degree of fatty infiltration of hepatocytes.

## CONCLUSION

Blood glucose levels were statistically significantly lower ($P<0.01$) in the puerperal cows than in the maximum lactation ones, suggesting an increased glucose uptake by the mammary gland at the beginning of lactation and a reduced degree of gluconeogenesis in the liver, as induced by the development of fatty infiltration and liver cell degeneration.

Significantly lower ($P<0.05$) blood triglyceride values and low values of total cholesterol in the puerperal cows suggest their being accumulated in the liver cells, as opposed to the cows a during the maximum lactation period.

Significantly lower ($P<0.01$) blood levels of total protein, albumin and urea were found in the puerperal cows, which suggested the decreased synthetic capacity of liver cells in the early lactation cows.

Blood bilirubin levels were significantly higher ($P<0.01$) in the cows at the beginning of lactation than in the those during maximum lacatation, which clearly indicated the decreased excretory capacity of the liver.

Significantly increased ($P<0.01$) activities of AST, GGT and LDH in the blood of the puerperal cows clearly reflected the disturbed morphological and functional integrity of liver cells and the release of the intracellular enzymes into the blood as induced by fat accumulation in hepatocytes.

The analysis of the blood parameters as indicators of the functional capacity of liver cells suggested that puerperal cows revealed fatty infiltration and different degrees of liver cell degeneration, as opposed to the cows during maximum lactation, in which the morphological and functional capacity of hepatocytes was preserved.

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# FUNKCIONALNO STANJE ĆELIJA JETRE KOD MLEČNIH KRAVA U POSTPARTALNOM PERIODU I TOKOM LAKTACIJE

RADOJICA. ĐOKOVIĆ, ZORAN ILIĆ, SNEŽANA BOGOSAVLJEVIĆ-BOŠKOVIĆ, MILUN PETROVIĆ

**Izvod**

Cilj ovog rada je bio da se odrede odgovarajući parametri krvi, indikatori funkcionalnog stanja jetre kod grupe krava (n=15) u puerperijumu i kod grupe krava(n=15) u periodu od 90 do 100. dana laktacije. Koncentracije glukoze u krvi su bile statistički značajno manje ($P< 0.01$) kod grupe krava na početku laktacije u odnosu na vrednosti u krvi kod grupe tokom maksimalne laktacije, što ukazuje na smanjeni stepen glukoneogeneze u jetri. Kod grupe krava u puerperijumu utvrđene su značajno manje vrednosti ukupnih proteina ($P<0.01$), albumina ($P<0.01$), ureje ($P<0.01$) i triglicerida ($P<0.05$) u krvi što ukazuje na smanjenu sintetsku sposobnost ćelija jetre. Koncentracije bilirubina u krvi kod krava na početku laktacije su bile značajno veće ($P<0.01$), što jasno ukazuje na smanjenu ekskrecionu sposobnost jetre. Značajno povećane ($P< 0.01$) aktivnosti AST, GGT i LDH u krvi kod grupe krava posle telenja jasno ukazuju na narušen morfološki i funkcionalni integritet ćelija jetre i oslobađanje ovih intracelularnih enzima u krv. Na osnovu dobijenih rezultata može se zaključiti da kod krava na početku laktacije je prisutna masna infiltracija i degeneracija ćelija jetre različitog stepena, za razliku od grupe krava tokom maksimalne laktacije kod kojih je očuvana morfološka i funkcionalna sposobnost hepatocita

**Ključne reči**: krava, protein, lipid, bilirubin, enzim.

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# HEAVY METALS CONTENT IN THE SELECTED SOILS AND FRUITS IN MONTENEGRO AND ESTIMATION OF THEIR DAILY INTAKE THROUGH FRUITS CONSUMPTION

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*SUMMARY: Levels of Pb, Ni, Cr and Cd, in soil and fruits (Rubus idaeus - raspberry and Vaccinium myrtilus - blueberry ) at 26 locations in Montenegro were examined. Heavy metals content in the samples was determined by AAS method. In the soils, the content of heavy metals was below pollution threshold, except at one location (Kolašin region). In the berries, heavy metals content was mostly within normal range for plants and guideline values for fruits. The calculated daily intakes of metals through selected fruits consumption are found to be below the recommended tolerable daily intakes proposed by FAO/WHO.*

***Key words:*** *Heavy metals, soil, food, contamination, blueberry, raspbery, daily intake.*

## INTRODUCTION

The major public concern for quality of food and risk, regarding the consumption of products contaminated by pesticides, heavy metals and/or toxins, has been constantly increasing (D'Mello, 2003). Some trace elements, such as: Cd, Hg, Pb and As, of both natural and anthropogenic sources are related to human health condition. Heavy metals, in general, are not biodegradable, have long biological half-lives and have potential for accumulation in different body organs, leading to unwanted side effects (Jarup, 2003). The general population is exposed to trace elements mainly by ingestion of drinking water and food and by inhalation of air (Kabata-Pendias and Mukherjee, 2007). Food contamination with heavy metals depends on several factors amongst which are soil

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concentration, soil chemical and physical properties, human activities (such as: irrigation with contaminated water, addition of low quality sewage sludge, fertilizers and metal-based pesticides, industrial emission, transport, the harvesting process, storage, and/or at the point of sale) and plant species and cultivars.

Among trace elements, lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) appear to be primarily involved in dietary exposure to chemical contaminants (Gunderson, 1995). Lead and cadmium are among the most abundant heavy metals and are particularly toxic. Excessive content of these elements in food is associated with a number of diseases and are implicated in carcinogenesis, mutagenesis and teratogenesis. On the other hand, nickel and chromium have been recently introduced as essential elements, but can be toxic at high level of exposure. Studies on humans and experimental animals have shown that trivalent chromium has an essential role in the maintenance of normal glucose and fat metabolism in humans and animals, while nickel may have some beneficial effects at low levels of exposure (NAS/IOM, 2003).

There is plenty of information on heavy metals in vegetables and estimations of their daily intake through vegetable consumption worldwide (Kumar et al., 2009; Maleki and Zarasvand, 2008; Radwan and salama, 2006), but there are limited data regarding heavy metals in fruits, particularly blueberry and raspberry. Keeping in view the potential toxicity, persistent nature and cumulative behavior of heavy metals, as well as the increasing consumption of fruits, there is necessity to analyze these food items to ensure that the level of heavy metals meets the international requirements. This study, therefore, presents data on the level of heavy metals (Pb, Ni, Cr and Cd) in selected soils and fruits from Montenegro, as well as the calculated daily intakes of these metals through selected fruits consumption.

## MATERIAL AND METHODS

The soil and berries (Rubus idaeus - raspberry and *Vaccinium myrtilus* - blueberry) samples were collected at 26 locations in Northern Montenegro (regions: Bjelasica mountain, southern hillside of Lisa mountain, Pljevalja, river Lim valley and river Tara valley) from the end of July to the end of August 2008, during maturity period.

Blueberry samples were labeled by the letter B and ascending numbers. Raspberry samples were labeled by the letter M and ascending numbers. After collection, fruit samples were frozen up to -18°C and brought to the laboratory. Before the analyses, fruits were defrosted, then oven-dried (80ºC) for 15 hours, and grounded to powder. Heavy metals content in the plant material was measured by AAS, after digestion in acids: $HNO_3$ and $HClO_4$, with the addition of $H_2O_2$. Detection limits were: 0.006 μg Pb $ml^{-1}$; 0.003 μg Ni $ml^{-1}$; 0.004 μg Cr $ml^{-1}$ and 0.005 μg Cd $ml^{-1}$.

Soil samples were collected from the depth of 30 cm by digging an adequate hole close to each sampling plant. For shallow soils, sampling depth was up to parent material. After collection, samples were labelled to correspond to the berries samples, then placed in polythene bags, brought to the laboratory, air-dried (20ºC), and sieved through a 2 mm stainless-steel mesh. Pseudo-total metal concentrations in soil samples were quantified by atomic adsorption spectrophotometry (Varian SpectrAA 2002 FS), in the acetylene/air flame, after digestion using $HNO_3$ conc. + $H_2O_2$ (US EPA Method

3050). Detection limits, calculated as analytic concentration greater than three times the standard deviation, obtained after eight measurements of the blank solution, were 0.007 μg Pb $ml^{-1}$; 0.003 μg Ni $ml^{-1}$; 0.005 μg Cr $ml^{-1}$ and 0.007 μg Cd $ml^{-1}$.

The results obtained are presented as the average value and standard deviation derived from three replicants of each sample.

## RESULTS

Pseudo-total content of heavy metals: Ni, Cr, Pb, and Cd, in the soils (Table 1) was below pollution threshold at all locations, except Ni and Cr concentrations at M14 (152.9 mg $kg^{-1}$ and 133.2 mg $kg^{-1}$, respectively).

Table 1. Heavy metals in the soils (mg $kg^{-1}$)

| Locality | Label | Ni | Cr | Pb | Cd |
|---|---|---|---|---|---|
| | | **Blueberry** | | | |
| **Bjelasica** | **B1** | 9.2±0.24 | 13.3±0.28 | 39.6±1.10 | 1.11±0.01 |
| | **B2** | 18.0±0.33 | 13.7±0.22 | 39.4±0.09 | 0.25±0.01 |
| | **B3** | 9.9±0.26 | 10.3±0.21 | 66.1±1.13 | 0.26±0.02 |
| | **B4** | 13.6±0.42 | 14.5±0.31 | 61.7±0.08 | 0.41±0.02 |
| | **B5** | 3.0±0.12 | 6.4±0.17 | 34.1±0.69 | 0.05±0.004 |
| **Lisa** | **B6** | - | - | - | - |
| | **B7** | 7.4±0.18 | 8.5±0.22 | 60.1±1.10 | < d.l. |
| | **B8** | 4.3±0.12 | 9.3±0.35 | 36.8±0.78 | < d.l. |
| **Bihor** | **B9** | 20.0±0.52 | 20.0±0.39 | 43.6±0.56 | 0.05±0.003 |
| | **B10** | 2.1±0.11 | 5.3±0.16 | 43.3±0.62 | < d.l. |
| **Pljevlja** | **B11** | - | - | - | - |
| **Average** | | 9.72 | 11.28 | 47.20 | 0.355 |
| | | **Raspberry** | | | |
| **Lim riv. valley** | **M1** | 25.9±0.32 | 19.1±0.52 | 32.1±0.55 | 0.05±0.005 |
| | **M2** | 28.5±0.24 | 22.9±0.44 | 32.5±0.42 | < d.l. |
| | **M3** | 28.7±0.31 | 17.1±0.27 | 27.7±0.33 | < d.l. |
| | **M4** | 27.0±0.28 | 20.6±0.37 | 31.4±0.39 | < d.l. |
| | **M5** | 30.4±0.36 | 20.2±0.51 | 26.8±0.51 | < d.l. |
| **Bjelasica** | **M6** | 25.6±0.17 | 41.5±0.45 | 88.6±1.98 | 2.92±0.09 |
| | **M7** | 18.0±0.29 | 13.7±0.34 | 39.4±0.64 | 0.25± |
| | **M8** | 27.0±0.22 | 21.3±0.38 | 68.9±1.73 | < d.l. |
| | **M9** | 13.6±0.19 | 14.5±0.23 | 61.7±0.98 | 0.41±0.03 |
| **Bihor** | **M10** | - | - | - | - |
| | **M11** | - | - | - | - |
| **Tara riv. valley** | **M12** | 50.2±0.25 | 45.1±0.55 | 69.8±1.13 | 0.15±0.008 |
| | **M13** | 54.2±0.31 | 48.2±0.48 | 60.0±1.23 | 0.05±0.002 |
| **Kolašin** | **M14** | 152.9±0.71 | 133.2±1.32 | 24.7±0.85 | < d.l. |
| **Pljevlja** | **M15** | - | - | - | - |
| **Average** | | 40.17 | 34.49 | 46.99 | 0.64 |
| **Max. allowable conc.*** | | 20-60 | 50-200 | 20-300 | 1-5 |

Source: * - Bowen, 1979.

Heavy metal content in both blueberry and raspberry samples (Table 2), expressed on dry weight bases, is within normal range for plant tissue. Significantly higher Ni content was found only in blueberry at location B8.

Table 2. Heavy metals in the fruits (mg kg- 1 d.w.)

| Locality | Label | Ni | Cr | Pb | Cd |
|---|---|---|---|---|---|
| | | Blueberry | | | |
| Bjelasica | B1 | 1.0±0.02 | 0.7±0.04 | 1.9±0.11 | 0.10±0.002 |
| | B2 | 1.5±0.07 | 0.9±0.04 | 3.2±0.23 | 0.16±0.003 |
| | B3 | 1.5±0.06 | 1.2±0.08 | 1.6±0.11 | 0.11±0.001 |
| | B4 | 3.1±0.09 | 0.2±0.03 | 3.9±0.18 | 0.16±0.002 |
| | B5 | 1.5±0.04 | 0.8±0.05 | 3.9±0.21 | 0.16±0.002 |
| Lisa | B6 | 1.5±0.10 | 0.9±0.05 | 8.4±0.57 | 0.11±0.001 |
| | B7 | 1.3±0.08 | 1.4±0.03 | 3.3±0.14 | 0.11±0.002 |
| | B8 | 51.2±0.56 | 1.1±0.06 | 4.5±0.16 | 0.64±0.01 |
| Bihor | B9 | 2.0±0.11 | 1.1±0.05 | 4.1±0.21 | 0.10±0.001 |
| | B10 | 1.6±0.10 | 1.0±0.07 | 2.4±0.13 | 0.05±0.003 |
| Pljevlja | B11 | 1.6±0.09 | 1.2±0.06 | 10.5±0.38 | 0.11±0.002 |
| Average | | 6.169 | 0.9618 | 4.323 | 0.1645 |
| | | Raspberry | | | |
| Lim riv. valley | M1 | 9.9±0.29 | 0.3±0.02 | 2.7±0.12 | 0.43±0.003 |
| | M2 | - | - | - | - |
| | M3 | 3.9±0.14 | 0.3±0.02 | 6.0±0.40 | 0.27±0.002 |
| | M4 | 6.4±0.18 | 0.4±0.03 | 2.8±0.22 | 0.38±0.002 |
| | M5 | 9.6±0.29 | 0.8±0.06 | 3.0±0.21 | 0.48±0.005 |
| Bjelasica | M6 | 0.05±0.002 | 0.4±0.04 | 2.1±0.31 | 0.11±0.002 |
| | M7 | 6.0±0.41 | 0.2±0.01 | 3.0±0.18 | 0.38±0.001 |
| | M8 | 1.9±0.05 | 0.4±0.02 | 2.3±0.17 | 0.32±0.001 |
| | M9 | 4.1±0.10 | 0.8±0.09 | 3.6±0.20 | 0.59±0.007 |
| Bihor | M10 | 1.7±0.08 | 0.6±0.01 | 2.4±0.16 | 0.11±0.002 |
| | M11 | 3.2±0.11 | 0.8±0.07 | 2.8±0.13 | 0.32±0.004 |
| Tara riv. valley | M12 | 2.7±0.13 | 0.7±0.03 | 4.4±0.25 | 0.21±0.001 |
| | M13 | - | - | - | - |
| Kolašin | M14 | 10.0±0.39 | 0.6±0.04 | 2.55±0.19 | 0.27±0.002 |
| Pljevlja | M15 | 3.6±0.26 | 0.9±0.03 | 3.5±0.26 | 0.48±0.003 |
| Average | | 4.849 | 0.5492 | 3.188 | 0.3346 |
| Normal range in plants* | | 0.02 -5 | 0.03-14 | 0.2-20 | 0.1-2.4 |

Source: * - Kabata-Pendias and Mukherjee, 2007.

Estimation of heavy metal intake requires information on the levels of metals in food and the amount of food consumed (NAS/IOM, 2003). Calculation of an estimated daily intake of heavy metals in this study is based on the average metal content in the berries expressed on the fresh matter basis. In order to obtain average metal concentrations in fresh berries, the average concentrations of metals in dry matter were divided by 10,

based on the assumption that berries contain at least 90 % of water. Therefore, if a person consumes 200 g of fresh blueberry per day, they will then ingest 123.38 μg Ni $day^{-1}$, 19.24 μg Pb $day^{-1}$, 86.45 μg Cr $day^{-1}$, 3.29 μg Cd $day^{-1}$; or 96.98 μg Ni $day^{-1}$, 10.98 μg Pb $day^{-1}$, 63.77 μg Cr $day^{-1}$ and 6.69 μg Cd $day^{-1}$ by consumption of 200 g of fresh raspberry (Table 3).

Table 3. Estimated daily intake of heavy metals through consumption of the selected fruits

| | Ni | Pb | Cr | Cd |
|---|---|---|---|---|
| **Blueberry** | | | | |
| Average concentration (mg $kg^{-1}$ f.w.) | 0.6169 | 0.09618 | 0.4323 | 0.01645 |
| Estimated daily intake (μg $day^{-1}$) | 123.38 | 19.24 | 86.46 | 3.29 |
| **Raspberry** | | | | |
| Average concentration (mg $kg^{-1}$ f.w.) | 0.4849 | 0.05492 | 0.3188 | 0.03346 |
| Estimated daily intake (μg $day^{-1}$) | 96.98 | 10.98 | 63.76 | 6.69 |

## DISCUSSION

Heavy metals accumulation in soils is of concern in agriculture due to adverse effects on food quality, plant growth and environmental health. Pseudo-total content of heavy metals: Ni, Cr, Pb, and Cd, in the soils under this investigation corresponds to unpolluted and un-serpentinic sites (Kabata-Pendias and Mukherjee, 2007). High concentrations of both Ni and Cr at the same location – M14 imply to their natural sources, although it does not exclude potentially high accumulation of these elements in plant tissue.

Heavy metal accumulation in the food chain can be highly dangerous to human health. These metals enter the human body mainly through two routes: inhalation and ingestion, and the ingestion being the main route of exposure. Since dietary intake of food may constitute a major source of long-term low-level body accumulation of heavy metals, the detrimental impact becomes apparent only after several years of exposure. Regular monitoring of heavy metals from effluents, sewage, in vegetables and other food is essential for preventing excessive buildup of the metals in the food chain (Islam et al., 2007).

It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals) (Islam et al., 2007). Taking into consideration that results of the present study are given on the basis of dry weight and that most fruits contain at least 90 % of water, they show low level of contamination of selected fruits with heavy metals, compared with permissible levels given by FAO, WHO and EC. According to Commission regulation (EC) No 1881/2006, maximum level of Pb in berries is set on 0.2 mg $kg^{-1}$ of fresh weight, while Cd level is 0.05 mg $kg^{-1}$ f.w.

In order to establish certain food quality criteria, as well as to assess trace

element risk level, several governmental and private organizations have made a set of recommendations, such as Acceptable Daily Intakes (ADIs) and Provisional Tolerable Daily Intakes (PTDIs) proposed by World Health Organization (WHO), and Reference Dose (RfD) derived by USEPA, which represents an estimate of the daily exposure to which human population may be continually exposed over a lifetime without an appreciable risks of deleterious effects of heavy metals.

Our estimated daily intake of heavy metals by the studied fruits is below limit intakes set by FAO/WHO on the basis of body weight for an average adult (60 kg body weight). PTDI for Pb , Ni, Cr and Cd are 214 µg, 300 µg, 200 µg and 60 µg, respectively (Joint FAO/WHO Expert Committee on Food Additives, 1990). Thus, the consumption of an average amounts (around 200 g) of these foodstuffs does not pose a health risk for the consumer.

For Cr, as essential element for humans and animals, the US National Research Council has recommended a dietary intake for adults of 50–200 µg Cr (III) day–1 (NRC, 1989). It is usually considered that almost all the chromium in food is in the trivalent form (MAFF, 1999). Ingestion of Cr by consumption of an average amount of the fruits from the present study falls within the proposed interval. Consumption of an average amount of blueberry and raspberry studied also corresponds to an average daily intake of nickel (130 µg person $^{-1}$day$^{-1}$, Kabata-Pendias and Mukherjee, 2007).

## CONCLUSION

The present study provides additional data on heavy metals content (Pb, Ni, Cr and Cd) in soils and berries (blueberry, raspberry) from Montenegro and also help in risk assessment of consumer exposure to heavy metals through selected fruits consumption. Soil contamination with heavy metals has not been found, except at one location. Heavy metals content in blueberry and raspberry was mostly within normal range for plant material and within guideline values for fruits. Daily intakes of these metals through selected fruits consumption are found to be below the recommended tolerable daily intakes proposed by Joint FAO/WHO Expert Committee on Food and Additives and may not pose a health hazard to consumers. Nevertheless, regular survey of heavy metals in fruits, as well as in other foodstuff, is recommended in order to evaluate whether any health risk does exist, to ensure food safety and to protect users from food that might be threat to their health.

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# SADRŽAJ TEŠKIH METALA U ODABRANIM ZEMLJIŠTIMA I VOĆNIM KULTURAMA CRNE GORE I PROCENA NJIHOVOG DNEVNOG UNOSA PUTEM KONZUMIRANJA PLODOVA

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**Izvod**

U radu je ispitivan sadržaj: Pb, Ni, Cr i Cd u zemljištu i plodovima borovnice (*Vaccinium myrtilus*) i maline (*Rubus idaeus*) sa 26 lokacija u Crnoj Gori. Sadržaj teških metala u sakupljenim uzorcima određen je AAS metodom. Sadržaj teških metala u zemljištu bio je ispod praga kontaminacije, izuzev na lokaciji Kolašin. Sadržaj teških metala u plodovima borovnice i maline nalazio se u intervalu normalnih vrednosti za biljke, kao i u okviru predloženih granica za bobičasto voće. Procenjeno je da se dnevni unos teških metala putem konzumiranja plodova nalazi ispod vrednosti dnevnog unosa koji je preporučen kao prihvatljiv od strane FAO/WHO.

**Ključne reči:** Teški metali, zemljište, hrana, kontaminacija, borovnica, malina, dnevni unos.

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# COMPARATIVE RESEARCH OF HYPERBARIC OXYGEN TOXIC EFFECT ON CNS AND ERYTHROCYTES

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*SUMMARY: This study describes a process of erythrocytopoiesis and changes in erythrocytes connected to toxic effects of oxygen, especially the membrane of erythrocytes. The experiment included 60 animals - Mus musculus which were exposed to effects of hyperbaric oxygen. The obtained results demonstrate that in cases of acute oxygen poisoning in hyperbaric conditions symptoms connected to CNS were most expressed. Detailed analyses performed by electronic microscopes revealed occurrence of changes in the shape of erythrocytes. Characteristic discoid shape of erythrocytes is changed so that acanthocytes, stomatocytes, echinocytes occur. Such shapes are reversible while these changes are most expressed in the 36$^{th}$ and 38$^{th}$ minute of exposure to hyperbaric oxygen under the pressure of 3.5 ATA. In the 40$^{th}$ minute of exposure to hyperbaric oxygen erythrocyte membrane rupture occurs, whereas these changes are irreversible. Such membrane ruptures occur in 30% of erythrocytes. Due to staging and occurrence of convulsions it is possible to stop the process and prevent destruction of erythrocytes.*

***Key words**: CNS, RBC, hyperbaric oxygenation, oxygen, toxicity*

## INTRODUCTION

Oxygen is the second most common gas forming the normal external air (20.93 percent), preceded only by nitrogen (78.10 percent). Oxygen is vital to sustain life. The partial pressure of oxygen, in inspired air, at sea level is about 160 mm Hg. Priestley, who discovered the oxygen, was himself amongst the first to suggest that there may be adverse effects of this ‘pure air’, when, in 1775, he observed a candle burning out faster in oxygen than in air, and wondered if the animal powers might ‘be too soon exhausted in this pure kind of air’. The CNS effects of oxygen toxicity are called ‘Bert effect’ named after Paul Bert, who, in 1878, demonstrated convulsions in larks exposed to 15-20 ATA

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(atmosphere absolute air). The so-called 'Smith effect' is the pulmonary effects of oxygen toxicity, named after J Lorain Smith, who, in 1899, while trying to reproduce 'Bert effect', noticed fatal pneumonia in rats after 4 days of exposure to 73% oxygen at 1 ATA. The clinical settings in which oxygen toxicity occurs are broadly divided into two groups; the first group, in which the patient is exposed to very high concentrations of oxygen for short duration, like in HBOT, and the second group, in which lower concentrations of the gas are used but for longer duration. These two can result in the so-called 'acute' and 'chronic' oxygen toxicity, respectively. The acute toxicity has predominant CNS effects, while chronic toxicity has predominant pulmonary effects (Dharmeshkumar et al, 2003).

The aim of this paper was to compare neurotoxicity and toxic effect of hyperbaric oxygen on RBC membrane.

## MATERIAL AND METHODS

The experiment was conducted on a group of 60 laboratory mice of Mus musculus species, which were exposed to hyperbaric oxygenation conditions. The control group (without hyperbaric conditions) was comprised of the equal number of experimental mice, and with the same biological characteristic, as the mice from the experimental group. The mice were 3-5 weeks old. In a special chamber they were exposed to 100% oxygen, during 40 minutes, at 3.5 ATA. 40 minutes and 3.5 ATA are enough for manifestation of convulsion (before research). Blood samples were taken from vein system of mice. Prepared samples were examined by transmission electronic microscope (TEM "Philips-500" and JSM-T20) (2500X and 10000X).

Statistical processing of obtained results included determination of: arithmetic mean (moment at which convulsion syndrome started), standard deviation, coefficient of variation, t-test for proportion (analysis of proportion of changed RBC during hyperbaric condition).

## RESULTS

The experimental animals showed convulsion while being exposed to hyperbaric pressure at 3.5 ATA. Table 1 represents the time when convulsion occurs. We calculated the mean value of time when convulsion occurred, which is 39.98 min.

The ensuing important question was: when did the general change in red blood cells from circulation happen – before or after convulsion?

Table 1: Time of convulsion occurrence
*Tabela 1: Vreme pojave konvulzija tokom izvođenja ogleda*

| Time of convulsion occurrence *Vreme pojave konvulzija* (min) | Number of experimental animals *Broj eksperimentalnih životinja* | Percent *Procenat* (%) |
|---|---|---|
| 39.00 | 7 | 11.67 |
| 39.50 | 8 | 13.33 |
| **40.00** | **32** | **53.33**** |
| 40.50 | 6 | 10 |
| 41.00 | 7 | 11.67 |
| Σ | 60 | 100 |

Table 2. The number of changed RBC contour regarding the time and kind of changes (/100 RBC)
*Tabela 2. Prikaz broja promenjenih eritrocita vezanih za vrstu i vreme promena kod ispitivane i kontrolne grupe, brojano na 100 eritrocita*

| Time *Vreme* (min) | Kind of RBC change / *Vrste promena eritrocita* | | | | | | | | Uncha nged RBC *Nepro menje ni ER* |
|---|---|---|---|---|---|---|---|---|---|
| | Acanthocyte *Akantociti* | | Stomatocyte *Stomatociti* | | Echinocyte *Ehinociti* | | Membrane rupture *Ruptura membr.* | | |
| | Contr. *Kontr* | Exam. *Ispit.* | Contr. *Kontr* | Exam. *Ispit.* | Contr. *Kontr* | Exam. *Ispit.* | Contr. *Kontr* | Exam. *Ispit.* | |
| 32. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **100** |
| 34. | 0 | **2** | 0 | 0 | 0 | 0 | 0 | 0 | **98** |
| 36. | 0 | **15** | 0 | **30** | 0 | 0 | 0 | 0 | **55** |
| 38. | 0 | **10** | 0 | **10** | 0 | **40** | 0 | 0 | **40** |
| 40. | 0 | 0 | 0 | 0 | 0 | **60** | 0 | **30** | **10** |

Table 3. Statistical analysis of proportion of changed RBC during hyperbaric condition
*Tabela 3. Statistička analiza razlike proporcija promenjenih eritrocita tokom hiperbaričnih uslova*

| Time *Vreme* (min) | Statistical value for t-test *Tablične vrednosti za t-test proporcije* | | Significance level *Nivoi značajnosi* |
|---|---|---|---|
| | $Z_{0.05}$ = **1.96** | $Z_{0.01}$ = **2.58** | |
| 32. | 1.96 | 2.58 | **0.00** |
| 34. | 1.96 | 2.58 | **1.43** |
| 36. | 1.96 | 2.58 | **9.00*** |
| 38. | 1.96 | 2.58 | **12.24*** |
| 40. | 1.96 | 2.58 | **30.00*** |

## DISCUSSION

During oxygen poisoning, changes occur at the cellular level, tissue level and organic level. Pathophysiology and clinical findings imply: change in glucose, lactose and fructose oxidation; shackle of haemoreceptors in aorta and glomus caroticum; increase of vagal tonus and decrease of heart minute volume; dilatation of pulmonary blood vessels; constriction and dilatation of CNS and renal blood vessels; convulsions, pulmonary oedema, alveo-capillary blockade (Dekleva, 1989; Hakan et al, 2007). Research of oxygen toxicity symptoms is very important, because oxygen is used in many medical treatments (Edremitlioglu et al 2005; Tomur et al 2005).

Acute oxygen poisoning causes oxygen convulsion or oxygen spasm in animals. This happened during hyperbaric conditions, when using oxygen at high pressure at level 3 ATA or higher. First, we can see light tremor with fasciculation of muscles in face and nape region. Second, there is the extended convulsion and status epilepticus with superficial breathing. At the final level, arrhythmia and coma happen (Belić, 1996). In later experiments, convulsive syndrome was asserted by EEG procedure and findings (Kurasako et al, 2000; David Henry, 2008). In literature there are many pathophysiology

explanations for this: change in CNS circulation and blood flow, change in NO metabolism, change in NADPH metabolism, oxidative stress, cytoskeleton changes, etc. (Kurasako et al, 2000; Nie et al, 2006; D'agostino et al, 2009).

Changes in RBC membrane, which happen during hyperbaric oxygen poisoning, are completely different from changes during diver type of anaemia. In toxic oxygenation there is the change in phospholipid arrangement in RBC membrane, and this part of RBC membrane becomes non-rigid (Byers et al, 1992; Lambert et al 1989; Lambert et al 1990). These changes are the result of mice exposure to hyperbaric conditions at level 3.5 ATA. For development of irreversible changes in RBC membrane certain amount of time has to elapse.

The changes of RBC membrane were not found in the first 32 minutes after hyperbaric exposure. The first altered form of RBC – acanthocytes, was detected in blood in 34$^{th}$ minute after the exposure. The number of acanthocytes was not increasing until 36$^{th}$ minute, and after that, their number decreased. In 40$^{th}$ minute acanthocytes were not found. Due to inconsiderable changes in acanthocytes membrane, RBC function is probably retained.

In 36$^{th}$ minute stomatocyte cells were discovered, the number of which was reduced in the samples that followed, including the samples of 40$^{th}$ minute, when the shape of RBC were not found. In this case, RBC function is probably retained, as well.

RBCs with very serious changes in membrane are echinocytes. Echinocytes represent 60% of RBC in 40$^{th}$ minute of hyperbaric condition. Erythrocytes vitality at this time is very disputable. When compensator mechanism of RBC membrane is used, it can be seen that there is a rupture in RBC wall, and the leak of matter from erythrocite inside. Erythrocytes function is damaged, and this finding indicates an irreversible process, and RBCs are definitely lost (Belić, 2008).

In these experimental conditions, general irreversible change in RBC population occurred simultaneously with convulsion. The next research needs to examine the possibility of RBC protection by EEG inspection, and CNS protection by measuring antioxidative enzyme activity from RBC.

## CONCLUSIONS

Oxygen is toxic in concentrations higher than the physiology concentration. RBCs (erythrocytes) are very sensitive to acute oxygen toxicity. Fundamental changes occurred in RBC membrane, and these changes are characteristic for oxygen toxicity. Oxygen toxicity increases in function with hyperbaric exposure time. Irreversible changes in RBC membrane are simultaneous with convulsion with experimental mice.

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# UPOREDNO ISPITIVANJE TOKSIČNOSTI HIPERBARIČNOG KISEONIKA NA CNS I ERITROCITE

BRANISLAVA BELIĆ, MARKO R.CINCOVIĆ

**Izvod**

Urađen je eksperimentalni rad na grupi od 60 eksperimentalnih životinja Mus musculus, koje su izlagane dejsvu hipebaričnog kiseonika. Rezultati pokazuju da su kod akutnog trovanja kiseonikom, u hiperbaričnim uslovima najviše izraženi simptomi vezani za CNS. Konvulzije su bile izražene između 39. i 40. minuta izlaganja životinja hiperbaričnom kiseoniku. Detaljne analize pomoću elektronskog mikroskopa su pokazale da na eritrocitima dolazi do promene oblika. Eritrociti gube karakterističan diskoidan oblik, javljaju se akantociti, stomatociti i ehinociti. Ovako izmenjeni oblici su reverzibilni, a promene su najizraženije u 36. i 38. minutu izlaganja uticaju hiperbaričnog kiseonika pod pritiskom od 3. 5 AT. U 40 minutu izlaganja uticaju hiperbaričnog kiseonika dolazi do ruptura membrana eritrocita, a te promene su ireverzibilne. Rupture membrana su nađene u 30% eritrocita. Zahvaljujući etapnosti i pojavi konvulzija moguće je proces zaustaviti i sačuvati eritrocite od propadanja.

**Ključne reči**: CNS, eritrociti, hiperbarična oksigenacija, kiseonik, toksičnost

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# GENOTYPING OF NEWCASTLE DISEASE VIRUS STRAINS, ALLOCATED IN UKRAINE IN 1967-2007 (GENOTYPES 1, 2 AND 4)

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*SUMMARY: The aim of this work is molecular epidemiology study of Ukrainian isolates of Newcastle disease virus. The research was conducted using molecular biology and cladistics methods. Viral isolates were genotyped with molecular techniques by Aldous E. method. Analyzed viruses were divided into 3 genetic lineages – 1, 2 and 4, in accordance with the modern classification. The lineage 1 was presented by 3 strains (2 from chicken and 1 from turkey) allocated in 1967 and 2005-2006, the lineage 2 was represented by 8 isolates from chicken, while the lineage 4 was subdivided into 4b and 4d isolates from chicken.*

***Key words:*** *genotype, molecular epidemiology, Newcastle disease virus, phylogenetic analysis RNA, sequencing*

## INTRODUCTION

Newcastle disease virus (NDV, avian paramyxovirus type 1 (APMV-1), is taxon of genus *Avulavirus*, subfamily *Paramyxovirinae*, family *Paramyxoviridae*, order *Mononegavirales* in accordance with modern classification (Lamb 2000; Mayo 2002).

NDV causes disease in chicken, associated with different types of disorders and clinical signs. It can vary from mild or asymptomatic disease to very acute and mass affection of high amount of poultry. The list of potentially affected species includes a high number of domestic, synantropic and wild birds, which can be latent virus-keepers or can demonstrate huge mortality disease (Alexander 1991).

Acute forms of Newcastle disease cause increased economic losses in poultry and

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have the tendency of panzootic spread. For the elaboration of effective schemes for virus eradication in poultry it is necessary to make and realize rapid diagnostics strategy of the infection. A lot of different serological, virological and molecular techniques are described for this purpose. In accordance with O.I.E. recommendations typical scheme of diagnostics consists of virus isolation in incubated chicken embryos, to be followed by identification of NDV antigen in serological tests (HI, ELISA etc.). More sensitive and rapid identification of pathogen can be done by using molecular techniques. It includes PCR, Real-time PCR, RFLP, and sequencing. The last one is a most informative test, which can provide researcher with information about virus pathogenity, viral genotype and viral source (Alexander 1991; Collins et al. 1993; Vallat 2008).

Aldous E. method of virus genotyping represents F-gene partial sequencing phylogenetic analysis data. In accordance with his classification, 6 viral genotypes were described. Three of them, 1, 2 and 6, are presented by strains with different levels of pathogenity (1 – asymptomatic disease, 2 – different types of clinical signs, 6 – low pathogenic) and have no subtypes. The genotypes 3 and 4 include 4 sub-lineages (3a-3d and 4a-4d), the genotype 5 – forms 5 subgroups (5a-5e), showing strains with very different levels of pathogenity (Aldous et al. 2003; Liu et al. 2007).

Genotyping methods provide researchers with molecular epidemiology experimental data, showing territorial, host-specific, time and pathogenity relations between NDV strains. This information can be used for elaboration of effective virus spread prevention means and NDV eradication strategy design.

Seventeen isolates of NDV from different pathotypes and allocation period were characterized in molecular study for F gene sequencing and phylogenetic analysis.

## MATERIALS AND METHOD

**Viruses**. Seventeen isolates of NDV were studied within this study (Table 1).

**RNA extraction and PCR.** RNA extraction from extraembryonal fluid and clinical material was done with RiboSorb-100 commercial kit, designed by ASRI of Epidemiology (Russia). The extracted samples were used for the reverse transcription reaction. This step was managed by commercial kit Reverta-L, produced by the same manufacturer. Amplification of the samples, used for sequencing, was done with PCR-Master-mix contained 2.5 mM $MgSO_4$, 0.25 mM each dNTP, and 5 U of Taq-polymerase, manufactured by ASRI of Epidemiology (Russia). The primers described by Aldous E. for amplification of F gene (partial sequences, ~400 bp region) were used (Aldous et al. 2003). Detection of PCR products was done in 1.5 % agarose gel, stained with 0.9 % solution of ethidium bromide.

**Sequencing**. Sequencing of the amplified products after purification (Qiagen DNA Purification kit) was performed by ABI-technology using Big dye terminator kits in normal conditions. Direct sequencing was done with both forward and reverse primers and purified by Rosche columns for sequencing products. Gel-screening of obtained cDNA-fragments was supplied with ABI-Prism series DNA-Sequencer. Obtained chromatograms correction was done in accordance with sequencing data of previous studies with BioEdit free software, v. 7.1.0.5 (Aldous et al. 2003).

**Phylogenetic analysis**. Phylogenetic analysis of the sequenced fragments was

done including reference nucleotide sequences of each NDV genotype and subgenotype, using Neighbor-Joining algorithm in free software Mega 4. Classical view dendrogrames were analyzed for each subtype.

Table 1 List of analyzed NDV strains
*Tabela 1. Sojevi NDV uključeni u istraživanje*

| Strain | Cleavage site | Lineage | Acc. number |
|---|---|---|---|
| NDV/Ch/Kegichovka/2004 | RRQKRF | 4b | Non-published |
| NDV/Ch/Kiev/02/2001 | RSKKRF | 4b | Non-published |
| NDV/Tr/BCh/1967 | GKQGRL | 1 | Non-published |
| NDV/Ch/Kupyansk/2003 | RRQKRF | 4d | Non-published |
| NDV/Ch/Kharkiv/2006 | RRQKRF | 4d | Non-published |
| NDV/vitapest | GRQGRL | 2 | Non-published |
| NDV/clone_30 | GRQGRL | 2 | Non-published |
| NDV/Ch/Lugansk/01/2003 | RRQKRF | 2 | Non-published |
| NDV/Ck/Pokrov/01/1987 | GRQGRL | 2 | Non-published |
| NDV/Ch/PMV/93 | GRQGRL | 2 | EU780912 |
| NDV/Ch/Lypova_dolyna/2003 | GRQGRL | 2 | EU780901 |
| NDV/Ch/Borky/2003 | GRQGRL | 2 | EU780900 |
| NDV/Ch/Lubotyn/2003 | GRQGRL | 2 | EU780898 |
| NDV/Ch/Ivano-Frankivsk/01/07 | RRQKRF | 5d | EU780892 |
| clone_UA | GRQGRL | 2 | EU780891 |
| Muskovy_duck/14/2005 | GKQGRL | 1 | EU780899 |
| Wild_duck/01/2006 | GKQGRL | 1 | Non-published |

## RESULTS

Both samples were amplified in classical PCR to recognize 400 bp fragment of viral RNA. All specimens showed positive reaction, which gave possibility to identify viral presence in these materials. After purification and quantification of our samples, amount of cDNA-amplicones was 41-72 ng/µl, which was enough to make sequencing.

Analysis of prepared chromatograms showed 8 sequencing variants of PCR products.

The sequvar 1 included strain from turkey origin. Allocated in 1967, this strain was characterized as lentogenic with classical methods by Prof. German et al (unpublished).

The sequvar 2 contained two isolates from wild ducks (Muskovy duck/14/05 and Wild duck/01/2006).

The sequvar 3, characterized as lentogenic, consisted form strains LaSota clone UA/2003, clone 30/2002, NDV/ch/Pokrov/08/1987, NDV/vitapest/2003.

The group 4 and 5 of viruses were presented by lentogenic isolates Lypova dolyna, Lubotin, Borky (2003), PMV, and mesogenic NDV/ch/Lugansk/112/2003, respectively.

The sequvariant 6 and 7 contained one isolate per each (Kegichovka and Kiev, velo- and mesogenic respectively).

The 8$^{th}$ group was presented by two velogenic isolates form chicken – Kupyansk and Kharkiv.

Analyses of variability for sequenced locus let us determine pair-wise distances. The first sequvar, which was isolated from turkey, was highly similar in comparison to ducks' viruses. Distances between them were about 0.06-0.07.

The sequvar 2 isolates was quite similar inside the group. The distance between them was 0.01. The closest was the first variant of sequenced viruses, and the most different was NDV/ch/Ivanofrankovsk/01/07 (0.27) and the viruses of the 6th and 9th sequvars (0.25-0.26).

The isolates from the group 3 had no differences inside the pool, they were the most similar to group 4 (0.01) and the most different from the viruses of the 11th and 12th clades (0.22-0.28).

The 4th group viruses demonstrated high level of similarity to the clade.

The 5-8$^{th}$ groups had polymorphic sequences in comparison to the inside pool (approx. 0.20) and outside (0.19-0.23), and the most different from the viruses of 2nd group (0.28).

The dendrogram, constructed from reference strains' sequences for F gene each genotype showed, that analysed NDVs belong to genotypes 1 (n = 3, sequvar 1, 2), 2 (n = 9, sequvars 3-5), 4b (n = 2, sequvars 6, 7), 4d (n = 2, sequvar 8).

The first genotype NDV was presented by 3 Ukrainian strains. The isolate NDV/Tr/BCh/1967, allocated in 1967, was highly similar to the Italian strain AV 941/99 3430 (AY175737), that was described by Aldous. These strains had only one mismatch in nucleotide sequences and had similar pathogenity. Ukrainian strain was isolated from domestic fowl, but Italian was derived from pigeon origin. Two other strains of this genoproup, isolated from ducks, had high level of F gene sequence homology to NDV strain AV 620/00 nor (AY175750), described by Aldous, isolated from domestic fowl (Aldous et al. 2003). High similarity was observed also between Ukrainian strains and Ulster2C/67 (M24694) derived viruses (Figure 1). Analyzed strains were allocated in 2005-06, and the divergence between Ukrainian strains, which were used in this study, was about 5 %.

Divergence level of group of sequences was not so high – up to 4 %, divergence between strains and the closest genetic neighbors in group showed rate in 0.25-0.7 %.

The Ukrainian viruses of genotype 2 from sequvar 3 were isolated from chicken origin and were similar to group of lentogenic viruses, isolated in Europe, like AV 1185/96 G91 (AY175693) with divergence less than 0.5 %. Other isolates of chicken origin (sequvars 4 and 5) demonstrated divergence level 2% and more. The virus NDV/Ch/Lugansk showed high level of similarity to duck and chicken isolates from India,

Indonesia, and other Asian countries (conservative rate of sequences 99.6 %). NDVs Borky, Lypova dolyna, PMV and Lubotin was highly similar to sequvar 3 viruses (homology 99.2 %) and viruses isolated in Asia. Also, these isolates were similar to all LaSota-like strains. Strains of this group were isolated during rather different periods (1987-2003).

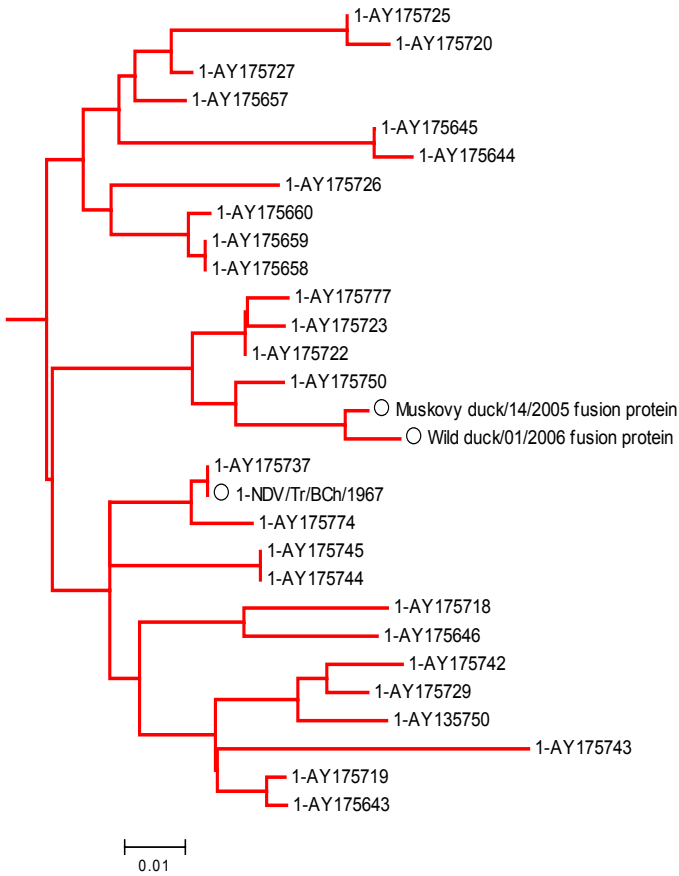


Figure 1. Subtree of phylogenetic relations between Ukrainian genotype 1 isolates and reference NDVs (○ – Ukrainian virus)

*Shema 1. Filogenetsko podstablo ukrajinskih izolata genotipa 1 i referentnih virusa ND (○ – ukrajinski virusi)*

The lineage 4 viruses (n = 4) were derived from chicken origin in 2001-2004. Topographically tree, derived from 4 genotype viruses, contained 3 sequvars of NDVs, isolated in different years. These viruses had high level of nucleotide homology to different NDVs. 4b isolate Kiev showed homology to Italian strain from wild birds AV 434/00 2736 (AY175759) from 2000, 4b virus Kegichovka was close neighbor with AV 316/98 ARABIA (AY175760), allocated in Saudi Arabia in 1998. Two other strains, 4d viruses Kupyansk and Kharkiv, were closely related to 4d isolates form Hon-Kong

(AY175673) and from Bulgaria (AY135742). Nucleotide divergence of the listed viruses in the group was about 0.1, 0.9 and 0.1-1.1 %, respectively.


Figure 2. Phylogenetic comparison between Ukrainian NDV isolates, world-wide isolated genogroup 2 and reference strains of NDV (○ – Ukrainian viruses).

*Shema 2. Filogenetsko poređenje ukrajinskih izolata NDV, drugih izolata NDV širom sveta u genogrupi 2 i referentnih sojeva NDV (○ – ukrajinski virusi).*

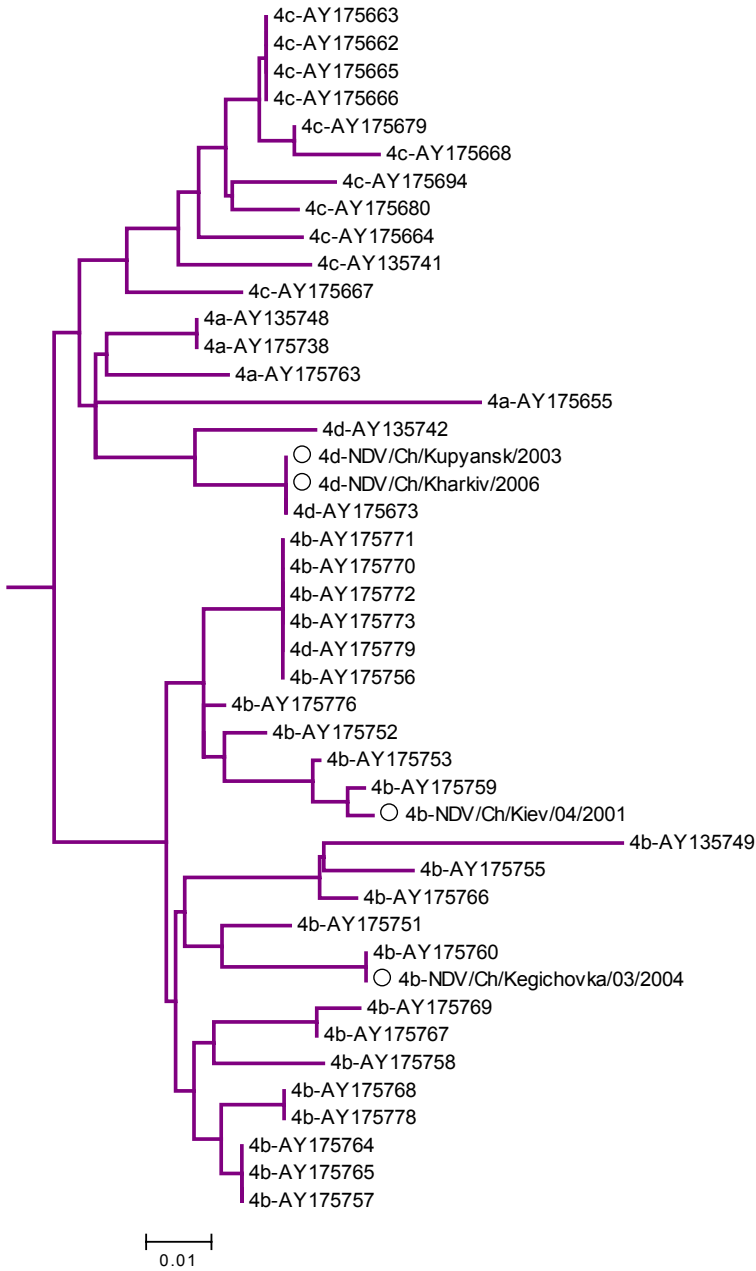


Figure 3. Subtree of phylogenetic relations between Ukrainian genotype 4 isolates and reference NDVs (○ – Ukrainian virus)

*Shema 3. Filogenetsko podstablo ukrajinskih izolata genotipa 4 i referentnih virusa ND (○ – ukrajinski virusi)*

## DISCUSSION

Ukraine is situated on the way of mass migration for wild birds from Europe to Asia and back. Such situation can explain viral spread processes and evolution of their populations. Host list of Newcastle disease virus includes a lot of species of wild birds, waterfowl, and domestic fowl.

In accordance with Aldous E. classification, 6 genotypes of virus has wide dissemination all over the world. Obtained data show circulation of 4 types of NDV: 1, 2, 4 (4b, 4d) in different species (Aldous et al. 2003).

Type 1 viruses were allocated from turkeys and ducks in 1967 and 2005-2006, and they are completely identical with in comparison with data of previous researchers. Also, these strains affect ducks and chicken. But in our case, it was also derived from turkey origin. Inside branch, divergence level was up to 10 % matching genotyping data of previous researchers (Seal et al. 1995; Aldous et al. 2003).

Type 2 isolates were allocated from chicken origin in different years from 1987 to 2003. They also have different pathogenity levels that were described by previous authors (Cattoli et al. 2001; Aldous et al. 2003; Otim et al. 2004; Lindh et al. 2008). Also, we have observed higher levels of conservatism inside clade. Divergence levels were not more 5 % inside group.

Ukrainian strains of the 4th genotype were subdivided into 4b and 4d isolates, which is quite typical for former Soviet Union countries lineage 4 viruses. Such situation was described also by Russian scientists (Pchelkina 2007b; Pchelkina 2007a). Moreover, isolates of these groups showed high similarity to NDVs from Asia (Aldous et al. 2003; Qin et al. 2008; Tan et al. 2008). All these viruses had high level of pathogenity and presented typical clinical signs in affected poultry. Diversity polymorphism of nucleotide sequences was up to 6 %, which is typical for this group. Source of virus from 4b genotype was untypical because it usually originates from pigeons, but our viruses were derived from chicken.

Analyzed viruses (n = 17) were classified as 4 different types representatives, with different sources. All isolates were characterized as derivates of previously described viruses and genotypes progenitors from European and Asian countries.

## CONCLUSION

The molecular genetic analysis of 17 NDV isolates shows the circulation of 4 genotypes: 1, 2 and 4 (4b, 4d) in different birds' species in Ukraine. The genetic lineage 1, presented by 3 strains (2 from chicken and 1 from turkey) allocated in 1967 and 2005-2006 demonstrated high level of similarity (95 %) with Ulster strain. The lineage 2 was represented by 8 isolates from chicken with 98-99.6 % homology with Asian strains of NDV. The lineage 4 is subdivided to 4b and 4d isolates from chicken, and is strongly related to Middle Asia and China strains. All isolated viruses were host-ordinary, and demonstrated different levels of pathogenity (from lento- to velogenic pathotype) and different geographical origin.

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## GENOTIPIZACIJA SOJEVA VIRUSA NEWCASTLE BOLESTI IZOLOVANIH U UKRAJINI OD 1967. DO 2007. (GENOTIPOVI 1, 2 I 4)

ANTON GERILOVYCH, BORYS STEGNIY, ALEKSANDAR POTKONJAK, VITALIY BOLOTIN, OLEXIY SOLODYANKIN

**Izvod**

Cilj ovog rada je molekularno epidemiološko istraživanje izolata virusa Newcatle bolesti (NDV) u Ukrajini. U radu su korišćene metode molekularne biologije, kao i metode koje se primenjuju za izradu filogenetskih stabala. Genotipizacija izolata NDV je izvršena primenom metode po Aldous E. Na osnovu savremene klasifikacije, ispitivani izolati NDV su klasifikovani u tri genogrupe (1, 2 i 4). U prvoj genogrupi nalaze se tri soja NDV (dva izolovana iz živine i jedan izolovan iz ćuraka, poreklom iz Ukrajine, 1967., 2005. i 2006. godine), u drugoj genogrupi nalazi se osam izolata NDV izolovanih iz živine, a u četvrtoj genogrupi nalaze se NDV izolovani iz živine, smešteni u dve podgrupe 4b i 4d.

**Ključne reči:** genotip, molekularna epidemiologija, virus Newcastle bolesti, filogenetska analiza RNK, sekvencioniranje.

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# A FIELD EVALUATION OF ANTHELMINTICS FOR CONTROL OF CYATHOSTOMES OF HORSES IN ALBANIA

REZART POSTOLI, DHIMITËR RAPTI, BEJO BIZHGA, GERTA DHAMO[1]

*SUMMARY: The anthelmintic efficacy of ivermectin (IVM), and fenbendazole (FBZ) alone was evaluated for the control of strongyles of equids during the grazing season and included 48 horses. The faecal egg count reductions (FECR) were evaluated 14 days after application. Ivermectin and the fenbendazole did not show significant differences during the trial, both groups exhibiting an FECR of >90% 14 days after application. The results of our study indicate that BZ resistance was not present on the farm investigated, as shown by a FECR test.*

***Keywords**: cyathostomes, fenbendazole, ivermectin, anthelmintic resistance, fecrt*

## INTRODUCTION

Strongyle nematodes are the most important internal parasites of the equines. Infection of equines with strongyles (nematodes of the superfamily *Strongyloidea*) is a widespread and serious cause of ill health throughout the world. Since the mid 1980s a widespread and frequent use of effective anthelmintics has resulted in a decreased occurrence of *Strongylus vulgaris* both in Europe and the USA (Herd, 1990; Nilsson et al., 1989) Unlike the situation of *S. vulgaris*, anthelmintic exposure has not had a similar impact on the small strongyles (subfamily *Cyathostominae*).

Control of these parasites is complicated by the presence of larval stages that are refractory to many commonly used anthelmintics, as well as by their ability to develop resistance to anthelmintic agents. The development of anthelmintic resistance (AR) of small strongyles has become a limiting factor in equine parasite control programs throughout the world. Widespread resistance to several anthelmintics by cyathostomin nematodes has become a major threat to equine health (Kaplan et al., 2004). This is partly attributed to benzimidazole resistance which is a widespread problem in cyathostome populations. Resistance to the benzimidazoles (BZ) was first reported in 1965 and since

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that time, BZ-resistant cyathostomes have been recorded in many countries (Lyons et al., 1999). Surveys on the occurrence of AR in Europe — Sweden (Nilsson et al., 1989), Denmark (Craven et al., 1998), Netherlands (Boersema et al., 1991) and Germany (Bauer et al., 1986), have shown that BZ resistance in cyathostomes is the rule rather than the exception.

The number of equines in Albania declined gradually from 1990 until now, due to the changes in agriculture, transportation and communication. Recent years have seen the expansion of interests in the equine field to include participation in national events, and particularly more in using horses for pleasure. Unfortunately, very little research on equine parasites has been done in Albanian regions. The traditional method for detection of anthelmintic resistance under field conditions is to perform a faecal egg count reduction test (FECRT). Resistance of cyathostomes to BZ anthelmintics has not been recorded in horse herds in Albania (Postoli and Munguli, 2004). Since no further information on AR of small horse strongyles in Albania was available, this finding prompted a trial of the occurrence of AR as determined by FECR test.

The objectives of the present study were first, to determine the presence and distribution of horses passing egg of cyathostomes and other strongyles second, to investigate the current status of resistance of cyathostomes to the commonly used anthelmintics by use of a FECR test.

## MATERIAL AND METHODS

The trial was conducted at a commercial breeding and racing farm in Peza commune, Tirana prefecture, during the 15 May-30 July 2008 grazing season and included 48 horses. The farm had a history of moderate to severe parasitic infections. The animals grazed on permanent horse pastures throughout the year. Their ages ranged from less than one to 20 years of age. The horses were divided into three age categories, foals (less than one year), young horses (one to three years) and adults (greater than three years) with 16, 15 and 17 horses in each age group, respectively. The criteria for this farm to be included in the trial were that no anthelmintic treatment had been undertaken for at least 8 weeks prior to the testing. The horse owners/managers were instructed to collect fresh faecal samples from horses on the farm, place them individually in plastic bags, evacuate the air and promptly send these samples to the laboratory.

In order to divide them into comparable groups, the horses were ranked according to age and pretreatment faecal egg counts, and then assigned ramdomly to a treatment group. Ivermectin 1.87 per cent (Vectin) was used at the recommended doze rate of 0.2 mg/kg. For fenbendazole an gel formulation at a doze rate of 10 mg/kg (Panacur) daily for 5 days was used. All the treatments were given orally. In order to calculate the appropriate doze for each animal, weight of the horses was estimated by measuring the heart girth, that is, the circumference of the chest directly behind the forelegs.

The horses were allocated to three groups. The group treated with ivermectin had 17 horses, with fenbendazole 16 horses and those in third group with 15 horses were kept as controls. Faecal samples were taken on days 14, 28, 42, 56 and 70 after treatment.

At the first farm visit, faecal samples were collected from each horse and stored at 50C in a cooler box until laboratory examination. Rectal faecal samples were taken

when possible, otherwise a sample of fresh faeces was collected from the floor of the stable (the horses were individually housed) or from the ground of the pasture (only faeces that were seen to be passed from individual horses were taken). The samples were processed within 24 h after faecal collection. Faecal egg counts were carried out using a modified McMaster technique based on 3 g of faeces (Anon 1986) with a minimum detection level of 50 eggs per gram of faeces (EPG). In order to estimate the proportion of cyathostomin eggs, pooled samples from each farm were incubated at 25ºC for at least 10 days. Third stage larvae were then collected by means of the Baermann procedure, preserved in Lugol's solution and identified either as Strongylus spp. or Cyathostominae spp. by morphological criteria according to Thienpont et al. (1979).

FECRs were calculated according to the recommendations for detection of AR in horses of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992). Based on this, worms are considered resistant when FECR < 90%, and arithmetic means (AM) are used in the calculations.

FECR% = (AM post-treatment control epg − AM post-treatment epg ) / (AM post-treatment control epg ) × 100

## RESULTS

The arithmetic mean strongyle egg counts before the treatments ranged from 790 to 982 and were not significantly different among the treatment groups. The efficacy of both ivermectin and fenbendazole was 100 per cent up to 28 days after treatment (Table 1). Since egg count reduction after an effective anthelmintic treatment should be at least 90% (Coles et al., 1992), BZ resistance was not detected in this horse population. The result of both ivermectin and fenbendazole was 100 per cent FECR 14 days after application.

Table 1: Efficacy of fenbendazole and ivermectin against naturally acquired strongyle infections in horses

| Days after treatment | | 14 | 28 | 42 | 56 | 70 |
|---|---|---|---|---|---|---|
| Groups | Arithmetic mean faecal egg counts expressed as eggs per gram of faeces | | | | | |
| Control | 790 | 520 | 632 | 965 | 1061 | 860 |
| Fenbendazol | 982 | 0 | 0 | 590 | 932 | - |
| Percent reduction (%) | - | 100 | 100 | 38.8 | 12.2 | - |
| Ivermektin | 890 | 0 | 0 | 25 | 570 | 622 |
| Percent reduction (%) | - | 100 | 100 | 97.4 | 46.3 | 27.7 |

Larval differentiation showed mixed infections in the samples taken before treatment; cyathostome larvae were predominant, but larvae of *Strongylus vulgaris*, were also observed. No larvae of Cyathostominae were present in the samples taken after the treatments.

## DISCUSSION

The results of these studies confirmed the efficacy of ivermectin and fenbendazole against strongyle infectious in horses. The results of our study indicate that BZ resistance was not present on the farm investigated, as shown by a FECR test. In Albania, an earlier report (Postoli and Munguli, 2004) did not revealed the presence of BZ resistant cyathostomes on two selected stud farms, and together with the results of the current study this suggests the lack of widespread AR of horse strongyles in the naïve horse population. The majority of the horses included in our study had been treated with BZ products one to two times per year for at least three years. BZs are the most commonly used anthelmintics in Albanian horse herds, due to the lower price of the drug compared to the avermectin/milbemycin products available on the market. In Australia, Kelly et al. (1981) reported that BZ resistant strongyles had only emerged on farms where horses had been treated more than three times per year. It is therefore unexpected to detect in our study the resistance with treatment regimes of only one to two doses per year. One general reason that clearly plays a role to the high efficacy of BZ products is an almost complete lack of information by horse owners and stable managers concern the equine parasite control, with minimal involvement by veterinarians. Several horse farms in Albania do not usually perform a control worm program (apart of their level management) and, thus, the Albanian cyathostome populations are not subjected to a high selection pressure by anthelmintic drugs. Their parasites have not been under any anthelmintic pressure for selection of resistance nor for the removal of susceptible species. In fact it is generally accepted that the selection pressure generated by abuse of anthelmintic drugs can be responsible for the appearance of resistance. These data suggest again that the higher the frequency of antiparasitic treatments the higher the probability of emerging drug resistant cyathostomes as previously demonstrated elsewhere, e.g. Australia (Kelly et al., 1981), Eastern Europe (Varady et al., 2000) and USA (Kaplan et al., 2004).

The efficacy of ivermectin was high: FECR was 100% up to 28 days after treatment. Post-treatment samples for FECR test are usually collected around 10–14 days after drug administration (Coles et al., 1992).

This study determined the ERPs of ivermectin and fenbendazole. This period can be defined as “the period after an anthelmintic treatment during which no eggs or small but acceptable numbers of eggs are excreted”. However, the meaning of a small or acceptable number is arbitrary and varies, according to the autor, from 100 to 200 epg (Herd et al., 1990; Uhlinger 1991). In the present study the ERP observed after treatment with ivermectin was about twice that after treatment with fenbendazol. The longer ERP observed for ivermectin provides a substantial benefit for controlling strongyle infections in horses. Furthermore, the use of ivermectin may reduce the frequency of treatments which may help to slow down the rate of development of resistance. The study of Borgsteede et al. (1993) demonstrated that nine weeks after treatment with ivermectin the egg output passed 90% of the reduction level. Once AR has occurred in a strongyle population, a reversion to anthelmintic susceptibility is not to be expected (Uhlinger and Johnstone, 1984). The lack of prevalence of a single family resistance (BZ) in equine cyathostomes in Albania suggests that it is not necessary to restrict the therapeutic use of this family and to exclude it from strategic treatment regimes in the region. Given its

previous limited usage resistance to fenbendazole is not likely to occur in horse herds in Albania in next few years, as it is seldom used for treatment of horses. Although there is some evidence that resistance to this drug may become apparent after 10–13 exposures to the drug. In this study ivermectin was effective against horse strongyles and may thus be a suitable candidate to replace BZ anthelmintics. On farms with a history of BZ resistance an annual rotation of tetrahydropyrimidines and macrocyclic lactones might be adopted in an attempt to minimize the rate of selection of AR.

Although the predominant species of parasites in both groups were cyathostomes, there was a greater diversity of parasites within the horse herd. This herd of horses has been experiencing a natural host parasite relationship.

Our findings in the regularly treated herd of horses, before and after treatment, indicate the predominant nematodes were cyathostomes. More attention is now being given to this group of nematodes that can cause a sudden onset diarrhea, a protein-losing enteropathy and some types of colic. Control strategies for improvement of parasite control, other than by chemical means alone, need to be adopted. Having a basic understanding of the life cycle of these parasites, using epidemiologic principles to determine appropriate timing of treatments, evaluating anthelmintics on every farm, incorporating pasture management into the overall handling and care for any herd will lead to improvements in the health of the horses and control of these parasites.

## CONCLUSION

The emergence of a degree of resistance of strongyles to FBZ in a segment of the cyatostomes in this population is suggested in the future. These data call for a geographically and numerically broader investigation of horse farms in all regions and for the development and implementation-among veterinarians, and owners of a plan to retard the expansion of these anthelmintic resistant populations and control these important parasites. Given the huge impact that anthelmintic resistance can and will have on welfare and the health of horses (Coles et al., 1992), it is of paramount importance that veterinarians take an active and leading role in planning and monitoring the efficacy of the worm control programs for horses. Without some intervention and education of farm managers and veterinarians in alternate anthelmintics treatment strategies it is likely that this phenomenon will spread.

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# TERENSKA ISPITIVANJA ANTIHELMINTIKA U KONTROLI CYATHOSTOMA KOD KONJA U ALBANIJI

REZART POSTOLI, DHIMITËR RAPTI, BEJO BIZHGA, GERTA DHAMO

**Izvod**

Efikasnost antihelmintika ivermektina (IVM) i fenbendazola (FBZ), ispitivano je tokom sezone ispaše, kod 48 konja. Redukcija broaj fekalnih jajajšaca (FECR) je ustanovljena 14 dana posle aplikacije preparata. Nije ustanovljena značajna razlika u delovanju ivemerktina i fenbedazola, jer je, kod obe grupe 14 dana posle tretmana, ustanovljena redukcija broja fekalnih jajašaca za > 90% . Rezultati istraživanja pokazuju da BZ rezistenicija nije bila prisutna na ispitivanoj farmi, što je pokazano FECR testom.

**Ključne reči:** cyathostome, fenbendazol, ivermectin, antihelmintici, otpornost, FECR.

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# REPLACING INORGANIC MINERALS WITH ORGANIC FORMS AT REDUCED LEVELS IN GROWING PIGS

JULES A.TAYLOR-PICKARD, LODE NOLLET, RONY GEERS[1]

*SUMMARY: Feeding lower levels of minerals in a more bioavailable form is important in reducing potential pollution from the manure resulting from intensive pig farming, as well as maintaining higher tissue reserves in the animals, facilitating growth and health. A trial using 136 crossbred male and female pigs was conducted to determine the effect of replacing inorganic minerals in the diet with organic forms (Bioplex and Sel-Plex, Alltech Inc, USA) at lower levels on growth performance and carcass characteristics. Diets were based on commercial wheat-soy formulations. The control treatment contained inorganic minerals (100 ppm zinc sulphate and iron sulphate, 15 ppm copper sulphate, 40 ppm manganese oxide and 0.3 ppm sodium selenite), whereas the test diet contained chelated minerals, with zinc, iron and manganese included at lower levels (40 ppm zinc, 40 ppm iron, 15 ppm copper, 20 ppm manganese and 0.3 ppm organic selenium from selenised yeast). Body weight and feed intakes were measured from weaning (~22 kg) to slaughter weight at 102 days after the start of the trial (~105 kg). Carcass characteristics were measured at slaughter. The results showed that growth performance was maintained and, in the case of gilts, increased for those fed the organic mineral diet. Slaughtering yield was not significantly different between treatments. Meat quality parameters were not significantly affected by the replacement of inorganic minerals with lower levels of organic forms, although backfat thickness and percentage lean meat were significantly improved ($P<0.05$) for the pigs fed the organic mineral diet The data demonstrated that lower levels of organic forms of minerals can be used in growing pig diets without any loss in performance, and benefits in growth and meat characteristics can be obtained.*

***Key words**: pigs, organic minerals, organic selenium, replacement.*

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Original scientific paper/ *Originalni naučni rad*

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## INTRODUCTION

Minerals are important for maintaining correct growth in growing animals. The current paper is concerned with the comparison of feeding different forms of manganese, iron, copper, zinc and selenium to growing pigs.

Manganese is involved in promoting immune function, as a component of antioxidant enzymes and for wound healing. Zinc plays a role in skeletal growth, immunity, metabolism, protein synthesis and nucleic acid metabolism as well as cellular repair and the utilisation of the antioxidant vitamins A and E. Copper is required for the development of connective tissue and blood vessels, and is also involved in production of functional red blood cells. Iron is necessary as a cofactor in enzyme reactions and is central to the transport of oxygen circulation in the blood, via haemoglobin. Selenium is a key antioxidant mineral, which works in concert with other antioxidants to maintain membrane integrity, reduce moisture loss from tissues and reduce the risk of oxidative damage. These minerals are hence recognised as necessary for correct and efficient growth in animals.

Minerals are available, broadly, in two forms; inorganic (such as oxides and sulphates) and organic (chelated to small peptides or amino acids via chemical or biochemical means). Various experiments have shown improvements in productive performance in animals receiving minerals that have been chelated to certain peptides. This is due to improved uptake from the digestive tract and better distribution and utilisation within the body (Coffey *et al*, 1994). Feeding organic mineral forms may result in higher circulating plasma concentrations (Hahn and Baker, 1993) or increased storage in tissues (Apgar *et al*, 1995). Two forms of Se are used in animal feed; inorganic (fed as sodium selenite or selenate) or organic (e.g. yeast-derived seleno-amino acids). Dietary seleno-amino acids are actively absorbed across the intestine via a specific sodium-dependent transport system (Wolffram *et al*., 1986, 1989). Inorganic selenate is absorbed by co-transport with sodium ions, whereas selenite is passively absorbed from the intestine. Animals are considered unable to synthesise seleno-methionine from inorganic Se (Sunde, 1990).

Replacing standard levels of inorganic minerals in animal diets with low levels of organic forms is currently of particular interest in animal nutrition. The mineral recommendations for animal feeds is typically based on trials conducted several decades ago (Leeson, 2005), which, due to modern genotypes and production methods, may be rendered obsolete. Several animal studies have shown that performance can be maintained in animals fed much lower levels of organic minerals compared to the inorganic levels normally used. The additional benefits available from the reductions in the amounts of undesirable elements, such as zinc, excreted by the animals is of great importance in countries where soil and water mineral imbalances (due to pollution) are an environmental concern and are subject to legal control. There is currently little published research relating to the role of organic forms of Se and other minerals in growing pigs. The following experiment compares the performance and carcass quality of pigs from weaning to slaughter weight fed either standard levels of inorganic minerals or lower levels of organic minerals in feed.

## MATERIALS AND METHODS

One hundred and thirty-six Pietrain x HYPOR weanling pigs weighing, approximately 22 kg at the start of the trial, were used, housed in 12 pens containing six pigs per pen (68 males and 68 females in total). Pigs were selected by bodyweight for each pen to maximise homogeneity of the groups, and giving an equal average weight per animal for each pen at the start of the trial. For the starter period (d1-30), the pens measured 2 m x 2 m, arranged with six on each side of a central corridor. During the grower and finisher period (d 31-102) the pigs were housed in larger pens, measuring 2 m x 2.5 m. The pigs were slaughtered on d 102, as it has been shown that further fattening does not improve carcass parameters (Kosovac *et al*., 2007). Throughout the trial period pigs were housed on heated metal slatted floors, with *ad libitum* access to feed and water. All animals were dewormed using Narpenol on d 1 and with Ivomec at d 64 of the trial. They were vaccinated against Aujeszky disease on d 1 and 35.

The pigs received a wheat-soyabean meal based diet formulated into three phases, starter (20-40 kg), grower (40-70 kg) and finisher (70-105 kg). The dietary treatments included a control diet, formulated with inorganic minerals (Bioplex™, Alltech Inc, USA), and a test diet, which was formulated with lower levels of chelated zinc, manganese and iron and the same levels of copper and selenium in its organic form (Sel-Plex™, Alltech Inc, USA) (Table 1).

Table 1. Dietary mineral treatments in three phases of wheat-soyabean meal based pig diets
*Tabela 1. Dodatak minerala u hrani svinja u tovu baziranoj na pšenici i sojinoj sačmi*

| Mineral level (mg/kg)<br>*Nivo minerala (mg/kg)* | Control diet (inorganic)<br>*Kontrolna hrana (neorganski minerali)* | Treatment diet (organic)<br>*Tretman (organski minerali)* |
|---|---|---|
| Copper/ *Bakar* | 15 | 15 |
| Zinc /*Cink* | 100 | 40 |
| Manganese/ *Mangan* | 40 | 20 |
| Iron/ *Gvožđe* | 100 | 40 |
| Selenium/ *Selen* | 0.3 | 0.3 |

Live weight and feed intake was recorded on days 1, 31, 64 and 102 of the trial, and used to calculate average daily gain (ADG) and feed conversion ratio (FCR). Carcass quality and yield was evaluated at the slaughter house, and included carcass weight, percentage slaughter efficiency, ham quality, backfat thickness, lean meat yield, meat type and the meat building index (MBI). Data was analysed by the GLM and t-test procedures of the SAS system for Windows release 6.12, and confidence limits for significance were set at 5%. Data was corrected by using starting weight as a covariate in the statistical analysis. The economics, in terms of cost of the feed and value of the meat produced, were calculated and compared for each diet.

## RESULTS

There were no significant differences (Table 2) between body weights or average daily gain of pigs fed either diet during the trial. The pigs fed the organic mineral diet had numerically better FCR, equating to a decrease of 0.07 in feed conversion ($P>0.05$).

Table 2. Growth and feed coversion ratio (FCR) of pigs fed either inorganic or organic minerals
*Tabela 2. Porast i konverzija hrane sinja u tovui hranjenih organski vezanim ili neorganskim mineralima*

| Period (d) *Period (d)* | Body weight[1] (kg) *Telesna masa (kg)* | | Average daily gain (g/d) *Prosečan dnevni prirast* | | FCR *Konverzija hrane* | |
|---|---|---|---|---|---|---|
| | Inorganic control *Neorganski minerali* | Organic minerals *Organski minerali* | Inorganic control *Neorganski minerali* | Organic minerals *Organski minerali* | Inorganic control *Neorganski minerali* | Organic minerals *Organski minerali* |
| 1 | 22.6 | 22.6 | - | - | - | - |
| 1-31 | 45.3 | 44.0 | 734 | 689 | 2.07 | 2.09 |
| 32-64 | - | - | 883 | 895 | 2.60 | 2.49 |
| 1-64 | 74.5 | 73.5 | 811 | 795 | 2.37 | 2.32 |
| 65-102 | - | - | 797 | 841 | 3.60 | 3.52 |
| 1-102 | 104.0 | 104.6 | 798 | 803 | 2.84 | 2.77 |

[1]Body weight was recorded at the start of each diet phase and at the end of the trial (102 d)
*[1]Telesna masa je merena na početku svake faze ishrane i na kraju ogleda (113 dana)*

Carcass parameters evaluated several aspects of meat and ham quality, alongside slaughtering yield. The type number (the conformation of the ham joints) and Meat Building Index (calculated as the ratio of meat content and conformation) were calculated from the carcass data. Lower figures for these parameters indicated more desirable meat characteristics. All carcass data was corrected for initial body weight recorded on day one of the trial (Table 3). The only significant differences ($P<0.05$) observed between dietary treatments for any carcass parameters was seen in the organic mineral fed pigs, where backfat thickness was significantly lower resulting in more lean meat.

Table 3. Carcass parameters of growing pigs fed either inorganic or organic forms of minerals (data corrected for body weight at d1 of trial)
*Tabela 3. Klanični parametri svinja u tovu hranjenih organski vezanim ili neorganskim mineralima (korigovano na telesnu masu na početku ogleda)*

| Carcass parameter<br>Klanični parametri | Inorganic control<br>Neorganska kontrola | Organic minerals<br>Organski minerali |
|---|---|---|
| Carcass weight (kg)/ Masa trupa (kg) | 82.5 | 81.8 |
| Slaughter efficiency (%)/ Randman (%) | 77.1 | 76.9 |
| Ham width (mm)/ Širina šunke (mm) | 200 | 200 |
| Ham angle (º)/ Ugao šunke (º) | 43.7 | 44.4 |
| Backfat thickness (mm)/debljina leđne slanine (mm) | 13.5[a] | 12.2[b] |
| Lean meat (%)/Krto meso | 60.0[a] | 60.7[b] |
| Type number[1]/ Broj tipa | 2.01 | 1.98 |
| Meat Building Index (MBIc)[2] | 3.47 | 3.34 |

Means not sharing a letter differ significantly (P<0.05)
*Vrednosti u istom redu sa različitim slovima su značajno različite (P<0.05)*
[1] Conformation of the hams; [2] Ratio between meat content and conformation of the pig
*[1] Konformacija šunki; [2] Odnos između sadržaja mesa i konformacije*

## DISCUSSION

Replacing inorganic minerals with lower levels of organic forms did not adversely affect the performance or carcass composition of finisher pigs. The pigs receiving the organic mineral diet showed numeric improvements in FCR, backfat thickness and lean meat production. This suggests that the form of minerals may have the same level of relevance as the energy-protein ratio regarding performance and carcass quality in pigs (Kovcin *et al*., 2007). Improvements in growth performance were also observed in a previous trial, conducted in the same manner (Taylor-Pickard, in press), where pigs receiving organic minerals at the same lower levels showed significantly higher average daily gain, better slaughter yield, ham width and meat classification.

## CONCLUSION

Growth performance in growing and finishing pigs can be maintained, and, in the case of gilts, improved by replacing inorganic minerals with organic minerals at lower levels of inclusion. Meat quality was not altered by this replacement, and some benefits in carcass composition were evident. Using the strategy of replacing inorganic minerals with lower levels of organic forms is useful in reducing the potential output of minerals in manure, which can lead to pollution and, in many countries, requires more problems of disposal from intensive production systems.

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## ZAMENA NEORGANSKIH MINERALA NIŽIM NIVOOM ORGANSKI VEZANIH MINERALA U ISHRANI SVINJA U PORASTU

JULES A.TAYLOR-PICKARD, LODE NOLLET, RONY GEERS

### Izvod

U radu su dati rezultati ogleda u koji je uključeno 136 meleza svinja (Pietrain x HYPOR) sa ciljem da se utvrdi efekat zamene neorganskih minerala u hrani prasadi (Fe, Cu, Zn, Mn, Se) nižim nivoom minerala u organski vezanoj formi (Bioplex and Sel-Plex, Alltech Inc, USA). U ogledu je korišćena komercijalna hrana na bazi pšenice i soje. Rezultati su pokazali da nije došlo do pogoršanja proizvodnih rezultata upotrebom nižih nivoa organskih vezanih minerala, dok je kod nazimica čak došlo i do povećanja performansi. Klanične karakteristike trupova se nisu razlikovale između tretmana, izuzev debljine leđne slanine koja je smanjena i procenta krtog mesa koji je povećan kod grupe sa organski vezanim mineralima. Ovi rezlutati ukazuju na to da se upotrebom organski vezanih minerala može sniziti njihov nivo učešća u smešama bez negativnog uticaja na performanse, čime se smanjuje njihova upotreba u ishrani svinja i potencijalno smanjuje nivo njihovog izlučivanja u spoljašnju sredinu.

**Ključne reči:** svinje, organski minerali, organski selen, zamena.

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# DISAPPEARANCE OF AZOXYSTROBIN IN CUCUMBER

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*SUMMARY: The paper deals with the monitoring the azoxystrobin residues in cucumber up to the expiry of PHI of 4 days. During the years 2008 and 2009 a cucumber was treated with Quadris 25% SC in the amounts of 0.75 and 1.0 l/ha. The determination of residues was carried out with GC-ECD. The obtained from 0.29 to 0.34 mg/kg in the applied 0.75 l/ha of the compound and from 0.45 to 0.48 mg/kg for the amount of 1.0 l/ha. The values were below MRLs as regulated in Serbia and the EU of 1.0 mg/kg of cucumber.*

***Key words**: GC-ECD, azoxystrobin, cucumber, residues.*

## INTRODUCTION

It is known that in the conditions of the contemporary agricultural production over 30 % of the produce does not find its way to the consumers, whereas without the application of the chemical agents the loss would be doubled (Bursić *et al*., 2006). Therefore the application of the pesticides is essential for the increase of agricultural production. Due to their biocide activity and potencial risk consumers, the concentration of pesticides must be kept at the minimum in fruits and vagetables and has to be below the maximum residue limits (MRL) (Lazić *et al*., 2008).

A cucumber (*Cucumis sativus L*.) is one of the most significant vegetable crops in Serbia. If the procedures used in the industrial food processing and domestic cooking may reduce the pesticide residue levels dramatically (Hajšlova, 1999), fresh cucumber used as salad and/or pickles may contain high residue content and may not be submitted to possible thermic decomposition. With the aim of protecting a cucumber against the downy mildew, as the biggest problem in its protection, it is necessary to use quite a few various fungicides (Stojšin et. al., 2008). In recent times there has been an increased

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Original scientific paper / *Originalni naučni rad*

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interest in the application of the pesticides of "reduced risk" to people, non-target organisms and the environment and at the same time with a wide range of effects in comparison with the already registered pesticides. One of them is azoxystrobin, with the structure shown in Figure 1.

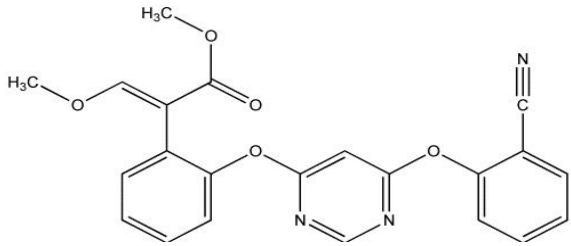


Figure 1. Chemical structure of azoxystrobin
*Slika 1. Hemijska struktura azoksistrobina*

The aim of this paper was to monitor the fungicide content from the application time to the expiry of the pre-harvest-interval (PHI) by the validated method (SHI, 2009; Lazić *et al*, 2008), of the gas chromatography (GC) coupled with ECD. The PHI in cucumbers is 4 days (Janjić and Elezović, 2008). The cucumber fruit was treated with Quadris 25% SC at the rates of 0.75 l/ha and 1.0 l/h. The MRLs for azoxystrobin in Serbia and the EU amounts to 1.0 mg/kg (Sl. Glasnik, 2002; Regulation EC, 2005).

## MATERIAL AND METHODS

Chemicals and Apparatus. The analytical standard of azoxystrobin of 99.7% purity was used (Dr. Ehrenstorfer GmbH, Germany). The stock standard solutions of azoxystrobin in the concentration of 100 µg/ml were prepared in toluene. The working standard solutions (0.01, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25 µg/ml) were obtained by dilution with toluene. All the solvents were of pesticide residue analysis grade (Carlo Erba, Milan, Italy), celit 545 (J.T. Bacer), carbo activatus (Laphoma).

The fungicide determination was carried out by the use of a GC-ECD (Hewlett Packard Gas Chromatograph 5890 Series II with splitless injection and capillary column SPB-5 (30m x 0.32 mm x 0.25 µm, Supelco, No.18441-03A)) and the temperature programme of azoxystrobin determination: the column temperature of 140 ºC maintained for 1 minute, the rise of 30 ºC /min to 195 ºC and then 40 ºC /min to 260 ºC hold for 15 min. The temperature of the injector was 230 ºC and of the detector 300 ºC. The total run time was 20 minutes.

Field Trials. The evaluation of the fungicide efficiency against cucumber downy mildew was conducted in the locality in Serbia, Čenej, on cucumber genotype Regal, 2008/2009. The treatments with Quadris 25% SC (250 g/l of azoxystrobin) in the concentration of 0.75 l/ha and 1 l/ha were sprayed with an atomizer using 600 l/ha of water.

Sampling. In both years the samples were collected at 0 (3 h post-application, after the deposit drying) and after 2 and 4 days after the application (from treated and untreated plots). Fruits (2 kg) were collected and placed in polyethylene bags, transported to the

laboratory immediately. Each sample was divided into five replicate sub-samples, and stored for a short time at -20 ºC until they were analyzed in the laboratory.

Extraction. The whole extracton was done by the Lentza-Rizos method, already used in the analysis of pesticide on grapes (2006). The azoxystrobin was extracted from 25 g of homogenized sample with the mixture of 50 ml of toluene and 25 ml of 2-propanol using an Ultra-Turax. The propanol layer was removed by rinsing twice with 2% $Na_2SO_4$ (125 ml x 2) solution and then pesticide - containing toluene layer was rinsed and cleaned up over the mixture of celit and active charcoal (1:3 w/w). The extract was evaporated to dryness and dissolved in toluene (1 ml).

Analysis. The retention time of azoxystrobin was 16.06 min. In order to avoid the matrix influence on the azoxystrobin standard, the standards prepared in matrix solution were used in the analysis.

Recovery assays. The samples of untreated cucumbers were fortified with the appropriate volumes of standard solutions, extracted in triplicate and analyzed by chromatography in duplicate. The concentration of standard solutions ranged from 0.01-1.25 μg/ml.

Validation. The detection limit (LOD) was determined as the lowest concentration giving a response of three times the average of baseline. The limit of quantification (LOQ) was determined as the lowest amount of a given pesticide giving a response of ten times the average of baseline (Araound, 2007). For the determination of LOD and LOQ cucumbers spiked with 0.01 μg/mL were used. The linearity in the response was studied with a standard solution prepared in matrix extract in the concentration ranging from 0.01 to 1.25 μg/ml.

## RESULTS

**Analytical method**. Figure 2 shows the chromatogram of the untreated cucumber extract. As it does not show any interfering peaks, no clean up was needed. Standard solutions were analyzed in the range from 0.01-1.25 μg/ml with good linearity for the ECD with the correlation coefficient of 0.996. LOD was 0.01 mg/kg which is below the EU MRLs. The quantification limit was 0.023 mg/kg, which is also considerably below the MRLs. The recovery for azoxystrobin in cucumbers was 93 - 101%.

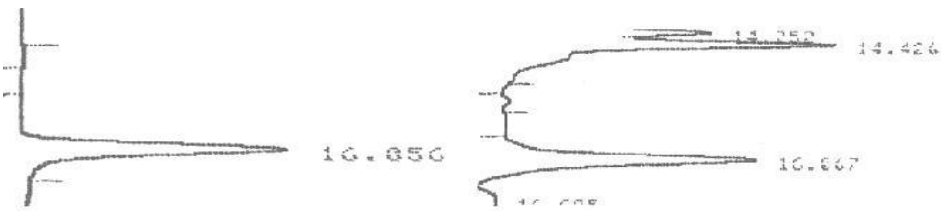


Figure 2. Chromatogram of standard and untreated sample spiked with 0.25 mg/kg of azoxystrobin

*Slika 2. Hromatogram standarda i spajkovanog uzorka sa 0,25 mg/kg azoksistrobina*

**Residue degradation.** The chromatograms of the analysed samples were shown in Figure 3.

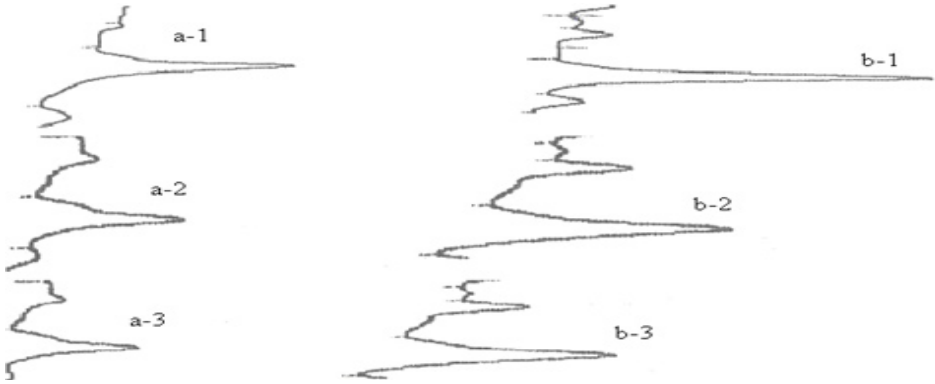


Figure 3. Chromatograms of analysed samples: a-application of 0.75 l/ha Quadris 25%, b-application of 1.0 l/ha Quadris 25%
*Slika 3. Hromatogrami analiziranih uzoraka: a-aplikacija 0,75 l/ha Quadris 25%; b-aplikacija 1,0 l/ha Quadris 25%*

1 – uzorci prikupljeni odmah nakon sušenja depozita, 2 – uzorci prikupljeni dva dana nakon tretiranja,i 3 – uzorci prikupljeni po isteku karence (4 dana)
*1-samples collected immediately after drying of deposit, 2-samples collected two days after treatment, 3-samples collected on the expiry of PHI (4 days)*

The azoxystrobin residue amount was calculated by the calibration curve, generated from the peak area obtained by the results of the matrix-matched standard solutions analysis. The table 1. shows the results obtained from the sample study in five replicates, as well as their mean value correlated to the amount of the applied compounds.

Table 1. Azoxystrobin content in cucumber samples
*Tabela 1. Sadržaj azoksistrobina u uzorcima krastavca*

| Quadris 25% | Godina *Year* | Dani nakon tretiranja *Days after treatment* | Ostaci (mg/kg) u ponavljanjima *Residues (mg/kg) in replicates* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | Prosek *Mean (mg/kg)* |
| 0.75 l/ha | 2008 | 0 | 2.15 | 2.12 | 2.02 | 2.09 | 2.17 | 2.11 |
| | | 2 | 1.12 | 1.02 | 1.09 | 1.03 | 1.09 | 1.07 |
| | | 4 | 0.39 | 0.31 | 0.35 | 0.29 | 0.36 | 0.34 |
| | 2009 | 0 | 2.10 | 2.15 | 2.16 | 2.04 | 2.02 | 2.09 |
| | | 2 | 1.20 | 1.06 | 1.32 | 1.19 | 1.08 | 1.17 |
| | | 4 | 0.25 | 0.29 | 0.24 | 0.34 | 0.33 | 0.29 |
| 1.00 l/ha | 2008 | 0 | 2.55 | 2.47 | 2.48 | 2.43 | 2.57 | 2.50 |
| | | 2 | 1.23 | 1.23 | 1.26 | 1.34 | 1.29 | 1.27 |
| | | 4 | 0.51 | 0.42 | 0.41 | 0.44 | 0.47 | 0.45 |
| | 2009 | 0 | 2.39 | 2.41 | 2.42 | 2.44 | 2.40 | 2.41 |
| | | 2 | 1.35 | 1.37 | 1.31 | 1.36 | 1.38 | 1.35 |
| | | 4 | 0.48 | 0.47 | 0.47 | 0.49 | 0.46 | 0.48 |

## DISCUSSION

A T-test assuming unequal variances showed no significant difference between the mean residues in cucumber for the two years of the study at the 95% confidence level. For both treatments, the residues in the samples collected after PHI were always below the MRLs for cucumber.

The applied amount of 187.50 g a.s./ha leaves an initial deposit of 2.07 mg/kg and 2.43 mg/kg for the applied amount of 250 g a.s./ha on average. Both amounts which were applied "drop" after the fourth day below the MRLs. According to Anand *et al*. (2008) the azoxystrobin residues in cucumbers, after the application of 31.25, 62.50 and 125 g/ha, dropped below the MRLs after the third day. According to their studies, on the application of 250 and 500 g a.s./ha the residue values were below the MRLs only after the fifth day (the fourth day was not analysed).

## CONCLUSIONS

The chromatographic method used for the quantification and analysis of azoxystrobin residues has the recovery value of 98-101%, with LOD of 0.01 mg/kg and LOQ of 0.023 mg/kg. When evident monitoring the azoxystrobin degradation, the decrease in its content is observes over the days with the final value below the MRLs of 1.0 mg/kg on the expiry of the PHI (on the fourth day). The study results show that the health-safe products with the pesticide residues below the MRLs can be obtained by the application of pesticides in accordance with the standard agricultural practice.

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## NESTAJANJE AZOKSISTROBINA SA KRASTAVCA

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### Izvod

Rad obuhvata praćenje ostataka azoksistrobina u krastavcu do isteka karence od 4 dana. Tokom 2008. i 2009. godine, izvršeno je tretiranje krastavca preparatom Quadris 25% SC u količinama 0,75 i 1,0 l/ha. Uzorkovanje je izvršeno odmah nakon sušenja depozita, dva i četiri dana nakon tretiranja. Određivanje ostataka izvršeno je GC- ECD. Dobijene vrednosti nakon isteka karence, kretale su se od 0,29 do 0,34 mg/kg kod primenjenih 0,75 l/ha preparata i 0,45 do 0,48 mg/kf za količinu od 1,0 l/ha. Vrednosti su bile ispod maksimalno dozvoljenih količina propisanih u Srbiji i EU od 1,0 mg/kg krastavca.

**Ključne reči**: gc-ecd, azoksistrobin, krastavac, ostaci.

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# HERBICIDAL EFFICACY IN WEED CONTROL IN CROPS CORN

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*SUMMARY: Field trials were carried out in 2004, 2005 and 2006 in corn in the region of Pančevački rit. The examined herbicides were: alahlor (Alahlor 480), atrazin (Atrazin 500), nikosulfuron (Motivell), prosulfuron+ primisulfuron-methyl (Ring 80 WG), EPTC + dihlormid (Eradicane 6-E), metolahlor (Dual), linuron (Afalon), dikamba-dimethylamonium (Banvel 480S). The data suggest that all applied herbicides could be successifully used for controlling broad – leaved weeds in corn crops in the region of Pančevački rit.*

***Key words**: corn, weed flora, herbicides, weed control, Pančevački rit.*

## INTRODUCTION

Because of the negative effects of weeds on the crop of corn, for their suppression is used a number of herbicides. When the decision on the selection and application of herbicides should be taken into account the stage of development of corn hybrids sensitivity, part of the weed flora, climatic conditions, time and manner of implementation. The choice of herbicides and/or their combinations are constantly changing due to the discovery of new active substances. Before applying herbicides to the weed flora composition of crops, as well as the effects of previously applied herbicides in broader environmental conditions (Konstantinović, 1999; Konstantinović et al. 1996, 2005; Nestorović, 2002, 2005, 2008). The aim of this study was to determine which herbicides to be successfully used for this purpose.

[1] Original scientific paper / *Originalni naučni rad*

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This paper presents the results of investigation in research projects Ministry of Science and Environmental Protection of the Republic of Serbia:

1. State and changes in biological and geological diversity of modified regions on the example of the central parts of Serbia (No 1864) financed by 01.01.2002. to 31.12.2005.
2. Diversity fossil and recent flora and fauna of Serbia - evaluation of the degree of diversity and vulnerability assessment as indicators of the protection of natural values (No 146023) funded by 01.01.2006.

## MATERIALS AND METHODS

Research conducted in the maize crop in the locality Pančavački rit in 2004, 2005 and 2006, according to the methods OEPP/EPPO (1998). The trials were placed in random block design in four repetitions, on plots 25 $m^2$ area.

Followed by the effect of applied herbicides (Table 1) to all the weed species found 30 days after applying herbicides counting individuals of each species $m^2$. Efficacy of herbicide efficiency coefficient is expressed in relation to the number of individuals of each species with the control variants.

Table 1. Applied herbicides.
*Tabela 1. Primenjeni herbicidi..*

| Variants<br>*Varijante* | Active substance<br>*Aktivna supstanca* | Preparation<br>*Preparat* | Amount applied of preparation<br>*Količina primene preparata* |
|---|---|---|---|
| A | alahlor + atrazin | Alahlor 480 + Atrazin 500 | 4 l/ha + 1.5 l/ha |
| | nikosulfuron + prosulfuron+primisulfuron-metil | Motivell + Ring 80 WG + Trend 90 | 1 l/ha + 30 g/ha |
| B | atrazin | Atrazin 500 | 1.5 l/ha |
| | nikosulfuron | Motivell | 1 l/ha |
| C | EPTC + dihlormid | Eradicane 6-E | 4 l/ha |
| | metolahlor + linuron | Dual + Afalon - tečni | 1 l/ha + 2 l/ha |
| | dikamba-dimetilamonijum | Banvel 480S | 0.5 l/ha |
| D | EPTC + dihlormid | Eradicane 6-E | 4 l/ha |
| | atrazin | Atrazin 500 | 2 l/ha |
| | nikosulfuron | Motivell | 1 l/ha |
| E | alahlor + atrazin | Alahlor-480 + Atrazin-500 | 4 l/ha + 1.5 l/ha |

## RESULTS AND DISCUSSION

The weed flora of maize crop is rich and varied, typically hilling crops. The maize crop is set forth 37 types that do not have equal importance in weeds grow (Table 2).

Weed flora consists mainly of corn plants therophytes (56.76%), and because of deeper and better before sowing processing and applications between the lines cultivation and hilling, thus reducing the direct involvement geophytes and hemicryptophytes. Among therophytes, which to us are about 60% of all identified species (Konstantinović and Marković, 2000; Konstantinović and Meseldžija, 2002), prevailing those grow in spring and late summer ripen. Hemicryptophytes (second with 27.03% representation identified species) were the most common types of vegetative reproduction through roots shoots. The geophytes, which consists of 8.11% the life spectrum, the most represented species from the rhizome for vegetative propagation. Due to intense vegetative reproduction, the

greatest dangers are exactly the kind hemicryptophytes and geophytes.

Based on the results (Table 2) may conclude that the most difficult to suppress corn crop species *Abutilon theophrastii, Agropyrum repens, Cirsium arvense, Convolvulus arvensis, Rubus caesius, Equisetum arvense, Sorghum halepense.*

Table 2. The average efficiency of herbicides in maize (2004-2006).
*Tabela 2. Prosečna efikasnost herbicida u kukuruzu (2004-2006).*

| Weed species *Vrsta korova* | Variants *Varijante* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control *Kontrola* | A | | B | | C | | D | | E | | Average *Prosek* | |
| | No/m$^2$ | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % |
| *Abutilon theophrastii* Med. | 9.00 | 1.50 | 82.61 | 2.67 | 69.09 | 1.25 | 85.89 | 2.42 | 73.02 | 3.17 | 65.13 | 3.33 | 75.15 |
| *Agropyrum repens* (L.) P. B. | 31.83 | 7.00 | 77.28 | 9.25 | 71.37 | 5.50 | 83.01 | 5.92 | 81.49 | 3.92 | 54.71 | 11.25 | 78.67 |
| *Agrostemma githago* L. | 0.33 | - | - | 0.17 | 33.33 | - | - | - | - | - | - | 0.17 | 33.33 |
| *Alopecurus pratensis* L. | 4.50 | 1.33 | 66.60 | 1.67 | 62.51 | 0.67 | 85.70 | 0.42 | 91.84 | - | - | 1.72 | 78.46 |
| *Amaranthus retroflexus* L. | 19.83 | 1.08 | 94.59 | 1.17 | 61.48 | 0.58 | 97.11 | 0.75 | 96.47 | 0.92 | 95.53 | 4.30 | 95.35 |
| *Ambrosia artemisiifolia* L. | 17.46 | 3.92 | 77.39 | 4.17 | 75.39 | 2.08 | 87.72 | 1.92 | 88.61 | 2.00 | 88.51 | 5.26 | 83.53 |
| *Anchusa arvensis* (L.) M. B. | 0.58 | - | - | - | - | 0.17 | 23.81 | 0.25 | 19.05 | - | - | 0.25 | 25.40 |
| *Avena fatua* L. | 3.92 | 1.50 | 62.64 | - | - | 1.00 | 76.07 | 0.33 | 26.98 | 0.25 | 28.57 | 1.84 | 72.92 |
| *Capsella bursa-pastoris* (L.) Med. | 10.50 | 1.33 | 57.51 | 1.92 | 53.99 | 1.17 | 59.12 | 1.00 | 60.48 | 0.83 | 60.86 | 2.79 | 58.39 |
| *Chenopodium album* L. | 25.17 | 2.58 | 89.44 | 2.42 | 90.28 | 0.50 | 97.81 | 0.92 | 96.22 | 2.25 | 91.37 | 5.64 | 93.02 |
| *Chenopodium murale* L. | 1.50 | 0.33 | 25.93 | 0.33 | 25.93 | 0.25 | 27.78 | 0.33 | 25.93 | 0.25 | 27.78 | 0.50 | 26.67 |
| *Cirsium arvense* (L.) Scop. | 7.67 | 2.58 | 65.67 | 3.42 | 55.06 | 2.42 | 68.13 | 2.58 | 65.28 | 2.67 | 64.80 | 3.56 | 63.79 |
| *Consolida regalis* Gray. | 2.92 | - | - | - | - | - | - | - | - | 0.75 | 49.21 | 1.83 | 49.21 |
| *Convolvulus arvensis* L. | 7.83 | 1.67 | 48.19 | 3.00 | 57.74 | 1.67 | 76.66 | 2.75 | 61.21 | 2.17 | 69.87 | 3.37 | 65.84 |
| *Cynodon dactylon* (L.) Pers. | 5.33 | 1.75 | 36.67 | - | - | 2.25 | 56.54 | 2.42 | 54.13 | 1.08 | 45.19 | 2.94 | 58.36 |
| *Echinochloa crus-galli* (L.) Beauv. | 6.58 | 2.25 | 65.84 | 1.42 | 43.89 | 1.08 | 82.63 | 1.08 | 82.63 | 0.83 | 53.33 | 2.51 | 76.70 |
| *Equisetum arvense* L. | 7.83 | 3.92 | 50.99 | - | - | 0.75 | 90.48 | 0.75 | 90.67 | - | - | 3.31 | 78.77 |
| *Fumaria officinalis* L. | 1.42 | 0.75 | 31.48 | 0.50 | 43.52 | - | - | - | - | - | - | 0.44 | 55.00 |
| *Galium aparine* L. | 1.00 | - | - | 0.42 | 41.67 | 0.58 | 25.00 | - | - | - | - | 0.67 | 25.00 |
| *Galium verum* L. | 0.67 | - | - | - | - | 0.25 | 8.33 | - | - | - | - | 0.64 | 13.89 |
| *Knautia arvensis* (L.) Coult. | 0.67 | 0.17 | 16.67 | 0.33 | 33.33 | - | - | - | - | - | - | 0.26 | 53.33 |
| *Lepidium draba* L. | 4.25 | 2.17 | 48.52 | 1.83 | 56.30 | 0.75 | 83.33 | 0.83 | 79.26 | 0.75 | 5.00 | 2.03 | 67.59 |
| *Nigella arvensis* L. | 0.33 | - | - | - | - | - | - | - | - | - | - | 0.08 | 33.33 |
| *Papaver rhoeas* L. | 2.33 | 1.00 | 57.50 | 0.17 | 91.67 | - | - | - | - | - | - | 1.17 | 74.58 |
| *Polygonum aviculare* L. | 2.42 | 0.58 | 16.67 | 0.67 | 48.25 | 0.83 | 43.97 | 1.00 | 39.37 | 0.33 | 23.81 | 1.21 | 42.12 |
| *Polygonum lapathifolium* L. | 5.00 | 1.75 | 65.18 | 1.42 | 41.44 | 0.33 | 24.44 | 1.42 | 71.04 | 1.42 | 41.37 | 2.39 | 66.61 |
| *Ranunculus acris* L. | 1.42 | 0.75 | 43.52 | 0.33 | 18.52 | - | - | - | - | - | - | 0.63 | 78.32 |
| *Rubus caesius* L. | 4.75 | 2.08 | 55.86 | 1.83 | 61.08 | 0.92 | 48.32 | 1.33 | 71.29 | 1.33 | 70.85 | 2.18 | 65.11 |

| Weed species *Vrsta korova* | Variants *Varijante* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control *Kontrola* | A | | B | | C | | D | | E | | Average *Prosek* | |
| | No/m$^2$ | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % | No/m$^2$ | ef. % |
| *Sorghum halepense* (L.) Pers. | 13.25 | 3.50 | 72.42 | 2.33 | 49.48 | 2.33 | 83.07 | 1.67 | 88.19 | 1.75 | 87.69 | 4.37 | 81.77 |
| *Stellaria media* (L.) Vill. | 7.75 | 2.25 | 68.45 | 2.00 | 69.78 | 1.58 | 79.71 | 0.92 | 85.97 | 0.33 | 95.70 | 2.58 | 79.92 |
| *Symphytum officinale* L. | 1.67 | 0.83 | 50.00 | - | - | - | - | - | - | - | - | 1.00 | 62.50 |
| *Verbascum blattaria* L. | 1.00 | 0.33 | 33.33 | - | - | 0.17 | 83.33 | 0.25 | 75.00 | - | - | 0.50 | 70.14 |
| *Vicia sativa* L. | 0.33 | 0.08 | 25.00 | - | - | - | - | - | - | - | - | 0.10 | 30.56 |
| *Xanthium strumarium* L. | 3.17 | 1.25 | 56.67 | 0.33 | 60.00 | 1.00 | 65.74 | 0.75 | 72.78 | 0.58 | 55.00 | 1.46 | 69.45 |
| number of individuals of weeds per m$^2$ *broj korovskih jedinki po m$^2$* | 227.21 | 53.58 | | 44.83 | | 32.50 | | 35.50 | | 30.42 | | 70.68 | |
| number of weeds species per m$^2$ *broj korovskih vrsta po m$^2$* | 30.33 | 25.67 | | 18.67 | | 23.00 | | 23.33 | | 17.00 | | 23.00 | |
| coefficient of efficiency (%) *koficijent efikasnosti (%)* | | | 76.10 | | 80.39 | | 85.67 | | 84.51 | | 86.66 | | 82.67 |
| lsd $_{0,05}$ = 4.099<br>lsd $_{0,01}$ = 5.181 | | | | | | | | | | | | | |

Alahlor showed good results in controlling species *Avena fatua, Amaranthus retroflexus, Capsella bursa-pastoris, Echinochloa cruss-galli, while weakly suppressing Abutilon theophrasti, Ambrosia artemisiifolia, Chenopodium album, Galium aparine, Papaver rhoeas, Polygonum lapathifolium, Xanthium strumarium.*

Atrazine well suppressing *Amaranthus retroflexus, Capsella bursa-pastoris, Chenopodium album*, and while not efficient enough to suppress *Abutilon theophrasti, Polygonum lapathifolium.*

Another problem in the production of maize is a species of weed *Sorghum halepense*. Applying herbicides from the group sulfonil urea scientific farming methods and appropriate measures can reduce the weedness of maize this kind. The fight against this weed must have long-term character, given the high level of weed control fields and the great potential of seeds and rhizome in land under cultivation layer. Efficient suppression of *Sorghum halepense* is only possible system of measures that would include the application of appropriate agricultural herbicides in all-vegetable crops (Drazić and Konstantinović, 1996). The mercantile corn can be applied herbicides from the group sulfonil urea, which are not Tell 75 WG and Tarot. Applying herbicides Tell 75 WG in the amount of 40 g/ha, with the addition Extravon 300 ml/ha, achieved with high efficiency to *Sorghum halepense* (Drazić, 1995).

Good efficacy in controlling many perennial weeds, especially turf species such as *Sorghum halepense* showed nikosulfuron, which is consistent with research Stefanović et al. (2003). Nikosulfuron good combat *Abutilon theophrasti, Amaranthus retroflexus, Chenopodium album, Echinochloa cruss-galli, Sorghum halepense, Polygonum lapathifolium, Sinapis arvensis,* and even the perennial species such as *Cirsium arvense and Convolvulus arvensis.*

Combination prosulfuron and primisulfuron-methyl good suppressing *Abutilon theophrastii, Amaranthus retroflexus, Ambrosia artemisiifolia, Chenopodium album, Polygonum lapathifolium, Sinapis arvensis, Xanthium strumarium*, satisfactory to control *Cirsium arvense and Convolvulus arvensis,* and suppressing low *Echinochloa cruss - galli and Sorghum halepense.*

Metolahlor good combat *Amaranthus retroflexus, Echinochloa cruss-galli*, a weak suppressing *Chenopodium album, Polygonum lapathifolium, Xanthium strumarium.*

Linuron good at suppressing weeds *Abutilon theophrastii, Amaranthus retroflexus, Capsella bursa-pastoris, Chenopodium album, Fumaria officinalis, Sinapis arvensis, Stellaria media, Polygonum lapathifolium* satisfying combat and combat weak species *Ambrosia artemisiifolia and Xanthium strumarium.*

Dikamba-dimetilamonium good combat *Amaranthus retroflexus, Ambrosia artemisiifolia, Chenopodium album, Polygonum lapathifolium, Xanthium strumarium,* while satisfying combat *Abutilon theophrastii, Cirsium arvense, Convolvulus arvensis, Polygonum aviculare, Sonchus arvensis*.

Application herbicides combination of applied immediately after sowing later update with other herbicides, showed better results in combating and annual and perennial species in comparison with using only during the growing season. If we take into account the resistance of perennial weeds and their ability to regenerate, it is clear that the maize crop only with herbicides can not solve the problem of weed control. In addition, having in mind the unpredictability of weather conditions, and in connection with this faster or slower growth of weeds and maize, it is understandable that the application of herbicides during the sowing of corn has an advantage (Stefanović et al. 2000, 2003; Malidža, 2001; Stefanović and Simić, 2002; Elezović et al. 2002).

## CONCLUSION

The weed flora of the maize crops found 37 species. Biological community is extremely therophytic character. Therophytes are 56.76% identified species. Because of the performance of intensive scientific farming methods measures reduced the share of hemicryptophytes and geophytes. The crops of corn herbicides tested were demonstrated satisfactory performance in controlling most weeds species. The biggest resistance to the tested combinations were evident herbicides type: *Abutilon theophrastii, Agropyrum repens, Abutilon theophrastii, Cirsium arvense, Convolvulus arvensis, Rubus caesius, Equisetum arvense, Sorghum halepense.*

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# EFIKASNOST HERBICIDA U SUZBIJANjU KOROVA U USEVU KUKURUZA

MARKO Lj. NESTOROVIĆ

**Izvod**

Ovim radom predstavljeni su rezultati trogodišnjeg istraživanja (2004-2006) efikasnosti herbicida u suzbijanju korova u usevu kukuruza na području Pančevačkog rita. Ispitivana je efifasnost sledećih herbicida: alahlor (Alahlor 480), atrazin (Atrazin 500), nikosulfuron (Motivell), prosulfuron+primisulfuron-metil (Ring 80 WG), EPTC + dihlormid (Eradicane 6-E), metolahlor (Dual), linuron (Afalon), dikamba-dimetilamonijum (Banvel 480S). Praćen je efekat primenjenih herbicida na sve utvrđene korovske vrste 30 dana posle primene brojanjem jedinki svake vrste. Efikasnost herbicida izražena je koeficijentnom efikasnosti u odnosu na broj jedinki svake vrste sa kontrolne varijante.

Korovska flora useva kukuruza je bogata i raznovrsna, tipično okopavinska. U usevu kukuruza utvđeno je 37 vrsta koje nemaju podjednak značaj u zakorovljavanju.

Korovsku floru kukuruza čine uglavnom biljke terofite (56,76%), a zbog dublje i kvalitetnije predsetvene obrade i primene međurednog kultiviranja i okopavanja, smanjujući tako direktno učešće geofita i hemikriptofita. Među terofitama, koje kod nas čine oko 60% svih utvrđenih vrsta, preovlađuju one koje niču u proleće a sazerevaju krajem leta. Hemikriptofite su druge po zastupljenosti sa 27,03% utvrđenih vrsta. Među geofitama, koje čine 8,11% životnog spektra, najviše su zastupljene vrste sa rizomima za vegetativno razmnožavanje. Usled intenzivnog vegetativnog razmnožavanja, najveću opasnost predstavljaju baš vrste među hemikriptofitama i geofitama.

U usevu kukuruza ispitivani herbicidi ispoljili su zadovoljavajuću do dobru efikasnost u suzbijanju većine prisutnih korovskih vrsta. Najveću otpornost prema ispitivanim herbicidnim kombinacijama ispoljile su vrste: *Abutilon theophrastii, Agropyrum repens, Cirsium arvense, Convolvulus arvensis, Rubus caesius, Equisetum arvense, Sorghum halepense.*

**Ključne reči:** kukuruz, korovska flora, herbicidi, suzbijanje korova, Pančevački rit.

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# EFFECTS OF PHYTASE ADDED INTO DIETS FOR FATTENING CHICKENS, ON THE LEVEL OF KALCIUM AND PHOSPHORUS IN BLOOD SERUM

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*SUMMARY: The aim of this study was to examine effects of adding phytase enzyme into the compositions for feeding fattening chickens, with different level of monokalcium phosphate, on the level of calcium and phosphorus in blood serum of chickens. The experiment of feeding included 220 chickens of Arbor Acres strain divided into two groups (110 chickens in each group). The first group was control group (K), without addition of phytase enzyme. The second group was the experimental group O-I which gained by diet enzyme phytase (0,1%) with double less level of monocalcium phosphate (MKF).*

*At the end of fattening period (42 days) sacrifice of chickens was done 10 chickens from each group. By sacrifice samples of blood were taken in which contents of calcium and phosphorus were determined. Gained results show that with addition of enzyme phytase (0,1%) into diets for chickens, by double less level of MKF, positive effects are reached meaning the contents of calcium and phosphorus in blood serum of chickens. The chickens of experimental group had bigger content of calcium (13,15mg%) (P<0,01), of total phoshorus (21,48mg%) (P<0,01); of inorganic phosphorus (7,02mg%) (P>0,05) in comparation to chickens to control group: calcium (11,95mg%) totally phosphorus (19,58mg%) and inorganic phosphorus (6,82mg%).*

***Key words:*** *chickens, phytase, calcium, phosphorus, blood serum.*

## INTRODUCTION

Metabolism of phosphorus that has origin from plant tissue is one of the most examined problems connected to minaral feeding. Considering that the diets for poultry are based maintly on nutrients of plant origin, the question applears in that measure

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do animals can use phosphorus from these sources. Only 30% of phosphorus in plant nutrients is in unphytinic form, and that ammount can be used by the side of animals. The rest 70% of phosphorus is connected to organic compounds, mostly in the shape of phytinic phosphorus (*Simons et al. 1990, Vogt, 1992*). Phytinic acid is firmly connected to essential minerals of diet, proteins and digestible enzymes, so it makes antinutritive factor (*Zhu et al. 1990*). *Matynka et al. (1990)* noticed low utilization of phytinic phosphorus at 3 weeks old chickens and it was only 3,40%.

Digestion of nutrient elements connencted to phytate could be increased considering hydrolisis of phytate molecule with phytase.

Application of microbic phytase in poultry and many effects of application from different views, quote in their labours many authors.

As criterion of phosphorus utilization content of calcium and phosphorus in blood serum, can be used.

In each 100ml of blood plasm can be found 3-5mg inorganic phosphorus. This is the fraction of largest interest at considering nutritive needs of animals in phosphorus, and its useful demonstrator for making sure phosphorus by diet, only if it is observed during longer time period.

*Perney et al. (1993), Radović et Bogosavljević-Bošković (2004*) confirm increasing of inorganic phosphorus of plasm, by addition of phytase into diets for chickens, at lower level of mineral phosphorus.

*Tyagi et al. (1998)* established significantly increasing the level of inorganic phosphorus in blood serum of Japanese quails, when into their diet phytase was added at decreased level of mineral phosphorus.

*Broz et al. (1994)* in their examinations established that the addition of phytase into the diets of fattening chickens could reach to significantly increasing of inorganic phosphorus concentration in blood plasm of chickens.

The aim of these experiments was to examine effect of phytase enzyme addition into diets for fattening chickens with different level of monocalcium phosphate. As criterion for determination of phosphorus utilization in this labour level of calcium and phosphorus in blood serum of chickens was used.

## MATERIAL AND METHODS

Experimental examinations included 220 chickens of Arbor Acres strain divided into two groups: K- control (110 chickens) and experimental group O-I (110 chickens). Chickens were fed with fully feeding mixture of some raw material composition. The only difference was addition of phytase enzyme and level of monocalcium phosphate into diet. Namely, K-group of chickens gained by diet monocalcium phosphate (1,4%), while O-I group gained double less level of monocalcium phosphate (0,7%) with addition of 0,1% phytase into diet. Standard technology of fattening was applied in lasting period of 42 days. At the end of fattening period sacrifice of 10 chickens out of each group was done. While sacrifice action, samples of blood were taken in whom content of calcium and phosphorus was established. Analysis were done in chemical laboratory of Institute for Animal Husbandry, Faculty of Agriculture in Novi Sad.

Statistically significance of differences was determined by analysis of variance, F

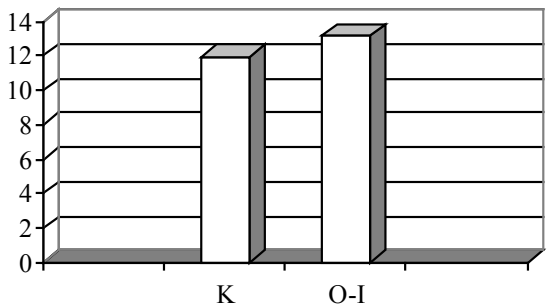
test as well as group test and Lsd test for single comparation for level of differences 5 and 1% in both cases.

## RESULTS AND DISCUSSION

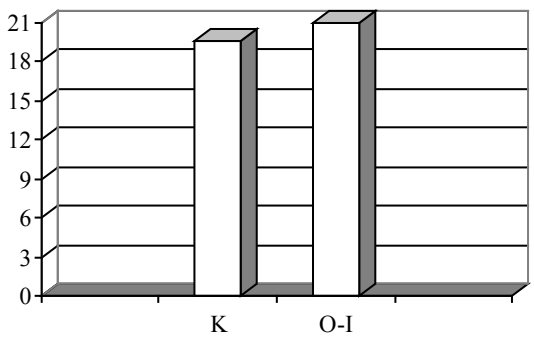
Results of these examinations (tab. 1) show that chickens which gained by diet 0,1% of phytase at decreased level of mineral source of phosphorus (monocalcium phosphate), had bigger level of calcium in blood serum (13,15mg%) in comparation to K-group (without additional phytase) with (11,95mg%) (P<0,01).

Table 1. Content of Ca of total and inorganic P in blood serum (mg %) (n=100)
*Tabela 1. Sadržaj Ca, ukupnog i neorganskog P u krvnom serumu (mg %) (n=100)*

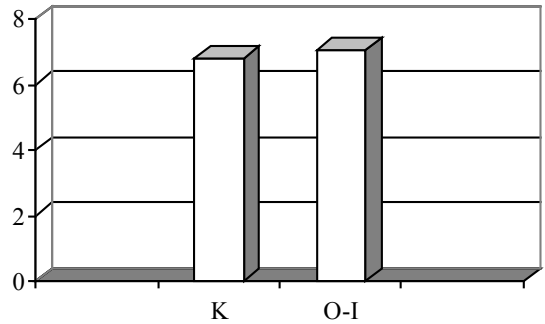
| Age in weeks *Starost u nedeljama* | Groups *Grupe* | Source of phosphorus *Izvor fosfora* | Calcium (Ca) *Kalcijum (Ca)* | Total phoshorus *Ukupni fosfor* | Inorganic phosphorus *Neorganski fosfor* |
|---|---|---|---|---|---|
| 6 weeks *6 nedelja* | K | MKF | 11,95 | 19,58 | 6,82 |
| | 0-I | MKF+phytase | 13,15 | 21,48 | 7,02 |
| | | | **P < 0,01 | ** P < 0,01 | P > 0,05 |




Graph. 1. Content of calcium in blood serum of chickens (mg%)
*Graf. 1. Sadražaj kalcijuma u krvnom serumu pilića (mg%)*




Graph. 2. Content of total phosphorus in blood serum of chickens (mg%)
*Graf. 2. Sadržaj ukupnog fosfora u krvnom serumu pilića (mg%)*




Graph. 3. Content of inorganic phosphorus in blood serum of chickens (mg%)
*Grafik 3. Sadržaj neorganskog fosfora u krvnom serumu pilića (mg%)*

Considering total phosphorus, chickens of O-I group with phytase added into diet had (21,48mg%) and K-group (0,0% phytase) (19,58mg%) (P<0,01).

Parameters for inorganic phosphorus are following: O-I group of chickens (7,02mg%) and control group (6,82mg%) (P>0,05).

For meutioned values which refer to parameters of blood, and were gained in this examination, could be said that they fit into normal physiological limits. *Perić Lidija (1996)* quotes serum which content in blood serum which moved from 9,87mg% to 11,8mg% with 6 weeks old. Content of inorganic phosphorus moved from 6,30-6,82mg%. Chickens gained as source of phosphorus dicalcium phosphate, monocalcium phosphate or phosphanol.

*Tyagi et al. (1998)* at feeding Japanese quals with decreased level of mineral sources of phosphorus into diet and addition of different levels of phytase, they fortified significantly increased content of inorganic phosphorus in blood serum. Broz et al. (1994) fortified significantly increased inorganic phosphorus in blood plasm when phytase was added into diets for chickens at decreased level of mineral phosphorus into diets for chickens, or when inorganic phosphorus is eliminated from diet.

*Perney et al. (1993), Miao et al. (1999), Radović et Bogosavljević-Bošković (2004)* in their examinations established positive effects of phytase added into diets for chickens to the content of calcium and phosphorus in blood serum.

## CONCLUSION

By perceiving results of many authors and by comparation to the results of these examinations, we conclude that phytase added into diet for fattening chickens at decreased level of mineral source of phosphorus in diet, had effects to increasing level of calcium in blood serum (P<0,01), total phosphorus (P<0,01). As for as level of inorganic phosphorus in blood serum, differences of average values had no statistical importance (P> 0,05).

Our opinion is that diets made this way of formulation with addition of phytase into diet, at decreased level of mineral source of phosphorus, should be applied in wide

production in aim to improve poultry production, as well as product quality and wellness of animals but in order to preserve life environment.

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# EFEKTI DODATE FITAZE U HRANU PILIĆA U TOVU NA NIVO KALCIJUMA I FOSFORA U KRVNOM SERUMU

VERA RADOVIĆ, S. FILIPOVIĆ, SNEŽANA BOGOSAVLJEVIĆ - BOŠKOVIĆ, V. DOSKOVIĆ

**Izvod**

Cilj ovog rada bio je da se ispitaju efekti dodavanja enzima fitaze u smeše za ishranu pilića u tovu, sa različitim nivoom monokalcijum-fosfata, na nivo kalcijuma i fosfora u krvnom serumu pilića. Ogledom ishrane obuhvaćeno je 220 pilića provenijence Arbor Acres, podeljenih u dve grupe (po 110 pilića u grupi). Prva grupa je bila kontrolna (K), bez dodatka enzima fitaze. Druga grupa je bila ogledna (O), koja je hranom dobijala enzim fitazu (0,1%) uz dvostruko niži nivo monokalcijum fosfata (MKF).

Na kraju tova (42 dana) izvršeno je žrtvovanje po 10 pilića iz svake grupe. Pri tome, uzeti su uzorci krvi, u kojima je utvrđivan sadržaj kalcijuma i fosfora. Dobijeni rezultati pokazali su da se dodatkom enzima fitaze (0,1%) u hranu za piliće, uz dvostruko niži nivo MKF postižu pozitivni efekti u pogledu sadržaja kalcijuma i fosfora u krvnom serumu pilića. Pilići ogledne grupe imali su veći sadržaj kalcijuma (13,15mg%) ($P<0,01$), ukupnog fosfora (21,48mg%) ($P<0,01$); neorganskog fosfora (7,02mg%) ($P>0,05$) u odnosu na piliće kontrolne grupe – kalcijum (11,95mg%) ukupni fosfor (19,58mg%) i neorganski fosfor (6,82mg%).

**Ključne reči:** pilići, fitaza, kalcijum, fosfor, krvni serum.

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# THE EFFECT OF AGE AND REARING SYSTEM ON THE PROPORTION OF CERTAIN MEAT CATEGORIES IN PROCESSED BROILER CARCASSES

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*SUMMARY: Given the established European Union regulations on the extensive i.e. traditional broiler rearing system as well as poultry breeding specificities in Serbia, an experimental study was conducted to examine slaughter characteristics of broilers as dependent on the fattening period and rearing system employed. Fattening included extensive fattening in a poultry house and free-range rearing. In view of the importance of the fattening period in non-industrial poultry production, fattening lasted 49, 56 and 63 days. At the end of each of the above fattening periods, the randomly selected broilers were slaughtered for quantitative and qualitative traits of the processed carcasses. This study presents results on the proportion of certain (1st, 2nd and 3rd) meat categories in processed broiler carcasses. In terms of the effect of the rearing systems analyzed and the gender of the examined broilers, the differences in the proportion of the first-category meat were small and statistically non-significant (P>0,05). Somewhat larger but equally non-significant differences were exhibited in terms of the fattening period, the highest and the lowest proportion of the first-category meat being produced by broilers aged 49 and 56 days, respectively (P>0,05). Similar results were reported on the proportion of the second- and third-category meat for the examined broilers.*

***Key words**: broilers, age, rearing system, meat categoriees.*

## INTRODUCTION

Poultry breeding systems have captured the attention of scientists and producers in

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many countries worldwide for a number of years. Efforts have been made to introduce innovative ideas and establish new technologies in poultry breeding for egg and meat production aimed at improving breeding conditions, environmental protection and enhancing the quality of poultry products. These efforts have been undertaken due to consumer demands for high-quality products and legal regulations on welfare in poultry breeding imposed by numerous groups of ecologists.

For several decades after the Second World War, poultry production had been practised on industrialized farms. This enabled high-volume production and pronounced profitability (Havestein et al. 1994, Remingnon et al. 1994), which resulted in a very high level of production and the overall volume of production reaching maximum limits of poultry biological potential and maximum consumption. It was then that the above demands for a change of breeding and production methods, particularly as regards poultry meat, ensued. Poultry breeding system is becoming increasingly important as is the fact that the modern poultry market is being radically transferred from a market where only price competitiveness existed to a market which gives an equal opportunity to quality competitiveness.

The above stated has resulted in the ever-increasing importance of non-industrial systems and organic production which are in some countries, such as those of the EU, strictly regulated under different directives (VO/EWG 1538/91 and VO/EG 1804/99) laying out minimum conditions required for satisfaction of non-industrial and organic poultry production standards (Ristić, 2003). Investigations aimed at improving the technology of non-industrial and organic poultry production are underway both in Serbia and worldwide and of high importance for future poultry production (Bogosavljević-Bošković et al. 2006, 2008).

Given the above and the importance of fattening length in non-industrial poultry production, a study was conducted to examine the meat quality of broilers bred under two different non-industrial systems (extensive breeding in a poultry house and free-range breeding) at different fattening lengths.

This paper gives results on the proportion of individual meat categories in processed carcasses of test broilers.

## MATERIAL AND METHODS

The trial material included a total of 200 one-day-old Cobb 500 broilers.

During the first 4 weeks, the broilers were bred in the same facility in a deep litter system.

On day 28, the broilers were divided into 2 groups, the first (trial group I) being kept in the poultry house at a stocking density of 12 chicks/m2 of floor space, the second one (trial group II) being provided with free range of 1m2/chick, apart from being reared at an identical stocking density.

The broilers were fed a complete fodder mixture containing 21% of proteins until day 28, a 19% protein mixture from days 28 to 42 and a mixture of ground maize and oats, mineral and vitamin supplements and a 30% complete fodder mixture from day 42 until the end of the fattening period.

Three fattening periods were employed, lasting for 49, 56 and 63 days.

Upon fattening, 24 broilers from each fattening period (12 broilers from each trial group), half males and half females, were slaughtered.

Appropriate weighings were made at slaughter and during processing of the slaughtered carcasses to examine slaughter traits. The broilers were, first, randomly selected and weighed, and then, manually slaughtered and defeathered. The following information was recorded: slaughter weight, warm processed carcass weight and cold carcass weight. Major slaughter by-products and abdominal fat were also weighed. Subsequently, the processed and cold carcasses were dissected into basic parts: breasts, drumsticks, thighs, wings, pelvis and backs (according to the 1981 Regulation on the Quality of Poultry Meat). The obtained data were used to determine the shares of the above parts in the processed carcasses, which were used to calculate the proportion of specific meat categories.

Testing of the significance of differences was conducted according to the following mathematical model of an analysis of variance:

$$Y_{ijkl} = \mu + BS_i + LF_j + G_k + (BSLF)_{ij} + (BSG)_{ik} + (LFG)_{jk} + (BSLFG)_{ijk} + e_{ijkl}$$

i.e. in a three-factor design of 2 x 3 x 2 (2 breeding systems – BS, 3 lengths of the fattening period – LF and 2 genders – G).

The tested parameters were subjected to the analysis of variance using Anova, Microsoft STATISTICA Ver.5.0., Stat Soft Inc. (1995).

## RESULTS AND DISCUSSION

The percentage of individual meat categories in the processed carcasses of the test broilers is given and discussed in this section.

The results on the trait for both male and female broilers of different age bred under the examined systems are given in Table 1.

The data given in Table 1 suggest that the percentage of the first-category meat was highest in the free-range broilers aged 49 days (63.01%) and lowest in the broilers reared in poultry-houses and slaughtered on day 56 of fattening (61.66%). The highest percentage of meat of the second and third categories in the processed carcasses was found in the non-free-range broilers aged 49 days. The percentage of the second-category meat was lowest in the non-free-range broilers aged 63 days. The lowest relative percentage of the third-category meat was identified in the free-range broilers at 63 days of age.

The analysis of significance of differences in the percentage of individual meat categories exhibited in terms of the effect of the breeding system employed, length of fattening and gender of the test broilers revealed that the stated differences were not statistically significant ($P>0.05$). However, the analysis and comparison of the coefficients of variation of the examined trait suggest that uniformity of both meat yield responses and percentage of individual meat categories in the processed carcasses was higher in the free-range broilers than in the non-free-range ones.

Table 1. Percentage of individual meat categories in the processed carcasses of the test broilers on days 49, 56 and 63 of fattening

*Tabela 1. Udeo pojedinih kategorija mesa obrađenih trupova brojlera ispitivanih grupa 49-og, 56-og i 63-eg dana tova*

| Group *Grupa* | Age, days *Starost, dana* | Gender *Pol* | | Category I *I kategorija* | Category II *II kategorija* | Category III *III kategorija* |
|---|---|---|---|---|---|---|
| | | | | % | % | % |
| Non free-range *Bez ispusta* | 49 | ♂ | $\bar{x}$ | 63.090 | 10.663 | 19.792 |
| | | | Cv | 1.051 | 3.198 | 4.562 |
| | | ♀ | $\bar{x}$ | 62.437 | 10.902 | 20.098 |
| | | | Cv | 2.583 | 7.274 | 5.180 |
| | | ♂+♀ | $\bar{x}$ | 62.764 | 10.782 | 19.945 |
| | | | Cv | 1.950 | 5.518 | 4.728 |
| | 56 | ♂ | $\bar{x}$ | 61.527 | 10.835 | 20.039 |
| | | | Cv | 4.138 | 4.762 | 4.681 |
| | | ♀ | $\bar{x}$ | 61.799 | 10.216 | 19.152 |
| | | | Cv | 1.354 | 6.725 | 3.655 |
| | | ♂+♀ | $\bar{x}$ | 61.663 | 10.525 | 19.596 |
| | | | Cv | 2.940 | 6.299 | 4.669 |
| | 63 | ♂ | $\bar{x}$ | 62.466 | 10.626 | 19.304 |
| | | | Cv | 2.220 | 10.536 | 3.455 |
| | | ♀ | $\bar{x}$ | 62.450 | 10.239 | 19.270 |
| | | | Cv | 2.684 | 5.391 | 5.246 |
| | | ♂+♀ | $\bar{x}$ | 62.458 | 10.433 | 19.287 |
| | | | Cv | 2.349 | 8.300 | 4.236 |
| Free-range *Sa ispustom* | 49 | ♂ | $\bar{x}$ | 62.971 | 10.574 | 19.841 |
| | | | Cv | 2.017 | 3.575 | 2.389 |
| | | ♀ | $\bar{x}$ | 63.056 | 10.438 | 19.861 |
| | | | Cv | 1.502 | 5.394 | 7.638 |
| | | ♂+♀ | $\bar{x}$ | 63.013 | 10.506 | 19.851 |
| | | | Cv | 1.696 | 4.407 | 5.400 |
| | 56 | ♂ | $\bar{x}$ | 61.329 | 10.999 | 19.639 |
| | | | Cv | 2.356 | 4.791 | 3.406 |
| | | ♀ | $\bar{x}$ | 62.357 | 10.512 | 19.438 |
| | | | Cv | 1.748 | 6.602 | 10.145 |
| | | ♂+♀ | $\bar{x}$ | 61.843 | 10.755 | 19.539 |
| | | | Cv | 2.155 | 5.960 | 7.206 |
| | 63 | ♂ | $\bar{x}$ | 63.557 | 10.889 | 18.788 |
| | | | Cv | 1.661 | 5.685 | 5.306 |
| | | ♀ | $\bar{x}$ | 62.263 | 10.125 | 19.336 |
| | | | Cv | 2.065 | 6.716 | 3.103 |
| | | ♂+♀ | $\bar{x}$ | 62.910 | 10.507 | 19.062 |
| | | | Cv | 2.082 | 7.014 | 4.380 |

There are substantial limitations on comparing the results of this study with literature data, given the combination of a number of factors that could have affected the obtained results to a greater of lesser degree. Nevertheless, the results of this study partially conform to those obtained by Lewis et al. (1997), Ristić (2003), Milošević et al. (2003), Bogosavljević-Bošković et al. (2006).

## CONCLUSION

The obtained results on the effect of age and breeding system on the percentage of individual meat categories in the processed carcasses of the test broilers suggest the following:

- The percentage of the first-category meat was highest in the free-range broilers at 49 days of age (63.01%) and lowest in the non-free-range ones at 56 days of age (61.66%).
- The percentage of meat of the second and third categories in the processed carcasses was highest in the non-free-range broilers at 49 days of age.
- Differences in the percentage of individual meat categories exhibited in terms of the effect of breeding system, length of fattening and gender of the test broilers were not statistically significant (P> 0.05).

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# UTICAJ UZRASTA I SISTEMA GAJENJA NA ZASTUPLJENOST POJEDINIH KATEGORIJA MESA U OBRAĐENIM TRUPOVIMA TOVNIH PILIĆA

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**Izvod**

Imajući u vidu pojedine ustanovljene propise Evropske unije za ekstenzivan, tj. tradicionalan sistem gajenja tovnih pilića, kao i specifičnosti u živinarstvu naše zemlje, organizovana su eksperimentalna istraživanja sa ciljem ispitivanja klaničnih karakteristika tovnih pilića u zavisnosti od dužine tova i sistema gajenja. Tov pilića organizovan je na dva načina, ekstenzivno u živinarniku i gajenjem uz korišćenje slobodnih ispusta. Obzirom na značaj dužine tova u neindustrijskoj živinarskoj proizvodnji, tov je trajao 49, 56 i 63 dana. Na kraju svakog od navedenih tovnih perioda pilići, odabrani metodom slučajnog uzorka, klani su radi ispitivanja kvantitativnih i kvalitativnih osobina obrađenih trupova.

U ovom radu prikazani su rezultati ispitivanja udela pojedinih kategorija mesa (I, II i III) u masi obrađenih trupova oglednih pilića. Razlike ispoljene u pogledu udela mesa I kategorije, a sa stanovišta uticaja ispitivanih sistema gajenja i pola oglednih pilića bile su male i nisu bile statistički značajne (P>0,05). Nešto veće razlike ispoljene su sa aspekta dužine tova (najveći udeo mesa I kategorije imali su pilići uzrasta 49 dana, a najmanji pilići starosti 56 dana), ali ni one nisu bile signifikantne (P>0,05). Slični rezultati ustanovljeni su i za ispitivane grupe oglednih pilića u pogledu udela mesa II i III kategorije.

**Ključne reči:** brojleri, uzrast, sistem gajenja, kategorije mesa.

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# EFFECT OF CANOPY DENSITY IN THE TRELLIS ON THE YIELD AND QUALITY OF GRAPES AND WINE OF THE CULTIVAR ‘LIZA’

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*SUMMARY: The objective of this study was to assess the effect of bud load and the green foliage they produce on the yield and quality of grapes and wine of the cultivar ‘Liza’. Three levels of bud loading, i.e., canopy density, were applied. The first variant of bud loading was 2 bud spurs and a cane with 12 buds, or 11.7 shoots per meter of canopy length. The second variant of bud loading was two canes each with 12 buds, or 20 shoots per meter of canopy length. The third variant of bud loading was two canes each with 17 buds, or 28 shoots per meter of canopy length. Influence of meteorological parameters on these cultivar features was also monitored. Ripening dynamics and grape and wine quality were observed through the test years. Based on long-term investigations and the results of this study, it was concluded that the increased bud load and canopy density, with the applied small plant distances in the row, decreased the (potential) bud fertility, grape yield, and grape and wine quality.*

***Key words:*** *grapevine, cultivar, green foliage, bud load, fertility*

## INTRODUCTION

For centuries, the grapevine had been grown using the gobelet or head training system and pergolas. With the invention of metal wire and its application in vine growing, some old training systems have been modified and new ones have been designed. In mid-19th century, the training systems diversified to include, in addition to stakes, posts and wires. At the beginning of the 20th century, theoretical backgrounds had been established for many well-known training systems, whose large-scale application started after the World War II. Ever since the introduction of the various training systems after the World

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War II, they became an object of study by a large number of researchers. The problem of photosynthetic activity of leaves in canopies of different densities was studied most intensively (Schaulis, 1982; Smart, 1985; Raynolds et al., 1985; Kliewer, 1982).

This topic has never been truly exhausted. It still attracts scientific interest because new grapevine cultivars are continually being developed, new training systems are being examined and new vineyards are being established in regions without tradition in grape growing (Cavallo et al., 1999; Pedroso et al., 1999; Scaglione and Pasquarella, 1999; Tarricone et al., 1999; Schwab et al., 2001; Ponchia et al., 2001; Murisier et al., 2001; Castro et al., 2005; Castro et al., 2007; Calo et al., 2007). As a result of long-term work on grape breeding at Faculty of Agriculture in Novi Sad, an array of promising cultivars has been developed. Complete agro-technical and crop protection recommendations must be supplied for cultivars judged to have a chance of being introduced in commercial production. These requirements defined one of the objectives of this study, i.e., to find which bud load and canopy density secure optimum performance of the cultivar 'Liza'.

## MATERIAL AND METHOD

*Facility*

Experiments were organized at the experimental vineyard of Faculty of Agriculture, Novi Sad, Serbia. The farm is located in the northern part of the Fruška Gora Mountain grape-growing region. The study was conducted in the period 1996 - 2004. The experimental vineyard, in which the cultivar 'Liza' was grafted on the rootstock V. berlandieri x V. riparia Kober 5BB, was established in 1987. The training system was the Karlovački type, with the planting arrangement of 2.8 x 1.2 m. For the needs of the experiment, the plants were retrained to the single Guyot system. The applied bud loads were: 2 + 12 = 14 buds per plant - the pruning by the single Guyot system, amounting to 11.7 shoots per meter of canopy length; 12 + 12 = 24 buds per plant - the pruning by the Karlovački system, amounting to 20.0 shoots per meter of canopy length; and 17 +17 = 34 buds per plant - the pruning by the Karlovački system, amounting to 28.3 shoots per meter of canopy length. The experiment was established in a random block design with three replications. The standard bud loading was two canes, each with 12 buds.

'Liza' is a new cultivar developed at Institute for Fruit Growing and Viticulture, Faculty of Agriculture, Novi Sad (Cindrić et al., 2000). It is a cross between the cultivars 'Kunleany' and 'Pinot Gris'. It is medium vigorous, has a fairly high sugar content in grapes and it is resistant to grey rot and low temperatures.

*Method of work*

Potential fertility was determined in May, when inflorescences were quite visible. Inflorescences were counted from the first to the twelfth or seventeenth node in the cane, depending on experiment variant. Their number was counted on 15 plants, and this served to determine the bud fertility coefficient. Particular attention was paid to green pruning, the practice of removing superfluous shoots and training preferred shoots in the desired position, to avoid excessive self-shading. Data on average cluster weight and sugar and acid contents in grape must were calculated from data obtained for one crate containing 100 to 130 clusters. Actual fertility, i.e., grape yield, was determined after harvest of the

entire experiment.

After pressing must from the samples, experimental wine was made in a micro wine-making plant. After a period of aging, the wine was scored by a group of professors of the Department using a 20-point scale.

*Environment*

The environmental conditions at the time of the experiment were highly variable and they significantly affected the fertility of winter buds not only of this but also of the other tested cultivars (Kuljančić et al., 2007). Experimental results showed that weather conditions in the period May-June have a major impact on fertility buildup for the subsequent year. If that period is rainy and cool, yield reduction in the subsequent year might range up to 50%. If the period is fairly warm and humid, the yield would be above-average.

## RESULTS AND DISCUSSION

*Potential fertility coefficient*

It should be mentioned here that the fertility coefficient values were invariably high, in consequence to special technological measures, which were applied at Sremski Karlovci experimental vineyard throughout the experimental period as they are applied today. The 1997 data are missing because of a computer failure.

As can be seen in Table 1, the tested treatments had statistically significant effects on bud fertility per length of productive element in the cultivar 'Liza'. It is evident that the dense canopy in variant 3, where 28.3 shoots developed per meter of canopy, was unfavorable for fertility buildup for the next year, as indicated by the value of the fertility coefficient which was 1.85. The observed changes in winter bud fertility were in agreement with Cindrić et al. (1984) who claimed that a successive reduction in the fertility of winter buds occurs in the case of dense canopies. Such effect of canopy density results from a change in micro-climatic conditions in the canopy. The increase in the number of shoots

Table 1. Potential fertility coefficient of the cultivar 'Liza'
*Tabela 1. Potencijalni koeficijenti rodnosti sorte liza (prosek / average 1996-2004)*

| Godine - *Years* | Varijante - *Variant* | | |
|---|---|---|---|
| | I | II | III |
| 1996 | 2,36 | 2,42 | 2,70 |
| 1997 | - | - | - |
| 1998 | 1,65 | 1,67 | 1,81 |
| 1999 | 2,30 | 1,89 | 1,65 |
| 2000 | 1,82 | 1,68 | 1,61 |
| 2001 | 2,58 | 2,27 | 1,82 |
| 2002 | 1,66 | 1,56 | 1,24 |
| 2003 | 2,58 | 2,23 | 1,99 |
| 2004 | 2,41 | 2,19 | 1,95 |
| Prosek/ *Average* | 2,17 | 1,99 | 1,85 |

| NZR/LSD | 0,05 | 0,22 |
|---|---|---|
| | 0,01 | 0,30 |

per meter of canopy causes significant increases in leaf area per grape plant (Reynolds et al., 1994), simultaneously with an increased shading (Castro et al., 2007). At the same time, the inside of the canopy receives less than 10% of the light falling on the outer surface of the canopy (Reynolds and Wardle, 1989). In cool years, temperature is higher inside a dense canopy than out side of it, which is favorable for grape plants and grapes. In warm years, however, the inside of a dense canopy is cooler and more humid than the outside, which is not good for plants.

*Average cluster weight*

The primordium of a future cluster form in a previous year, as mentioned in the paragraphs dealing with the environment, but its weight is determined by a combined action of the soil and microclimatic conditions in the current season.

Table 2. Average cluster weight (g) of the cultivar 'Liza'
*Tabela 2. Prosečna masa grozda (g) sorte liza (prosek / average 1996-2004)*

| Godine - *Years* | Varijante - *Variant* | | |
|---|---|---|---|
| | I | II | III |
| 1996 | 133 | 125 | 146 |
| 1997 | 110 | 111 | 104 |
| 1998 | 137 | 135 | 126 |
| 1999 | 152 | 160 | 128 |
| 2000 | 122 | 112 | 101 |
| 2001 | 149 | 149 | 135 |
| 2002 | 102 | 93 | 106 |
| 2003 | 120 | 125 | 125 |
| 2004 | 117 | 136 | 108 |
| Prosek/ *Average* | 127 | 127 | 118 |

| | | |
|---|---|---|
| NZR/LSD | 0,05 | 9,26 |
| | 0,01 | 12,75 |

As can be seen in Table 2, the dense canopy, with 28.3 shoots per meter of canopy, produced the lowest average cluster weight of 118 grams. The largest average weight (127 g) was achieved with 20.0 shoots per meter of canopy. These results are in good agreement with those of Reynolds et al. (1994) and Castro et al. (2007) who, working with similar canopy densities, obtained statistically significant reduction in cluster weight as the number of shoots per meter of canopy increased. It is obvious that a large number of leaves in variant 3 were in full shade or half-shade, which lowered their photosynthetic activity that resulted in reduced average weight of the cluster.

*Average grape yield*

The experiment setup itself included large differences in the number of buds per grape plant, so that it was aware in advance that the increased bud load will increase the yield of grapes. As shown in Table 3, statistically significant differences existed between variants 1 (1.13 kg/m2) and 2 (1.61 kg/m2), and variants 1 and 3 (1.78 kg/m2). Similar results were obtained by Reynolds et al. (1994) who studied the effect of shoot density on production characteristics of the cultivar 'Rhine Riesling'. It is important to note here

that the three times higher bud load in variant 3 rendered an increase in grape yield of only 50%, which is in agreement with the results of Shaulis (1982), Smart (1985) and Castro et al. (2007). These results indicate that a dense canopy significantly reduces the photosynthetic activity of grape leaves. According to Kriedemann (1968), a leaf that stayed in full or partial shade for two to three weeks in early spring, loses the capacity to increase its intensity of photosynthesis when exposed to light and it keeps behaving as if it had remained in the shade.

Table 3. Average grape yield (kg/m2) of the cultivar 'Liza'
*Tabela 3. Prosečan prinos grožđa (kg/m2) sorte liza (prosek / average 1996-2004)*

| Godine - *Years* | Varijante - *Variant* | | |
|---|---|---|---|
| | I | II | III |
| 1996 | 1,36 | 1,85 | 2,68 |
| 1997 | 0,99 | 1,71 | 1,70 |
| 1998 | 0,99 | 1,67 | 2,09 |
| 1999 | 1,39 | 1,65 | 1,74 |
| 2000 | 1,12 | 1,39 | 1,66 |
| 2001 | 1,34 | 1,71 | 1,73 |
| 2002 | 0,73 | 1,04 | 1,05 |
| 2003 | 1,05 | 1,53 | 1,52 |
| 2004 | 1,20 | 1,95 | 1,87 |
| Prosek/ *Average* | 1,13 | 1,61 | 1,78 |

| | | |
|---|---|---|
| NZR/LSD | 0,05 | 0,20 |
| | 0,01 | 0,28 |

*Average contents of sugar and acids*

Very early studies of grapevine physiology had indicated that yield of grapes and sugar content in must are negatively correlated. It means that, in most cases, low yields will be combined with increased sugar content in must, and that sugar content will go down as the yield increases.

Table 4. Average contents of sugar (%) and acids (g/l) in must of the cultivar 'Liza'
*Tabela 4. Prosečan sadržaj šećera (%) i kiselina (g/l) kod sorte liza (prosek / average 1996-2004)*

| Godine - *Years* | Varijante - *Variant* | | | | | |
|---|---|---|---|---|---|---|
| | I | | II | | III | |
| | Šeć. *Sugar* | Kis. *Acid* | Šeć. *Sugar* | Kis. *Acid* | Šeć. *Sugar* | Kis. *Acid* |
| 1996 | 22,4 | 10,8 | 21,5 | 10,4 | 20,4 | 10,1 |
| 1997 | 239 | 10,5 | 23,8 | 10,3 | 23,5 | 9,7 |
| 1998 | 22,2 | 10,0 | 21,6 | 9,7 | 21,9 | 9,1 |
| 1999 | 21,6 | 8,6 | 21,5 | 8,5 | 22,0 | 8,6 |
| 2000 | 20,7 | 7,8 | 21,2 | 7,4 | 19,7 | 7,3 |
| 2001 | 20,2 | 10,2 | 19,2 | 10,4 | 19,3 | 9,8 |
| 2002 | 23,3 | 8,9 | 22,3 | 9,0 | 22,2 | 8,9 |
| 2003 | 22,7 | 8,1 | 21,5 | 8,1 | 20,6 | 8,4 |
| 2004 | 19,7 | 10,7 | 19,7 | 10,2 | 19,1 | 9,8 |
| Prosek/ *Average* | 21,7 | 9,6 | 21,3 | 9,4 | 20,9 | 9,2 |

| | | |
|---|---|---|
| NZR/LSD Šeć./*Sugar* | 0,05 | 0,49 |
| | 0,01 | 0,67 |
| NZR/LSD Kis./*Acid* | 0,05 | 0,25 |
| | 0,01 | 0,34 |

This was confirmed in this study, but the explanation was associated not only with yield but also with the microclimate in a dense canopy. This is especially true for the temperature in the canopy, the change of which tends to change the material used in respiration. At low temperatures, plant metabolism relies on sugars while at high temperatures it relies more on acids. It is certain that the average daily temperature was lower in the dense canopy made by 28.3 shoot per meter of canopy length than in the canopy made by 11.7 shoots per meter length. Accordingly, the denser canopy metabolized mostly sugars by day and mostly acids by night.

The data in Table 4 speak in favor of this assumption – sugar and acid contents in must were significantly higher in variant 1 than in variant 3.

*Wine evaluation*

A paper dealing with the effect of weather conditions and the fertility of winter buds in grapevine (Kuljančić et al. 2007) stated that climatic factors have significant effects on grape yield and quality, thus on wine quality as well. Table 5 shows that the effect of the treatments, i.e., canopy density, on wine quality was close to being significant. The wines obtained from the grapes cultivated at the densities of 11.7 and 20.0 shoots per meter of canopy length had highest average scores regardless of the fact that their acid content was higher than that obtained in variant 3. This is in agreement with the results of Reynolds et al. (1994) who claimed that wine quality is in agreement with must quality. The factors at work were more favorable photosynthetic conditions and a more harmonious accumulation of all substances in grapes.

Table 5. Wine evaluation of the cultivar 'Liza'
*Tabela 5. Degustaciona ocena vina sorte liza (prosek / average 1996-2004)*

| Godine - *Years* | Varijante - *Variant* | | |
|---|---|---|---|
| | I | II | III |
| 1996 | 17,7 | 17,9 | 17,5 |
| 1997 | 18,8 | 18,6 | 18,2 |
| 1998 | 17,6 | 17,3 | 17,1 |
| 1999 | 18,2 | 18,9 | 18,5 |
| 2000 | 18,2 | 18,6 | 17,7 |
| 2001 | 16,5 | 16,3 | 16,0 |
| 2002 | 18,0 | 17,2 | 18,4 |
| 2003 | 17,7 | 18,1 | 17,5 |
| 2004 | 18,0 | 18,2 | 17,9 |
| Prosek/ *Average* | 17,9 | 17,9 | 17,6 |

| | | |
|---|---|---|
| NZR/LSD | 0,05 | 0,34 |
| | 0,01 | 046 |

## CONCLUSION

Based on our own findings and results of other researchers, this study confirmed the theoretical hypothesis that excessively dense canopies are not favorable for grape growing and that they should be avoided. This study indicates that with plant distances in the row below 1 m, densities of 11 to 12 shoots per meter of canopy length are recommended. With plant distances in the row over 1.0 m, the density up to 20 shoots per meter of canopy length can be used.

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# UTICAJ GUSTINE ZELENE MASE U ŠPALIRU, NA RODNOST I KVALITET GROŽĐA I VINA SORTE LIZA

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**Izvod**

Cilj ovog istraživanja je bio da se utvrdi uticaj opterećenja okcima i stvorene zelene mase iz njih, na rodnost i kvalitet grožđa i vina kod sorte Liza. Istraživanja su se odvijala od 1996. do 2004. godine, na Oglednom dobru Departmana za voćarstvo, vinogradarstvo, hortikulturu i pejzažnu arhitekturu, Poljoprivrednog fakulteta u Novom Sadu, u Sremskim Karlovcima. Ispitivana je novostvorena sorta liza, nastala u ovom departmanu ukrštanjem sorti kunleanji i burgundac sivi, a priznata 1991. godine. Primenjna su tri nivoa opterećenja okcima tj. tri gustine zelene mase u špaliru. Prvo opterećenje kondir od 2 okca i luk od 12 okaca tj. 11,7 lastara po dužnom metru špalira, drugo dva luka od po 12 okaca tj. 20,0 lastara po dužnom metru špalira i treće opterećenje dva luka po 17 okaca tj. 28,3 lastara po dužnom metru špalira. Pored uticaja gustine zelene mase i opterećenja rodnim okcima na moguću (potencijalnu) i stvarnu rodnost, praćen je i uticaj klimatskih činilaca na ova svojstva sorte. Tokom godina ispitivanja je praćena i brzina (dinamika) sazrevanja i kvalitet grožđa i vina. Na osnovu dugogodišnjeg posmatranja i dobijenih rezultata se može zaključiti, da sa povećanjem opterećenja i gustine zelene mase u špaliru, pri uobičajenim razmacima između čokota u redu, opada moguća (potencijana) i stvarna rodnost, kakvoća (kvalitet) grožđa i vina.

**Ključne reči:** vinova loza, sorte, zelena masa, opterećenje, rodnost.

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# DROUGHT PROTECTION OF WATERMELON SHOOT GROWTH BY ARTIFICIAL COVER MULCHES

PETER FERUS, SILVIA FERUSOVÁ, JÁN KÓŇA[1]

*SUMMARY: In order to explain how mulch regulates watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) shoot growth under dehydration, white and red polyethylene film and black non-woven textile were tested. Plants on black non-woven textile and red polyethylene foil showed the lowest shoot growth reduction, and this can be ascribed mainly to water saving properties of these cover mulches. However, important role could be played by modified light and temperature environment, as well.*

***Key words:*** *watermelon, artificial cover mulch, drought, shoot growth, water regime.*

## INTRODUCTION

Cultivation technologies of many vegetable crops (sweet pepper, tomato, cucumber, watermelon, etc.) include application of artificial cover mulches (Carranca, 2006). Besides the positive effect on biomass/fruit production (Diaz-Perez and Batal, 2002), they also improve fruit quality (Farias-Larios and Orozco-Santos, 1997), and regulate pest development (Diaz-Perez et al., 2007; Fortnum et al., 2000; Webster, 2005). However, in conditions of climate changes and global warming, drought protective properties emerge, as well (Xie et al., 2005).

Formulating practical rules for farmers requires great experimental effort. Studying effects on production and its quality must be accompanied by a detailed explanation of principles which enable further technological improvement. Proposing experimental design, more attributes regarding artificial cover mulch must be followed: material, porosity, thickness and colour. Colour determines light as well as temperature environment, which is modified by material with certain porosity and thickness, defining range of water and energy fluxes (Tarara, 2000). Therefore, soil and atmospheric

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parameterization is essential. An important element of the system is a species/genotype with concrete trait collection. Generally, space-time dynamics of environmental factors should be moved by mulch more closely to plant optimum. On the other hand, mulch-related shift to suboptimal conditions could contribute to plant hardening. Thus, to get a complete picture, plant physiological responses have to be recorded.

This study is intended to explain whether the principal factor enabling shoot growth of extremely drought tolerant watermelon plants (Yoshimura et al., 2008) is water saving ability of the mulch and whether it is provided by osmotic adjustment, or the if key factors are associated with changes in light and temperature environment.

## MATERIAL AND METHODS

*Plant material, cultivation and treatment*

Watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) seedlings of Paladin F1 hybrid (Sakata Seed corp., Japan) with 4 right leaves were prepared in June 2008, under external conditions using substrate AB Extra for Vegetables (Agro CS a.s., Czech Republic). Thereafter, they were transplanted into 5 litres-plastic pots with Potgrond H substrate (Klasmann-Deilmann GmbH, Germany) covered by 50 μm thick red (Maxithen HP 431641) or white (Maxithen HP 15101) coloured polyethylene film Bralen RA 2-63 (Plastika a.s., Slovakia), or by black non-woven textile (Milmar s.r.o., Czech Republic). To establish homogenous light conditions for whole above-ground plant part, the same material was laid under the pots (under non-covered control pots transparent plastic film was applied). During vegetation, water supply and pest management were provided.

*Physiological measurements*

After 20 days of growth, water was withheld to watermelon plants. At the beginning and the end of dehydration sequence (when sustained wilting was observed, after 8 days) these physiological parameters were determined:

- cumulative shoot length (CSL) (sum of main shoot and branches lengths), enabling calculation of CSL difference
- 2 p.m.-relative water content (RWC) of the youngest expanded leaf in main stem according to formula:

  $$RWC = ((w_{act} - w_{dw}) / (w_{sat} - w_{dw})) \,.\, 100(\%)$$

  where $w_{act}$ – actual sample weight (g), $w_{sat}$ – sample weight in water saturated state (g), and $w_{dw}$ – sample dry weight (g)
- 2 p.m.-osmotic potential (OP, MPa) of this leaf refractometrically; linear regression between index of refraction and calculated osmotic potential of sucrose solutions with increasing concentration at room temperature was:
  $y = -0{,}075x$

After dehydration cycle, leaf osmotic adjustment (OA, MPa) of the youngest expanded leaf in main stem was calculated following method of Morgan (1992), which specifies participation of drought-induced cell content concentration in osmotic potential:

$$OP_0 = OP_w ((RWC_w/100) / (RWC_d/100)) \quad (MPa),$$

where $OP_0$ – concentration effect in osmotic potential (MPa), $OP_w$ – osmotic potential of

well watered leaf (MPa), $RWC_w$ – relative water content of well watered leaf (%), $RWC_d$ – relative water content of dehydrated leaf (%)

$$OA = OP_d - OP_0 \quad \text{(MPa)},$$

where $OP_d$ - osmotic potential of dehydrated leaves (MPa).

*Statistical analysis*

Obtained data were submitted to analysis of variance (ANOVA) using application Statgraphics Plus v. 4.0 and MS Excel. LSD tests on confidence level 99 percent were performed for cumulative shoot length, CSL difference, relative water content, osmotic potential and osmotic adjustment. Between RWC at the end of dehydration and CSL difference, linear regression was drawn and coefficient of determination (R2) calculated.

## RESULTS AND DISCUSSION

Shoot growth of watermelon plants under sufficient water supply was positively influenced by mulch (Figure 1). However, the only significant difference in cumulative shoot length against non-mulched control plants was observed in white foil-mulched individuals (round 50 cm in average). Black non-woven textile and red foil had moderate effect on cumulative shoot length. Dehydration changed the shoot growth proportions in favour of red foil-mulched plants (in average, approximately 260 cm), but analyzing cumulative shoot growth difference, significant increase was exhibited only with plants on black non-woven textile (Figure 2).

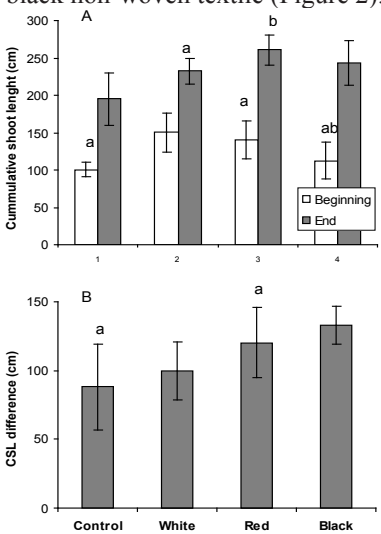


Figure 1: Cumulative shoot length (CSL) of mulched watermelon plants at the beginning and the end of dehydration cycle (A), and difference between them (B). Letters indicate statistically significant difference at P=0.01. Control – non-mulched plants, white – plants mulched by white polyethylene film, red – plants mulched by red polyethylene film, and black – plants mulched by black non-woven textile.

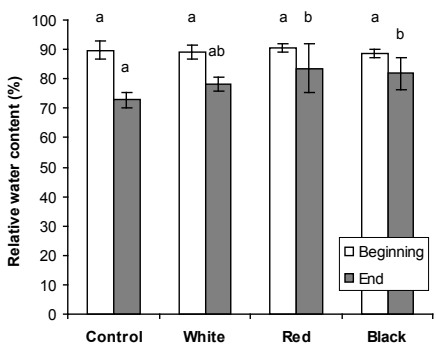


Figure 2: Relative water content of the youngest expanded leaf in main shoot of mulched watermelon plants, as influenced by dehydration. Letters indicate statistically significant difference at P=0.01. Control – non-mulched plants, white – plants mulched by white polyethylene film, red – plants mulched by red polyethylene film, and black – plants mulched by black non-woven textile.

Treatment equilibrium in leaf relative water content (RWC) at the beginning of dehydration (at about 90 %) moved to mulch-based fluctuation at the end of dehydration period (Figure 3). The highest values were found both in plants mulched by black non-woven textile and red foil (in average, 82% and 84 %, respectively). In non-mulched plants RWC fell to approximately 73 %. Similarly, although the initial leaf osmotic potential was equal in every treatment, after dehydration red foil- and black non-woven textile-mulched plants showed the highest values (Figure 4). However, on white plastic film the values were slightly lower than in non-mulched control. Subtracting participation of dehydration-mediated cell content concentration in final osmotic potential showed a large variability but no difference in mean leaf osmotic adjustment (OA) of watermelon plants (Figure 5), pointing to no influence of applied mulches. In spite of this, we found a correlation between relative water content and the cumulative shoot growth difference (coefficient of determination $R^2 = 0.57$, Figure 6).

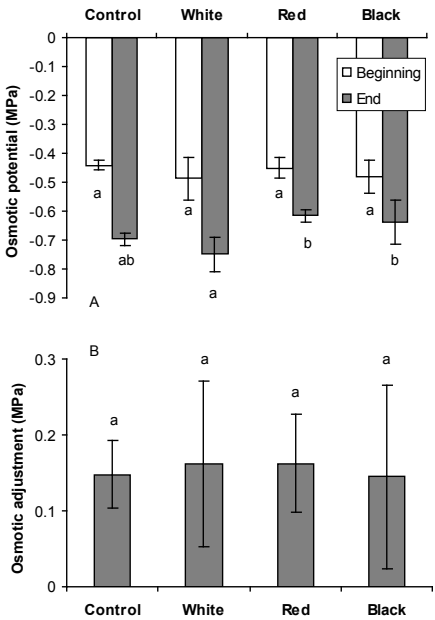


Figure 3: Changes in osmotic potential of the youngest expanded leaf in main shoot under dehydration, as modified by mulch (A), and osmotic adjustment of this leaf evoked by dehydration (B). Letters indicate statistically significant difference at P=0.01. Control – non-mulched plants, white – plants mulched by white polyethylene film, red – plants mulched by red polyethylene film, and black – plants mulched by black non-woven textile.

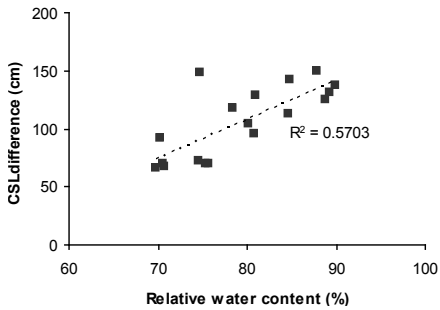


Figure 4: Linear regression between relative water content of the youngest expanded leaf in main shoot at the end of dehydration and cumulative shoot growth difference of watermelon plants mulched by different material.

Shoot growth is the result of genotype traits and a complex of physico-chemical and biological soil and atmosphere characteristics. Mulches markedly influence light, temperature and moisture environment in the field. Although in the first phase there was no limit of water supply in our experiment, light composition as well as temperature

dynamics near the mulch surface might affect the cumulative shoot length of watermelon plants.

Application of red plastic mulch enhances red light reflection, thus, increasing the proportion of red light at the shoot level. Due to better absorption and utilization efficiency of this light (Sanchez and Quiles, 2006), higher photosynthetic rate and assimilate production can be expected. On the other hand, changing ratio of red/far red (R/FR) light leads to reduced shoot elongation and branched habitus (Salisbury and Ross, 1991). In this context, wavelength of the reflected red light is also important, since for phytochrome activation followed by a growth response the specific light wavelength (660 nm) is required (Orzolek and Otjen, 2005). However, temperature could also play an important role. Relatively high colour intensity (Diaz-Perez and Batal, 2002), and low evaporation (Xie et al., 2005), attributed to this mulch, make watermelon plants prone to heat stress (Rivero et al., 2003), which limits photosynthesis and growth but accelerates tissue respiration (Wahid et al., 2007). Black non-woven textile represents a completely different mulching material. Dark colour with small reflection coefficient causes almost complete light absorption and transformation to long-wave radiation (Diaz-Perez and Batal, 2002; Diaz-Perez et al., 2007). Unlike polyethylene film, porous structure of this material enables cooling through evaporation. White plastic mulch might increase light input (of whole spectrum) and slightly increase root-zone temperature (RTZ), as well (Diaz-Perez et al., 2007). Therefore, reduced evaporation is expected. In comparison to non-mulched control, proportion of FR light could be markedly decreased.

Twenty days of shoot growth on distinct mulches under sufficient water supply revealed differences: the lowest values of cumulative shoot length were found in non-mulched plants, while significantly higher values were exhibited only in plants mulched by white polyethylene film. Saleh et al. (2003) demonstrated that among red, blue, violet and yellow-green plastic mulch, the highest cucumber (*Cucumis sativus* L.) plant length, total leaf area, total fruit weight as well as air temperature, humidity and soil temperature were achieved on red mulch. Ibarra-Jimenez et al. (2008) amend a positive relationship between RZT, as influenced by mulch, photosynthetic rate and fruit yield. In case of tomato *(Lycopersicon esculentum* L.), Diaz-Perez and Batal (2002) showed a strong negative correlation between light reflectance of mulching foil colour (decreasing from white, silver through grey, red to black) and root zone temperature, and a positive quadratic relationship of RTZ and vegetative top fresh weight, fruit yield, fruit number and individual fruit fresh weight. However, in our former greenhouse experiment (Ferus et al., 2009), no such regression was observed in watermelon plants. Thus, rhizosphere temperature might be a central element in aboveground growth regulation under mulching. For our present results, this is true only when we think of protective effect against excessive summer temperatures. The difference between day and night temperatures may also play a role in water melon shoot growth. Negative “DIF regime” (nights warmer than days) reduced watermelon internode length (Kwon et al., 1999). However, works describing energy balance in mulched canopies are missing.

Successive dehydration changes the complex interaction of mulched plants with their environment. Water deficit slows down/stops growth mainly through loss of turgor, essential for cell expansion (Larcher, 2003). In its later stages, when tissue cooling by transpiration (as well as photosynthesis) is decreased or ceased, a large heat stress can

also occur. On the other hand, mulches reduce non-effective evaporation and postpone development of water deficit (Kirnak et al., 2003; Kaya et al., 2005). In this context, plastic mulches can save more water than porous ones. However, the water-saving ability of mulched species/genotype is also important.

Watermelon exhibits an extraordinarily high drought tolerance. It is able to oscillate between survival strategies: at the early stage of drought stress, root development is significantly enhanced (drought avoidance), while at the later stage lignin synthesis-related proteins and molecular chaperones, increasing desiccation tolerance, are induced (Yoshimura et al., 2008). Moreover, there is a large diversity in watermelon growth intensity (Dittmar et al., 2009), and this parameter might determine its drought tolerance, as well. Vigorous (and probably less drought tolerant) hybrid Paladin F1 showed sustained wilting, reflected in reduced relative water content (RWC), after eight days of dehydration. However, black non-woven textile and red foil significantly alleviated water loss from the substrate as well as RWC of the youngest expanded leaf in the main stem. This phenomenon was not associated with osmotic adjustment, pointing to undergoing first survival strategy. Under severe drought, watermelon accumulates high concentration of osmoprotective citrulline, glutamate and arginine in leaves (Yokota et al., 2002).

The highest values of cumulative shoot length were recorded at the end of dehydration period, and its differences were observed in plants on red polyethylene foil and black non-woven textile – though significant increase in these parameters against the non-mulched control was found on red plastic foil and black non-woven textile, respectively. Relatively high determination coefficient of the regression between RWC and cumulative shoot length difference suggests the key position of mulch water saving ability in shoot growth stabilization under drought. Nevertheless, there is an important part of variability, which could be associated with other ways of growth control, possibly light and temperature. And it is also likely that the actual weather or local climate have relatively large participation in mulch-regulated shoot growth (Locher et al., 2005; Ibarra-Jimenez et al., 2008). In our experiment, the dehydration proceeded under relatively cold and changing weather.

## CONCLUSIONS

Among tested artificial cover mulches, shoot growth of watermelon plants was mostly preserved by black non-woven textile and red polyethylene foil under drought. In this context, their water saving ability represents the most important trait, though modifications in light and temperature environment can also play an important role.

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# ZAŠTITA RASTA IZDANAKA LUBENICE OD DEHIDRACIJE PRIMENOM VEŠTAČKIH PREKRIVAČA

PETER FERUS, SILVIA FERUSOVÁ, JÁN KÓŇA

**Izvod**

Ispitivan je uticaj belog i crvenog polietilenskog, kao i crnog netkanog tekstilnog prekrivača, na rast i zaštitu od dehidracije izdanaka lubenice *(Citrullus lanatus (Thunb.) Matsum & Nakai)*. Najmanje smanjenje rasta, ustanovljeno je kod biljaka prekrivenih crnim tekstilnim i crvenim polietilenskim prekrivačem. Ovo se može pripisti dobroj sposobnosti ovih prekrivača da spreče dehidraciju. Osim toga, važnu ulogu može imati i stepen propuštanja svetlosti, kao i ambijentalna temperatura.

**Ključne reči:** lubenica, veštački prekrivač, suša, rast izdanaka, vodni režim.

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# QUALITATIVE AND QUATITATIVE CONTENT OF ANTHOCYANINES IN THE FRUIT OF CORNEL (*Cornus mas* L.) IN POLIMLJE

MIODRAG JOVANČEVIĆ, ZORAN KESEROVIĆ, JASMINA BALIJAGIĆ[1]

*SUMMARY: In the paper the results of the qualitative and quantitative analyses of anthocyanins in the fruit of 17 different Cornel genotypes, determined by cellulose thin–layer chromatography method, are presented. Analyses of anthocyanins were carried out in 8 genotypes from Berane region and 9 from Bijelo Polje region. Genotypes are selected on the basis of the different shades of red, and are symbolized by first letters of the mentioned locations. The evaluated cornel population in Gornje Polimlje has shown, variability in quantitative and qualitative content of anthocyanins. There are 5 anthocianins contained in cornel genotypes of this location: Delphinidin – 3 – glukoside (Rf =18), Cyanidin – 3 – arabinoside (Rf = 27), Peonidin – 3 – arabinoside (Rf = 36), Peonidin – 3 – rhamnoside (Rf = 47) and an unidentified anthocyanin Rf = 7,5).*

***Key words:*** *cornel, genotype, fruit, anthocyanin.*

## INTRODUCTION

Cornel is a fruit culture very rarely cultivated in the world, but wild, this sort is spread over wide range of areas. On account of its attractive yellow colour it is also used in horticulture for park decoration.

The fruit of cornel has a high dietician value. It contain important nutrients for human nutrition in the fresh form, or through various confectionery products (Ninic-Todorovic, et al., 2005). As food it can be used fresh, or processed in a marmalade, jam, preserves etc. Harmonic content of chemical substances in the fruits and production of and cornel without the use of pesticides have a justifiable reason to continue work on the selection of fruit varieties that are recommended for organic production (Gološin et al., 2009). Cornel genotypes vary greatly in time ripening, largeness, colour, shape, taste the fruits and their nutritional value (Bijelić et al., 2007). Analysis of the fruit on pests and

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Original scientific paper/ *Originalan naučni rad*

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pathogens showed that the fruits are completely safe healthy food, because they did not previously treated by any means of pesticides. Further development of cornel selection from natural populations shall enable formation of direct collection and research to find optimal technologies for production of cornel nursery plants (Ninic-Todorovic, et al., 2005)

Cornel gives healthy food, with no use of pesticide and since it gives a good and regular harvest demanding no special agrotechnique, it is highly recommended to be cultivated for household use (Jaćimović, 1999).

The colored substances of cornel fruit are chlorophylls, carotinoides, anthocyanins. Antochyanins belong to the group of "flavonoide " unions. The colour of the cornel fruit skin in the natural population of Polimlje is rarely yellow, but various shades of red are highly present.

There is no evidence of anthocyanin content in cornel fruit, but taking into account the importance of its color for pomology, refinement, and recently chemotaxonomy too, the aim of this work is to identify the qualitative and quantitative content of anthocyanin in the fruit of cornel.

## MATERIAL AND METHODS

Analyses of anthocyanins were carried out in 2002 for 8 genotypes from Berane region and 9 from Bijelo Polje region. Fruits collected in 2001 and genotypes are selected on the basis of the different shades of red, and are symbolized by the first letters of the mentioned locations.

The extraction of anthocyanin from fruit was done by 0.1% by solution concentrated HCL in methanole during 24 hours. The extraction was done from 10 g of fruit using 10 ml of the mentioned solution.

Setting anthocyanin aside was done by means of one – dimensional paper chromatography on the paper. Whatma N 1, Tešović et al (1999), using a dissolvent: acetic – acid concentrated HCL – water (15: 3: 82 ). 50 ml of the extract was analysed.

The content of anthocyanin was determined by scale T-5. T signifies the content of anthocyanin in traces, while 5 stands for the highest content.

## RESULTS AND DISCUSSION

The average content of total anthocyanins in fruits of, selected cornel genotypes from the urban environment (site Polyclinic) was 55.88%, 35.89% minimum and 79, 22% maximum (Bijelić and sar., 2008), while the content of anthocyanins in the tested samples according to Gološin et al. (2009) amounted from 28 to 86 mg/100 g of fresh fruit

The different shades of red in fruit are the result of the various content and quantity of anthocyanin. This correlation, noticed by Tešović (1988) while investigating red raspberry, is characteristic to cornel (Table 1). The differences in the genotype have brought about different qualitative and quantitative content of anthocyanin in the fruit of cornel. So, two anthocyanins (*Delphinidin – 3 – glukoside and Cyanidin – 3 – arabinoside* ) are present in all 17 evaluated genotypes. Besides anthocyanins *Peonidin*

*– 3 – arabinoside and Peonidin – 3 – rhamnoside* are also contained in traces of fruit of genotypes IG–07, anthocyanin *Peonidin – 3 – arabinoside* in fruit of genotype IG – 08, while an unidentified anthocyanin is found to be present in the fruit of genotype BP – 04 (Rf – 7.5).

Table 1. Qualitative and quantitative analyses antocyanines fruit in cornel
*Tabela 1. Kvalitativan i kvantitativna analiza antocijana u plodu drijena*

| Nr<br>*R. Br.* | Genotype<br>*Genotip* | Delfinidin -3-glucoside | Cyanidin -3-arabinoside | Peonidin -3-arabinoside | Peonidin -3-rhamnosideide | Unidentified anthocyanins |
|---|---|---|---|---|---|---|
| 1 | IG-01 | T | 1 | - | - | - |
| 2 | IG-02 | 1 | T | - | - | - |
| 3 | IG-03 | T | 1 | - | - | - |
| 4 | IG-04 | 2 | 1 | - | - | - |
| 5 | IG-05 | 1 | 1 | - | - | - |
| 6 | IG-06 | T | T | - | - | - |
| 7 | IG-07 | 2 | 2 | T | T | - |
| 8 | IG-8 | T | 2 | - | T | - |
| 9 | BP-01 | 2 | 3 | - | - | - |
| 10 | BP-02 | 1 | 2 | - | - | - |
| 11 | BP-03 | T | 1 | - | - | - |
| 12 | BP-04 | 1 | 3 | - | - | T ? |
| 13 | BP-05 | 2 | 1 | - | - | - |
| 14 | BP-06 | 2 | 1 | - | - | - |
| 15 | BP-07 | T | T | - | - | - |
| 16 | BP-08 | T | 1 | - | - | - |
| 17 | BP-09 | 3 | 2 | - | - | - |

Rf – values of identified anthocyanins are as following:

1. *Delphinidin – 3 – glukoside* = 18
2. *Cyanidin – 3 – arabinoside* = 27
3. *Peonidin – 3 – arabinoside* =36
4. *Peonidin – 3 – rhamnoside* = 47
5. *Unidentified anthocyanins* = 7.5

Major and minor anthocyanins are found to be present in apple fruit skin (Sun et al, 1967; van Buren, 1970; Tešović et al, 1999). However, in contrast to apple, in cornel fruit one and the same anthocyanin is not the major representative of all genotypes. In that way, some genotypes had the highest content of *Delphinidin – 3 – glukoside* the others of *Cyanidin – 3 – arabinoside*, while the same quantity of both anthocyanins was presented in the fruit of few genotypes. Besides one and the same anthocyanin is not of the same importance for different sorts of fruit, so that *Cyanidin – 3 – arabinoside*, which is one of the major anthocyanins in fruit of cornel, is a minor anthocyanin in apple.

## CONCLUSIONS

The evaluated cornel population in Gornje Polimlje has shown variability in quantitative and qualitative content of anthocyanins.

- There are 5 anthocyanins contained in cornel genotypes of this location:

*Delphinidin – 3 – glukoside* (Rf = 18), *Cyanidin – 3 – arabinoside* (Rf = 27), *Peonidin – 3 – arabinoside* (Rf = 36), *Peonidin – 3 – rhamnoside* (Rf = 47) and an unidentified anthocyanin (Rf = 7,5).

- Depending on a genotype, in cornel fruit, anthocyanins *Delphinidin – 3 – glukoside* and *Cyanidin – 3 – arabinoside*, are quantitavely the most presented ones, so that they determine the shade of red in fruit in natural population.

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# KVALITATIVNI I KVANTITATIVNI SADRŽAJ ANTOCIJANA U PLODOVIM DRIJENA (*Cornus mas* L.) U POLIMLJU

MIODRAG JOVANČEVIĆ, ZORAN KESEROVIĆ I JASMINA BALIJAGIĆ

**Izvod**

U radu su, metodom uzlazne tankoslojne hromatografije, analizirani antocijani u 8 genotipova sa područja Berana i 9 genotipova sa područja Bijelog Polja. Populacija drijena u Gornjem Polimlju odlikuje se varijabilnošću u pogledu količine i kvaliteta sadržanih antocijana. U plodovima genotipova drijena izdvojeno je 5 različitih antocijana: *Delphinidin – 3 – glukoside* (Rf = 18), *Cyanidin – 3 – arabinoside* (Rf = 27), *Peonidin – 3 – arabinoside* (Rf = 36), *Peonidin – 3 – rhamnoside* (Rf = 47) and an unidentified anthocyanin (Rf = 7,5). Glavni antocijani koji kreiraju boju ploda u drijena su *Delphinidin – 3 – glukoside* i *Cyanidin – 3 – arabinoside.*

**Ključne reči:** drijen, genotip, plod, antocijani.

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# CHARACTERISTICS OF WALNUT POPULATION (*Juglans regia* L.) IN THE AREA OF ANDRIJEVICA, BERANE, BIJELO POLJE, PLJEVLJA AND ROŽAJE

MIODRAG JOVANČEVIĆ, JASMINA BALIJAGIĆ[1]

*SUMMARY: In this paper the results of stock-taking of walnuts from their natural populations in Andrijevica, Berane, Bijelo Polje, Pljevlja and Rožaje were presented. In this investigation, 245 samples were included. For description of genotypes the paper titled "Instruction suggestions for the investigations on the differences, similarities and stability of the walnut varieties" presented by UPOV from Geneva USA was used, while the evaluation of fruit quality was done according to Korać (1998). Rožaje has small walnut population, while in Berane, Bijelo Polje and Andrijevica there is a great number of various walnut populations. In the population of Bijelo Polje there is a higher number of genotypes with lower quality fruits related to the populations of Andrijevica, Berane and Pljevlja. For cultivation in the continental part of Montenegro BP 201 genotype is significant. It has characteristics of late vegetation, blooms in the third decade of May, and has nut weight of 9.99 g and kernel percentage of 44.04%. For generative rootstocks production the genotypes BA106 (6.02gr), PV14 (6.11gr) and A36 (7.96gr) are significant, because of their low nut weight, kernel percentage of over 50% and good germination.*

***Key words:*** *walnut, natural population, stock-taking*

## INTRODUCTION

Walnut (*Juglans regia* L.) is the most important kernel fruit in the world. Its commercial importance is multiple. The walnut fruit is used in human alimentation, chemical industry and medicine, and the walnut tree is a very valuable raw-material in wood processing industry.

The reasons for stagnation of nut cultivation in our country are, in the first place, low and irregular crops, as a result of frequent damages caused by frost, poor agricultural

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techniques, insufficient work on introduction of quality varieties and selections in production and uncontrolled generative reproduction of walnuts.

There is a big difference in biology of blooming and fruit quality in Juglans regia species, which allows the selection of these species and choice of genotypes adequate for different climate areas. From natural populations in our environment, or even wider, the quality selections were chosen by: Đurđević (1968), Jelenković (1974), Kuzmanovski (1980), Korać and Cerović, (1980), Bugarčić et al. (1985), Mišić et al. (1987). Therefore the aim of this paper is to isolate genotypes from their natural population, which will be successfully grown in ecological conditions in the area of Upper Polimlje.

## MATERIAL AND METHODS

The genotypes in natural populations which belong to towns in the north of Montenegro were investigated. These genotypes are: 37 genotypes from Andrijevica, 100 genotypes from Berane, 68 from Bijelo Polje, 14 from Pljevlja and 6 from Rožaje, during the period from 2000 to 2007. The genotypes were marked by capital letters of town names where certain populations are grown, and numbers according to the order of invention.

The evaluation of fruit quality was done using the method of Korać et al. (1998), and the statistical analysis was done according to Hadživuković 1984.

## RESULTS AND DISCUSSION

The characteristic of Rožaje is a small walnut population, around 20 trees. More numerous walnut population is in Pljevlja, where trees are grown in home gardens in towns more than in the village zone. Thanks to better climate conditions other towns (Berane, Bijelo Polje and Andrijevica) have more numerous and more various walnut populations.

Knowledge of the phenology of walnut varieties has higher importance than the phenology of other fruit species. The beginning of walnut vegetation is very different, so the earliest varieties start their vegetation before the latest ones even up to 60 days. It is much more related to other fruit species. This huge difference in blooming biology of Juglans regia species allows the selection of this species and choice of hybrids appropriate for different climate areas (Germain, 1979).

Late spring frost is particularly characteristic for the continental area of Montenegro. The frost had particularly noxious effect in spring 2001, when the damages caused by late spring frost affected all varieties. The exception was the selection of walnut novosadski kasni, recommended by Korać and Cerović (1980) for cultivation in areas exposed to frost, where the rate of damage was 30%. The frost, despite big damages in production, contributed to the selection of genotypes. That year, the genotype BP 201 was distinguished, with an important harvest, which was far higher even than the variety of novosadski kasni. The characteristic of this genotype is late vegetation, therefore it blooms in the third decade of May. Late vegetation was confirmed by the genotype BP 20 even in 2007 (Figure 1).

The sensitivity of walnuts to low temperatures is a variety characteristic, and it,

to a great extent, depends on outdoor conditions. During winter months, in conditions of continental area of Montenegro, strong winter frosts are almost a regular occurrence. These frosts contributed to reduction of walnut population to a small number of samples on the territory of Rožaje town.

The differences in genotype influenced the differences in fruit quality between populations, as well as within a particular population. However, there are important differences in nut weights between genotypes of the population of Rožaje and other investigated populations (Table 1). They are more influenced by the sea-level altitude, i.e. the climate in Rožaje, than by the properties of genotypes.

A very important difference in kernel percentage between genotypes of the population of Bijelo Polje and populations in Andrijevica, Berane and Pljevlja indicates weaker natural and anthropogenic selection, which enabled maintenance of trees with smaller kernel percentage in Bijelo Polje compared to other towns (Table 2).

Figure 1. The tree of BP 201 genotype on May10th, 2007 with the nut in the right corner and lines of different walnut genotypes in the left corner

*Slika1. Stablo genotipa BP201, 10. maja 2007 godine sa plodom u desnom uglu i različitim linijama oraha u levom uglu*

The characteristic of all investigated populations is that there are few hard nuts and big fruits with rough shell (big walnuts). This indicates that the man, as well as nature, made selection according to kernel percentage and the size of nut, i.e. leaving the trees with big fruits or soft shell untouched, and cutting and selling the trees with hard nuts as technical wood.

The lowest average nut weight and kernel percentage were registered in Andrijevica,

and the highest in Berane (Table 3). Variation of nut weight is more expressed than the variation of kernel percentage. These variations (of nut weight and kernel percentage) are the highest in the population of Berane, and the lowest in the population of Andrijevica. In 95% of cases the values of nut weight are within the range of 8.299-9.242 g for the population of Bijelo Polje and within the range of 4.985-8.215 g for the population of Rožaje. The values of kernel percentage in 95% of cases are within the range of 41.648-43.598% for the population of Berane, and 37.312-47.888% for walnut population of Rožaje.

For cultivation in the continental area of Montenegro, BP 201 genotype with nut weight of 9.99 g and kernel percentage of 44.04% is very interesting, Fig.2. This genotype has similar selection values (29/82 and 44/82) to the values recommended by Bugarčić et al. (1985) for cultivation in locations exposed to late frosts. The selection 29/82 has nut weight of 11.2 g and kernel percentage of 43.7%, while the selection 44/82 has nut weight of 9.1g and kernel percentage of 43.4%.

Table 1. Presence of investigated genotypes on the basis of fruit mass, in percents
*Tabela 1. Zastupljenost ispitivanih genotiova prema masi ploda u procentima*

| Fruit mass *Masa ploda (g)* | Locations / *Lokalitet* | | | | |
|---|---|---|---|---|---|
| | Andrijevica | Berane | Bijelo Polje | Pljevlja | Rožaje |
| > 14.0 | 0.00 | 3.00 | 1.47 | 0.00 | 0.00 |
| 13.6 – 14.0 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 13.1 – 13.5 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 |
| 12.6 – 13.0 | 0.00 | 1.00 | 1.47 | 0.00 | 0.00 |
| 12.1 – 12.5 | 0.00 | 1.00 | 1.47 | 0.00 | 0.00 |
| 11.6 – 12.0 | 5.40 | 3.00 | 4.41 | 14.29 | 0.00 |
| 11.1 – 11.5 | 2.70 | 2.00 | 1.47 | 0.00 | 0.00 |
| 10.6 – 11.0 | 0.00 | 3.00 | 4.41 | 0.00 | 0.00 |
| 10.1 – 10.5 | 8.11 | 3.00 | 5.58 | 0.00 | 0.00 |
| 9.6 – 10.0 | 5.41 | 8.00 | 10.29 | 7.14 | 0.00 |
| 9.1 – 9.5 | 10.81 | 8.00 | 14.71 | 7.14 | 16.66 |
| 8.6 – 9.0 | 16.22 | 7.00 | 13.26 | 21.43 | 0.00 |
| 8.1 – 8.5 | 8.11 | 12.00 | 10.29 | 7.14 | 0.00 |
| 7.6 – 8.0 | 13.51 | 7.00 | 5.88 | 14.29 | 0.00 |
| 7.1 – 7.5 | 10.81 | 11.00 | 7.35 | 7.17 | 16.67 |
| 6.6 – 7.0 | 8.11 | 8.00 | 4.41 | 7.14 | 16.66 |
| 6.1 – 6.5 | 2.70 | 10.00 | 1.47 | 0.00 | 16.67 |
| 5.6 – 6.0 | 0.00 | 7.00 | 8.82 | 0.00 | 16.67 |
| 5.1 – 5.5 | 2.70 | 2.00 | 1.47 | 14.29 | 0.00 |
| < 5.0 | 5.41 | 0.00 | 1.47 | 0.00 | 16.67 |
| St. Dev. | 1.77 | 2.66 | 1.95 | 2.08 | 1.54 |
| CV | 0.21 | 0.30 | 0.22 | 0.25 | 0.23 |

It is also important to mention A32genotypes, with nut weight of 9.5 g and kernel percentage of 55%, BP 1 with nut weight of 9.9 g and kernel percentage of 53%, and PV 01, with nut weight 9.7 g and kernel percentage of 51%. What prevents these genotypes from being recommended for cultivation in the continental area is their sensitivity to late spring frosts caused by early vegetation. However, they can be cultivated successfully in viticultural zone.

Lately, walnut scions grafted in room conditions have been mass-produced. As

rootstocks for grafting, the seedlings of common (domestic) walnuts have been used. The walnut genotypes from natural populations of Bijelo Polje with good germination are well known. The varieties grafted on these rootstocks grow well on both fertile and scarified soils, as well as on harder soils, limestone soils and give equalized seedlings (Jovančević and Balijagić, 1999). These demands have been satisfied only by genotypes with low nut weight and kernel percentage over 50%.

Table 2. Presence of investigated genotypes on the basis of kernel percentage
*Tabela 2. Zasupljenost ispitivanih genotiova prema randmanu jezgra*

| Kernel percentage *Randman jezgra (%)* | Locations / *Lokalitet* | | | | |
|---|---|---|---|---|---|
| | Andrijevica | Berane | Bijelo Polje | Pljevlja | Rožaje |
| >53.0 | 8.11 | 3.00 | 2.94 | 0.00 | 0.00 |
| 52.0 – 52.9 | 2.70 | 1.00 | 0.00 | 0.00 | 0.00 |
| 51.0 – 51.9 | 0.00 | 1.00 | 0.00 | 7.14 | 0.00 |
| 50.0 – 50.9 | 0.00 | 1.00 | 1.47 | 7.14 | 0.00 |
| 49.0 – 49.9 | 0.00 | 3.00 | 0.00 | 0.00 | 16.67 |
| 48.0 – 48.9 | 8.11 | 2.00 | 2.94 | 0.00 | 0.00 |
| 47.0 – 47.9 | 8.11 | 6.00 | 4.41 | 7.14 | 0.00 |
| 46.0 – 46.9 | 8.11 | 9.00 | 1.47 | 7.14 | 16.66 |
| 45.0 – 45.9 | 8.11 | 8.00 | 8.82 | 7.14 | 16.66 |
| 44.0 – 44.9 | 10.81 | 12.00 | 4.42 | 7.14 | 0.00 |
| 43.0 – 43.9 | 8.81 | 5.00 | 1.47 | 0.00 | 0.00 |
| 42.0 – 42.9 | 5.41 | 8.00 | 10.30 | 14.30 | 0.00 |
| 41.0 – 41.9 | 8.11 | 6.00 | 2.94 | 7.14 | 0.00 |
| 40.0 - 40.9 | 5.41 | 6.00 | 10.30 | 0.00 | 0.00 |
| 39.0 - 39.9 | 8.11 | 2.00 | 2.94 | 14.3 | 16.67 |
| 38.0 – 38.9 | 0.00 | 5.00 | 7.35 | 0.00 | 16.67 |
| 37.0 – 37.9 | 0.00 | 12.00 | 1.47 | 0.00 | 16.67 |
| 36.0 – 36.9 | 2.70 | 6.00 | 7.35 | 7.14 | 0.00 |
| 35.0 – 35.9 | 2.70 | 1.00 | 7.35 | 7.14 | 0.00 |
| < 35.0 | 5.41 | 3.00 | 22.06 | 7.14 | 0.00 |
| St. Dev. | 5.654 | 4.915 | 5.857 | 5.589 | 5.039 |
| CV | 0.129 | 0.115 | 0.148 | 0.132 | 0.118 |

Table 3. Minimum, maximum and average values of nut weight and kernel percentage in investigated populations
*Tabela 3. Minimalna, maksimalna i prosečna vrednost mase ploda i randmana jezgre kod ispitivanih genotipova*

| Locations *Lokalitet* | Mass of fruit / *Masa ploda* | | | Kernel percentage / *Randman jezgre* | | |
|---|---|---|---|---|---|---|
| | Minimum *Najmanja* | Maximum *Najveća* | Average *Prosjek* | Minimum *Najmanja* | Maximum *Najveća* | Average *Prosjek* |
| Andrijevica | 4.0 | 11.9 | 8.4 | 30.9 | 56.6 | 44.1 |
| Berane | 5.1 | 19.5 | 8.6 | 31.3 | 58.4 | 42.2 |
| Bijelo Polje | 4.7 | 15.1 | 8.8 | 26.3 | 55.4 | 39.7 |
| Pljevlja | 5.1 | 12.0 | 8.4 | 32.2 | 51.1 | 42.4 |
| Rožaje | 4.5 | 9.1 | 6.6 | 37.3 | 49.4 | 42.6 |

## CONCLUSIONS

Rožaje has small population with around 20 trees, while the walnut population in Pljevlja is more numerous. Other towns, Berane, Bijelo Polje and Andrijevica, have numerous and various walnut populations.

- In the population of Bijelo Polje genotypes with low quality fruits are more present than in the populations of Andrijevica, Berane and Pljevlja.
- For all investigated populations low presence of hard-shell nuts, known for difficulties in removal of the kernel (hard nuts), is typical.
- For cultivation in the continental area of Montenegro the genotype BP 201 is interesting. It has characteristics of late vegetation, blooms in the third decade of May, and has nut weight of 9.99g and kernel percentage of 44.04%.
- The genotypes A32, with kernel percentage of 55%, BP 01 (53%) and PV 01 (51%) are very suitable for cultivation in viticultural zones of Montenegro.
- For nursery production, i.e. for generative rootstocks (seedlings) the genotypes BA 106 (6.02g), PV14 (6.11g) and A36 (7.96g) are important. The characteristic of these genotypes are low nut weight, kernel percentage of over 50% and good germination.

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# KARAKTERISTIKE POPULACIJE ORAHA (*Juglans regia* L) U PODRUČJU ANDRIJEVICE, BERANA, BIJELOG POLJA, PLJEVALJA I ROŽAJA

MIODRAG JOVANČEVIĆ i JASMINA BALIJAGIĆ

**Izvod**

U radu su prikazani rezultati inventarizacije oraha iz prirodnih populacija Andrijevice, Berana, Bijelog Polja, Pljevalja i Rožaja. Ovim ispitivanjem obuhvaćeno je 245 uzoraka. Za opis genotipova korišćen je „ Predlog upustava za sprovođenje testova za razlike, sličnosti i stabilnost sorti oraha„ koje je dao UPOV iz Geneve USA, dok je ocjena kvaliteta ploda određena po Koraću (1982). Rožaje ima malobrojnu populaciju, dok, Berane, Bijelo Polje i Andrijevica, imaju brojnu i raznovrsnu populaciju oraha. U populaciji Bijelog Polja veća je zastupljenost genotipova sa plodovima manjeg kvaliteta u odnosu na populacije koje pripadaju opštinama Andrijevica, Berane i Pljevla. Za gajenje u kontinentalnom dijelu Crne Gore interesantan je genotip BP 201, koji se odlikuje kasnim kretanjem vegetacije, cvjeta u trećoj dekadi maja, ima masu ploda 9.99gr i randman jezgre 44.04%. Za proizvodnju generativnih podloga interesantni su genotipovi BA106 (6.02gr), PV14 (6.11gr) i A36 (7.96gr), koji se odlikuju malom masom ploda, randmanom jezgre preko 50% i dobrom klijavošću.

**Ključne riječi:** Orah, prirodna populacija, inventarizacija.

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# THE RISK OF INSECTICIDE SPRAY LIQUID APPLICATION DELAY

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*SUMMARY: The subject of the research is the determination of biological efficacy of insecticide (thiametoxam, cypermethrin) depending on water quality (hardness 4.5 i 32.6 do; pH 5.6-8.9) and storage duration (0, 24 and 72h) of spray liquids (sl). The thiametoxam efficacy just after preparation is almost 100%, and with the storage of sl (for 24h and 72h) it is 92 – 100% in all kinds of water. Immediately after preparation, the sl of cypermethrin showed efficacy of 33-85%, and in the alkaline–hard water it is on significantly lower level than in a different kind of water. With 24h storage of cypermethrin, the efficacy in all kinds of water is drastically reduced (4 – 58%) in comparison with the readily applied sl.*

***Key words:*** *thiametoxam, cypermethrin, water quality, spray liquid storage duration.*

## INTRODUCTION

The nature and application of pesticides, reffering to insecticides, include them in the group of substances of high risk, not only for the environment but for humans also. Based on many researches and achieved results, well-known risks that follow the group of mentioned substances have been summarised. In this paper, we are emphasising less known risk points such as: the duration of spray liquid storage in the reservoir until application, the quality of the water used for insecticide application, the choice of test insects for the determination of biological effects and the exposure duration, and also the time of effects assessment.

Water quality can lead to more rapid degradation of some pesticides or even to more distinctive persistence, which as a consequence, may have an alternation of biological effect.

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Original scientific paper / *Originalni naučni rad*

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There is almost no data concerning the effect of water quality on biological efficacy of insecticides, and also for the change of the spray liquid quality in the period from preparation to application, which under certain circumstances, may be prolonged (due to bad weather conditions, damage on application devices). Therefore, the subject of the research was the determination of biological efficacy of insecticides depending on water quality and storage duration of spray liquid in the reservoir.

According to Hock (2008) spray liquid storage in the reservoir of application device, may result in 50% degradation of the active ingredient or even more.

Water properties important for the quality of insecticide application are pH, hardness and electrical conductivity. Many pesticides are unstable in the alkaline medium and completely stable in the slightly acid one (Roberts and Hutson, 1999; Tomlin, 2006). The insecticide efficacy rapidly decreases if they are mixed with the alkaline water (Buss, 2006). Organophosphates, synthetic pyrethroids, carbamates and others are vulnerable to hydrolysis in the water with pH over 7, which as a consequence results in a more rapid degradation. Hydrolysis is very fast if pH of the water is 8 – 9. For every increase of the water pH by one unit, the speed of hydrolysis rises ten times (Yates, 2003; Pasian, 2004). According to Browning and Cartwright (2006) and Sparks (2006) high pH of water dictates more rapid pesticide degradation, especially at higher temperature. Based on the water quality mapping of the water used for pesticide application in APV during the years 2007/08, it has been concluded that this water differs in hardness – from very soft to very hard (0.63-34.7) and in pH – from neutral to moderately alkaline (7.11-9.3) (Vuković i sar., 2008). Water quality can influence biological effect of pesticides, mixing of pesticides, and their mixing with non – pesticide substances (complex fertilizers, surfactants, adjuvants, synergists) (Vuković i sar., 2009; Vukovic i sar., 2009a).

## MATERIAL AND METHODS

Researches were conducted during the years 2008 and 2009 under laboratory conditions, in the Department of Phytomedicine and Environmental Protection in the laboratory for pesticides in Novi Sad.

Biological effects of thiametoxam (Actara 25-WG 0.07 kg/ha) and cypermethrin (Cipkord 20-EC 0.3 l/ha), depending on the water hardness, water pH value (slightly soft /4.5 do/, pH 5.6- 8.9 and very hard water /32.6 do/, pH 5.6-8.7) and storage duration of spray liquid (immediately after the preparation, after the storage of one and three days), were determined by biotest through adult mortality of Colorado potato beetle (*Leptinotarsa decemlineata* Say.), the first generation from field conditions, locality of Čurug. Insect dipping method was applied (Inđić i sar., 1994). The trial was set in four replicates, number of adults per replicate is 30, gender relation 1:1, under temperature conditions of 23±2 oC and light regime light/dark 16/8h during the trial period. Insecticide effects were determined through dead and paralysed insects, and assessments were conducted 24, 48 and 72h after exposure. Insects were not fed during the trial period. Data was corrected for mortality in control by Schneider Orelli (Püntener, 1981). The significance of differences for efficacy was determined by analysis of variance (ANOVA).

## RESULTS

The efficacy of thiametoxam applied readily after preparation, was almost 100% in all kinds of water regardless of the water hardness and pH. The insect revival was registered only in the alkaline-soft water, during the mortality assessment after the exposure of 72h, referring to that insecticidal effect is on significantly lower level compared to effects of thiametoxam in the water of different quality.

Thiametoxam spray liquids one day old showed high efficacy (91.7-100%) and they are on the same significance level during 72h of the insect exposure.

By the application of the abovementioned three-day-old spray liquids in all kinds of water, the efficacy accomplished was 80.3 do 100%, and only after the exposure of 48h in the acid-soft water the efficacy was on significantly lower level than in other kinds of water, but the very next day the efficacy on the level of the other spray liquids was registered (table 1).

The cypermethrin efficacy readily after preparation of spray liquid after 24h of the insect exposure was between 50 and 85.2%, with a tendency of insect revival depending on exposure duration and pH of water. Significant decrease of efficacy was evidenced in the alkaline-hard water regardless of the exposure duration, and in the alkaline–soft water only after 72h of exposure.

After a one-day storage of cypermethrine spray liquid in the reservoir, the efficacy in all kinds of water decreased in comparison with the efficacy readily after preparation, and it was between 4.0 and 58%, which is regarded as insufficient. Yet, significant differences between treatments depending on water quality were not detected, while the efficacy is in the reverse proportion with the exposure duration.

Cypermethrin spray liquids after a three-day storage showed somewhat higher efficacy compared to spray liquids one day old, which could be interpreted by the presence of cypermethrin metabolites (table 2). Only in the acid-hard water, cypermethrin showed the efficacy on significantly higher level compared to other spray liquids, but still insufficient to justify its use in Colorado potato beetle suppression.

Table 1. Efficacy (%) of thiametoxam insecticide (Actara 25-WG) for L. decemlineata depending on water pH and hardness

*Tabela 1. Efikasnost (%) insekticida tiametoksama (Actara 25-WG) za L. decemlineata u zavisnosti od pH i tvrdoće vode*

| water for spray liquid preparation *voda za pripremu rt* | *applied readily after preparation* primenjen odmah po pripremi | | | *1 day later* 1 dan posle | | | *3 days later* 3 dana posle | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| *alkaline – soft* alkalna- meka | 100 **a** | 100 **a** | 96.7 **b** | 96.0 **a** | 97.0 **a** | 91.7 **a** | 99.0 **a** | 100 **a** | 100 **a** |
| *acid – soft* kisela- meka | 99.0 **a** | 99.0 **a** | 100 **a** | 95.0 **a** | 99.0 **a** | 100 **a** | 100 **a** | 80.3 **b** | 100 **a** |
| *neutral - soft* neutralna - meka | 99.1 **a** | 99.1 **a** | 100 **a** | 100 **a** | 100 **a** | 97.0 **a** | 92.0 **a** | 100 **a** | 100 **a** |
| *alkaline – hard* alkalna - tvrda | 98.9 **a** | 100 **a** | 100 **a** | 100 **a** | 100 **a** | 100 **a** | 99.0 **a** | 100 **a** | 100 **a** |
| *acid – hard* kisela - tvrda | 100 **a** | 100 **a** | 100 **a** | 99.0 **a** | 100 **a** | 98.0 **a** | 95.0 **a** | 98.0 **a** | 100 **a** |
| *neutral – hard* neutralna - tvrda | 100 **a** | 100 **a** | 100 **a** | 99.0 **a** | 100 **a** | 89.0 **a** | 96.0 **a** | 97.0 **a** | 100 **a** |
| LSD 5% | 2.20 | 1.64 | 2.48 | 6.87 | 3.62 | 14.0 | 5.78 | 18.3 | |

Table 2. Efficacy (%) of cypermethrin insecticide (Cipkord 20-EC) for L. decemlineata depending on water pH and hardness

*Tabela 2. Efikasnost (%) insekticida cipermetrina (Cipkord 20-EC) za L. decemlineata u zavisnosti od pH i tvrdoće vode*

| *water for spray liquid preparation* voda za pripremu rt | *applied readily after preparation* primenjen odmah po pripremi | | | *1 day later* 1 dan posle | | | *3 days later* 3 dana posle | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| *alkaline – soft* alkalna- meka | 74.0 **a** | 71.0 **a** | 56.0 **ab** | 50.0 **a** | 15.0 **a** | 5.2 **a** | 55.5 **a** | 27.3 **bc** | 14.5 **b** |
| *acid – soft* kisela- meka | 77.0 **a** | 83.0 **a** | 70.0 **a** | 46.0 **a** | 14.0 **a** | 4.0 **a** | 63.0 **a** | 31.0 **bc** | 23.0 **b** |
| *neutral – soft* neutralna - meka | 85.2 **a** | 81.0 **a** | 79.1 **a** | 45.1 **a** | 13.7 **a** | 9.0 **a** | 56.0 **a** | 13.0 **c** | 7.0 **b** |
| *alkaline – hard* alkalna - tvrda | 50.0 **b** | 47.0 **b** | 33.0 **b** | 58.0 **a** | 17.5 **a** | 11.5 **a** | 67.0 **a** | 34.0 **bc** | 24.7 **b** |
| *acid – hard* kisela - tvrda | 69.6 **a** | 76.0 **a** | 76.0 **a** | 40.0 **a** | 12.2 **a** | 8.0 **a** | 74.0 **a** | 76.0 **a** | 53.7 **a** |
| *neutral – hard* neutralna - tvrda | 79.2 **a** | 79.8 **a** | 72.0 **a** | 49.0 **a** | 10.0 **a** | 8.0 **a** | 70.5 **a** | 40.8 **b** | 26.1 **ab** |
| LSD 5% | 16.3 | 21.2 | 25.7 | 23.5 | 17.4 | 10.1 | 23.0 | 19.7 | 25.3 |

## DISCUSSION

Changes in biological effects, subject to spray liquid storage duration as a consequence of bad weather conditions or damage on the application devices, during which degradation of active ingredient is likely, and also depending on water quality, deserve further investigations, so that possible metabolites could be detected and differences in biological activity of insecticides, which are of high importance for plant protection, could be explained. Decreased biological activity induces treatment repetition, which increases: the content of harmful pesticide residues in agricultural products, the risk of contaminated products, the selection pressure on harmful organisms and the occurrence of their resistance to insecticides.

During the research of water hardness and the influence on insecticidal effect, Vuković *et al*. (2009) bring out the data on high susceptibility of Colorado potato beetle to thiametoxam in the slightly hard water. In the same water, by mixing thiametoxam with other components (azoxistrobin, mancozeb, complex fertilizer), insecticidal effect of thiametoxam did not change, and in the very hard water there was lower efficacy of thiametoxam in mixes with other components.

Similar results on the influence of mixes of insecticides (thiametoxam) and fungicides (mancozeb, folpet, metiram) on efficacy onto Colorado potato beetle depending on water pH (7.5-8) and on the efficacy of mixes chlorpyrifos+metalaxil+mancozeb and chlorpyrifos+folpet in water of different quality (the sink water and well water), showed that the efficacy of these mixes was influenced by water quality and mixture compounds (Inđić i sar., 2000; Klokočar i sar., 2000; Klokočar i sar., 2002).

Considering lots of data on decreased susceptibility of insects to substances from pyrethroid group, and the registered resistance in a number of generations of Colorado potato beetle, decreased efficacy of cypermethrin is a more often phenomenon in our region (Perić i sar., 1997). According to the research Inđić *et al*. (2009), insecticide

cypermethrin in 13 localities showed very high efficacy, while in 27 localities 2-50% of survived insects was registered, and in Padinska Skela locality insect survival of 86% was evidented.

These researches enclose possible combinations of water (hardness and pH) and formulations as water soluble granules (WG) and emulsion concentrate (EC), yet wettable powder (WP) and suspension concentrate (SC) are very much in use, and also, because of their physical and chemical properties, deserve researches as the above mentioned two.

## CONCLUSIONS

Based on the conducted researches, it can be concluded that thiametoxam showed high efficacy regardless of the water quality and duration of spray liquid storage. The cypermethrin efficacy was on significantly lower level in the alkaline-hard water, and also the duration of spray liquid storage significantly influenced the efficacy of this insecticide. Therefore, cypermethrin showed significantly lower efficacy after one day of spray liquid storage compared to the one applied readily after preparation. Considering the abovementioned, the standardization of basic requirements for quality of water used for insecticide spray liquid preparation and for their storage duration in the reservoir is necessary, in order to improve the application quality and health safety of agricultural products.

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# RIZIK ODLAGANJA PRIMENE RADNE TEČNOSTI INSEKTICIDA

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**Izvod**

Predmet istraživanja je bio određivanje biološke efikasnosti insekticida (tiametoksam, cipermetrin) zavisno od kvaliteta vode (tvrdoća 4,5 i 32,6 d°; pH 5,6-8,9) i vremena stajanja (0, 24 i 72h) radnih tečnosti (rt). Efikasnost tiametoksama odmah po pripremi je skoro 100%, a stajanjem rt (24 i 72h) je 92-100% u svim vodama. Odmah po pripremi rt cipermetrina ispoljile su efikasnost 33-85%, a u alkalnoj-tvrdoj vodi je na značajno nižem nivou nego u drugim vodama. Stajanjem cipermetrina 24h, efikasnost u svim vodama se značajno smanjila (4-58%) u odnosu na odmah primenjene rt.

**Ključne reči:** tiametoksam, cipermetrin, kvalitet vode, stajanje radne tečnosti

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# SETTING UP THE HACCP SYSTEM IN PRIMARY PRODUCTION*

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*SUMMARY: The primary agricultural production has its own specifics and is subjected to actual trend aiming to get safety alimentary products. This paper presents the requirements of constitution and application of this globally already excepted, international concept on food safety (HACCP) in primary agricultural production. The paper also provides a retrospective view on implementation and certification of HACCP concept in Serbia.*

***Key words:*** *primary agricultural production, food safety, HACCP*

## INTRODUCTION

According to the definition by Codex Alimentarius, HACCP is a program for identification, evaluation and control of hazards regarding food safety. The food safety is considered through the analysis and control of biological, chemical and physical hazards from “the field to the table”.

The Codex Alimentarius Commission, working within UN, with Food and Agriculture Organization (FAO), has adopted the first document which refers to setting up and tracking the critical control points (”Guidelines for the application of the Hazard Analysis Critical Control Point – HACCP system – ALINORM 93/13/A, Appendix II”) on its meeting in Geneva in 1993. This document was revised subsequently (in 1997) and annexes have been made in accordance with the requirements and changes in the field of food production (www.who.int ).

HACCP system implies setting up the responsibilities for all participants in food production chain. If there is a risk concerning consumers’ health, all of the participants are obliged to urgently undertake the necessary measures for preventing harmful

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Review paper / *Pregledni rad*

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consequences and inform the authorities on it.

Food safety is an imperative of modern agricultural production and food industry. In food production chain, which is to reach the final consumer, it is important to control the whole production process and to evaluate and consider each step as a possible risk in getting the safe product. Former strategy in food production was based on final production control (Savić et al., 2006).

After many incidents related to food quality, registered on both international and national level, the need for setting up common and obligatory standards for tracking and controlling the production process and food distribution has arisen. Primary producers, as well as all the others in food production chain, are obliged to certificate the production process and provide the market with safe products. Therefore, HACCP program should be introduced and applied in primary production of agricultural products and certified by the authorized certifying body (Savić, 2006).

HACCP program is a scientifically based concept for controlling processes of food products production and distribution, which enables:

- Identification and evaluation of all possible hazards of physical, chemical or micro-biological origin in all phases of food production process,
- Determination of measures necessary for prevention and control of these hazards,
- Providing successful and effective implementation of these measures.

When introducing HACCP system in firms engaged in the above mentioned activities, it is necessary to consider the peculiarities of primary production, but, on the other hand, certain general, basic principles of this program have to be enforced. (Food safety management system requirements for any organization in the food chain JUS ISO 22000:2006). These are:

- Principle 1. Hazard analysis (diagram of the course for each phase, recognizing hazards, making registration and determining controlling measures)
- Principle 2. Critical controlling points – CPP (decision-making tree)
- Principle 3. Critical boundaries (they provide control of each critical controlling point)
- Principle 4. System of tracking - monitoring
- Principle 5. Corrective measures which should be undertaken when monitoring indicates that some CCPs are not under control.
- Principle 6. Verification method for affirmation whether HACCP system is efficient (critical revision and tests).
- Principle 7. Making documentations which refer to all the procedures and records in accordance with these principles and their appliance (managing the documentations).

## DOCUMENTS OF HACCP PROGRAM

The phase of HACCP program certification can be attained in two ways. One way is implementation of standards automatically, and the other way is by consulting firms involved in this activity. Both ways imply complete cooperation of all participants in production, from hierarchical top in companies to the very executers. It is necessary

to engage all the participants in constitution and updating of documentation, which is to cover every phase of production, coordinated by HACCP team. Making appropriate documentation (each documents is coded) is important, as every operation needs to be registered.

Creation of HACCP Internal Rule, as the highest systematic document, is necessary as a joining form of all activities related to introduction of HACCP program.

A detailed insight of all operations in a productive process, and its realization through phases, has been presented in the diagram of course. The diagram of course is a document which comprises all phases in the production course, the documents every phase is based on, as well as a person responsible for realization of a specific phase.

Regarding systematic documents, systematic procedures should be made for: HACCP plan, monitoring, corrective measures, verification, managing documents and records, and realization of hygienic-sanitary measures. Each of these procedures specifies the activities and plans related to a certain field of activity and have obligatory points and sub-points. Each procedure comprises the following elements: code, procedure description, distributive list, object, field of appliance, relations to other documents, definitions, responsibility, course of creation and enclosures. (System of managing food safety, Yugoslav standard JUS ISO 22000:2006).

The procedure for realization of hygienic-sanitary measures is an important systematic document, which should meet a number of requirements:

- efficient and reliable controlling and maintaining hygienci-sanitary conditions
- prevention of unfavourable situations regarding hygienic-sanitary conditions
- clear defining of authorization and responsibility level regarding hygienic-sanitary requirements
- developing consciousness and approaches of employees to take operative role in controlling the hygiene.

Documentation which is operative and refers to a concrete *farming cultivar* (sweet-corn, rape etc.), vegetable crops (cucumber, paprika) or animal products (milk, meat) has to contain certain elements. The document ***product identification*** contains all basic information on a product regarding sort, kind, as well as who, where, and on which surface grows a certain plant or breeds a certain animal race.

The document ***product description*** has to have data on the name and composition of a product, method of storage, characteristics of the product, primary and transportation packaging, storage and transport conditions, declaration, expiry date, intended usage, instruction for use.

For each of farmed cultures or bred races, the production procedure needs to be made, where technological procedures of production are specified. For example, ***the production procedure*** of sweet-corn, among basic elements of each procedure, implies also the ***procedure course*** which thoroughly describes all phases in the production of the cultivar. After an authorized entity has made a decision on a timely start of production, it is time to start activities such as: basic cultivation, fore-sowing cultivation (which has its sub-phases: fertilization and preparation for sowing), sowing, care of crops (with sub-elements: plant protection, irrigation, crop supplemental feeding), harvest, control of products and transport directly from the field to food industry. Each of these

production process phases has its specific time, i.e. time of the year when it happens and meteorological conditions needed for gathering the maize of the required phase of maturity. Sweet-corn is specific as different sorts are adjusted seasonally, so there can be more gatherings during a vegetation period.

Each product must have *HACCP – a plan, course digram, list of hazards, risks analysis, identified critical points and corrective measures. Risks identification and hazards analysis* are made to determine the *control critical measures and critical boudaries*, in which the risk or products incompatibility can vary, so that the corrective measures could be undertaken, if they are above the permitted level.

## PECULARITIES OF PRIMARY AGRICULTURAL PRODUCTION

The primary production has spatial and time limitations and peculiarities regarding tracking and controlling the production process. All factors and participants in this process must be engaged in developing the consciousness on significance of monitoring and registering of all activities that lead to healthy-safe product. Field cultures, as well as livestock breeding, have their technological requirements, to which the productive practice (according to standards GLOBAL-GAP; www.globalgap.org) has to adhere. The primary production, spatially observed, spreads on huge surfaces and there are already means of monitoring and, if necessary, means of intervention in terms of certain corrective measures. During the year, each culture has its requirements, too, and each phase of vegetative cycle/animal development is tracked and registered. The primary production has its requirements regarding document and validation of each step of production.

The policies of primary agricultural producers have to be focused on realization of qualitative product. Declaration on quality and producers' decision to apply the policy of quality and apply the standards is base, while the starting point in setting up these standards is the decision of management, under the leadership of the general director, and that represents the most important point for success in setting up the quality system. As next step, it is inevitable to form an adequate, expert HACCP team. This step requires determination of the manager and members of the team, who are informed on production process. The HACCP team, in crop agricultural production, has to engage an agronomist, who tracks the production and all phases of growing, and engage agronomist for plant protection, required for applying adequate agrotecnical measures. The members in HACCP team for livestock production have to be experts in primary production.

## INTRODUCTION AND CERTIFICATION OF HACCP PROGRAM IN SERBIA

Protection, preservation and improvement of population's health is one of the priorities of Serbian government. One of the basic factors, which should be satisfied in this respect, is production of safety food.

In the period 1999-2003, the number of epidemics as well as the number of the diseased increased (Table 1.). In 2003, compared to 1999, there was an increase in the number of epidemics of alimentary bacterial poisoning, salmonellosis, botulism and

intestinal bacterial intoxications. Such disproportional increment of diseased number in epidemics of salmonellosis and intestinal bacterial intoxications is implication of consumption of food which had not been under constant supervision, i.e. had been unsafe.

The new legislative frame in Serbia, which is to regulate the field of food safety, will provide an overall approach in regulating rights, obligations and responsibilities of all participants in food chain (from „the field to the table", „from the farm to the table"). In this way, it will be possible to recognize, identify and inform all actors in food chain, including production of animal food, primary production, processing and food industry, storage, transport, sale and other forms of distribution, as well as control and supervision with their rights, obligations, authorizations and responsibilities.

Table 1. Epidemic of diseases transferable by food in Serbia in the period 1999-2003

| Diseases | | 1999 | 2000 | 2001 | 2002 | 2003 | Growth rate (%) |
|---|---|---|---|---|---|---|---|
| Alimentary bacterial poisoning | Number of epidemic | 36 | 46 | 31 | 53 | 44 | 5,14 |
| | Number of the diseased | 501 | 601 | 539 | 739 | 526 | 1,22 |
| Salmonellosis | Number of epidemic | 147 | 101 | 111 | 136 | 183 | 5,62 |
| | Number of the diseased | 1.060 | 1519 | 1151 | 1196 | 2054 | 17,98 |
| Dysentery | Number of epidemic | 7 | 12 | 6 | 6 | - | -5,00* |
| | Number of the diseased | 104 | 205 | 36 | 21 | - | -41,33* |
| Botulism | Number of epidemic | 2 | 3 | 7 | 2 | 4 | 18,92 |
| | Number of the diseased | 6 | 9 | 18 | 5 | 12 | 18,92 |
| Intestinal bacterial infections | Number of epidemic | 3 | 17 | 7 | 22 | 18 | 56,50 |
| | Number of the diseased | 18 | 166 | 80 | 522 | 223 | 87,61 |
| Brucelossis | Number of epidemic | - | - | 1 | - | 2 | - |
| | Number of the diseased | - | - | 21 | - | 9 | - |
| Trichinosis | Number of epidemic | 42 | 32 | 29 | 25 | 15 | -22,69 |
| | Number of the diseased | 614 | 297 | 452 | 229 | 159 | -28,66 |
| Total | Number of epidemic | 237 | 211 | 192 | 244 | 266 | 2,93 |
| | Number of the diseased | 2.303 | 2797 | 2297 | 2712 | 2983 | 6,68 |

Data source: Proposal of Strategy Draft on food safety in RS, 2009
* Growth rate calculated for years from 1999 to 2002

Big companies, small and medium enterprises, cooperatives, individual producers in primary agricultural production, processing and distribution of animal and plant origin products in Serbia are introducing or have already introduced and applied HACCP program. There is a relatively small number of the companies in the field of primary production of plant and animal origin: Agro Mobil, Bio Dvig, Centropak, Dren, Farma Radin Salaš, Farma Tomić, Jugokoka, Menta, Bilje Borča i ect. (www.poljoprivreda.info).

On the EU and the World Trade Organization markets, HACCP system became obligatory on 1st January 2006 (Council Directive 93/43/EEC); it became a precondition for doing business with partners on the international market. Serbia has accepted this concept of safety food, so since 1st January 2009, HACCP concept has become obligatory for producers and processors of animal products. The companies which did not get HACCP certificate by this term, would not be able to export and sell their products in Serbia.

Veterinarian inspection of General Inspectorate of Ministry of Agriculture, Forestry and Water Management RS has realized, in 2009, the control of objects for production of animal origin products on Serbian territory and has ascertained that 120 objects have HACCP program, while in 180 objects the program introduction is ongoing. If the Veterinarian Inspection ascertains that there is no contract for introducing the HACCP program in registrated objects, the Inspection will submitted an application for economic offence, as anticipated by law, and the final measure is closing such objects (Law on Veterinary RS – „Official Gazzete RS“, No. 91/05).

## CONCLUSION

HACCP represents an integrated preventive program, which ensures food safety in every phase of production and distribution process, which contributes to the decrease of health risk of population.

Primary agricultural producers, regarding both the domestic and foreign market, where they are to place their products, are becoming more and more aware of the significance of standardization and implementation of good production practice, adopted worldwide. The basic segments of HACCP standard are hazard analysis (HA) and determination of critical controlling points (CCP), compatiblity with ISO 9001 standards which imply registration of all productive activities.

The supporting assets Serbia allocates for HACCP program implementation for food safety function as primary support for producers, so that they make a decision to introduce this concept and get certificate on applying of this food safety concept. In this way, general awareness on food safety significance is raised to a higher level. It is very important that primary producers realizes the importance of safety food, which can provide him, if it is certified, with a competitive price and income by good placement both on international and domestic market.

Safety of alimentary products has to be a goal and obligation of each successfully oriented business system, small and medium enterprises and individual producers and food distributors.

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## USPOSTAVLJANJE SISTEMA HACCP U PRIMARNOJ PROIZVODNJI

NADA MIJAJLOVIĆ, MIRJANA SAVIĆ, LJUBO VRAČAR

### Izvod

Primarna poljoprivredna proizvodnja ima svoje specifičnosti i podleže savremenom trendu standardizacije u cilju dobijanja bezbednog prehrambenog proizvoda. U radu su prezentovani zahtevi u uspostavljanju i primeni sada već široko prihvaćenog međunarodnog koncepta o bezbednosti hrane (HACCP) u primarnoj poljoprivrednoj proizvodnji. Dat je osvrt na uvođenje i sertifikaciju HACCP koncepta u Srbiji.

**Ključne reči**: primarna poljoprivredna proizvodnja, bezbednost hrane, HACCP

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# NOURISHMENT OF GAME FROM THE CARNIVORA ORDER – DAMAGES AND BENEFITS IN HUNTING ECONOMY, FORESTRY AND AGRICULTURE*

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*SUMMARY: An overview of different types of damage caused in hunting grounds and agriculture in general by wildlife from the Carnivora order is given in this paper. Predators present one of the greatest problems for the hunting grounds, since they are destroying primarily progeny and eggs of game while searching for food. Most damage to the stock of game offspring is caused by jackal, who is in expansion in the recent years. Fox is feeding primarily on rodent-type vermin and predates on game offspring only in a small percent. Wolf may cause damage both on game and domestic animals stock. Wild boar may cause significant damage to game stock by predating on progeny of other game species. The brown bear may cause significant damage on corn and wheat crops. Unlike all aforementioned species, who are also scavengers, lynx feeds exclusively on living prey. One of measures for predator population control is considered hunting. Supplemental nutrition of carnivores by carrion is allowed by planning and construction of carrion baits.*

***Key words**: game, carnivora, damages, control.*

## INTRODUCTION

Beside economic benefits that man obtains from game and its positive influence on the habitat, it is also important to consider negative impact that game may produce, both in forestry and farming and on domestic animals and wildlife itself. Damages caused by game are usually caused by lack of, poor quality and inadequate distribution of food within the hunting grounds, as well as by disturbing of game and even by habits of the

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game (Popović, 2006-a).

Damages may be reduced or prevented by adequate management of the hunting grounds (Popović, 2006-b). Increase in natural food production within the hunting grounds and provision of additional food from other sources that is conducted during the period when damages occur are among the more important measures. (Đorđević et al. 2006; 2008). These procedures may help to maintain adequate game population (Popović et al. 2008), control losses (Popović and Bogdanović, 2001) and increase trophy value (Popović and Bogdanović, 2002; Popović and Gačić, 2006). A series of protective measures for farming and horticulture crops, orchards and vineyards, forests, domestics animals and beehives, as well as fencing the hunting grounds, which allows to breed game in a controlled environment, may be undertaken (Popović and Đorđević, 2009). High degree of wildlife control and protection is obtained in national parks (Beuković et al. 2006)

Wildlife from the carnivore order may cause specific type of damage both in hunting grounds and on livestock and beehives. „Predatory" type of damage may also be caused by wild boars, although this species is an omnivorous even-toed ungulate and not a carnivore (Beuković et al. 2006). Specific protective measures are required to ward against this type of damages. This overview is dedicated to that particular problem and possibilities for its resolving.

## TYPES AND EXTENT OF DAMAGE

Foxes and, in the recent years, jackals, are most numerous carnivores in our hunting grounds, followed by wolves, while examples of lynxes and bears are located primarily in the national parks (Beuković et al. 2007). Damages caused by carnivores are determined on the field, based on the remains (wildlife and domestic animals, beehives) or the gastric content of shot animals' (Đorđević et al. 2009).

Nutrition of foxes in our hunting grounds is based primarily on mouse-like rodents, which is highly advantageous for the agriculture. According to Popović and Đorđević (2009), daily meal of a fox contains at least 10-15 voles (*Microtus sp.*) with average mass of 25 grams per vole.

Through study of stomach contents of foxes in Hungary, Lanszki (2005) determined that 24.3% of meal samples comprised vole and that vole may form up to 37.3% of sample biomass. Still, rest of foxes' nutrition consists of game, primarily pheasant and rabbit, which represents a substantial harm to hunting economy. According to Popović (1996) and Beuković et al. (2009), beside unfavorable weather conditions, foxes are second principal cause of significant loss in rabbit progeny.

It is also thought that bad physical shape of pheasants from intensive production, raised in pheasantries and released in hunting grounds may be particularly beneficial to the fox (Pekeč et al. 2008; Popović and Stanković, 2009; Popović et al. 2009). According to Linnella (2007), eighteen year long research of roe deer in hunting grounds of Sweden and Norway showed that fox is the principal predator of fawns of up to three months of age. Fawn losses reached up to 50% and in some areas 100% of fawn losses were caused by foxes.

According to Popović (2007), fawn losses by foxes and stray dogs in Serbia are

lower (about 20%) while main cause of losses is anthropogenic factor – agricultural mechanization, traffic, poaching, snaring, fawn catching...

Fox is also an approved poultry predator and it was shown that it may induce losses in offspring of small livestock. Spreading and transfer of parasites and contagious diseases to other wildlife, domestic animals and even humans is a particular type of damage caused by foxes. Rabies is a dominant problem of this kind (Ristić et al. 2008).

Jackal expansion in Serbia is evident in the recent years, a problem significantly contributed by illegal landfills and disposition of dead farm animals against sanitary and veterinary regulations – directly in hunting grounds areas (Ćirović et al. 2008).

Carrion, primarily originating from domestic animals, as well as various slaughterhouse waste, such as bowels, skins, bones and entrails, dominate in jackal nutrition. Since jackal is a bad hunter its nutrition is comprised mostly of mouse-like rodents and game offspring. Based on analysis of stomach contents and meal remains, it was determined that jackals may cause significant damage to hunting grounds through destruction of fawns, young rabbits and earthbound bird nests. Jackals are also capable to organize in a group and hunt adult rabbits by use of drive and ambush (Pantelić, 2008).

A predominant carnivore, wolf may cause significant damage to wildlife and livestock. It was shown that wolves are capable to dwell in vicinity of men, in cattle-breeding areas (Kusak, 2002), in fields or along town outskirts. They may base their nutrition almost exclusively on livestock. For example, sheep, goats and dogs comprise 84% of wolves nutrition in Dalmatia region, but roe and red deer and, to a lesser extent, wild boar, comprise 77% of wolves nutrition in Gorski Kotar region (Štrbenac et al. 2005). According to Sidorovich et al. (2003), wild even-toed ungulates in Belarus comprise 80-88% of wolves nutrition.

Wolves cause substantial damage to hunting grounds by predating on wild even-toed ungulates, but that activity is also beneficial as it is mostly old, sickly and out-of-shape animals that wolves predate on (Ballard et al. 1981). Wolf's menu also includes stray dogs, sheepdogs and hunting dogs (Popović et al. 2008-b). Actual damage caused by wolves should nonetheless be estimated with some precaution, as livestock (primarily bovine) remains in wolf's refuse may have come from carrion and carrion baits.

Damages caused by bear are inflicted on plant cultures, wildlife, domestic and cultivated animals. Farming crops and orchards are most damaged by bears. Beehives in some countries are also menaced by bears, so specific averting measures such as electric fences are frequently utilized (Sillings et al. 1989). Animals rarely serve as food source for brown bear, which happens when there is lack of bear-edible plant food in the hunting grounds. In that case bear will firstly predate on roe and red deer and then attack flock of sheep, goats and grazing horses. Ultimately it may attack pens and stables and may even attack people. In ex Socialist Federate Republic of Yugoslavia, a bear attacked and killed one person in Plitvice, Croatia (Mihić, 1996). According to Popović (2006-a) bears killed three heads of large cattle and eight heads of small cattle in three observed hunting grounds („Šipovo", „Borja", „Ribnik"). As a rule, attacks occurred in early spring and late autumn, a consequence of decreased production of natural food resources in these periods. Further harm scored 12 beehives, 1.5 ha of oat and 46 trees of fruit in ripening phase. Certain amount of bear-induced damage may also occur in forestry as a result of peeling off of bark and destruction of saplings (Malik and Karnet, 2007).

Unlike other carnivores, lynx feeds exclusively on living prey, in average 3.3 kg daily. Damages caused by lynx are generally related to a significant reduction of population of wild even-toed ungulates in the hunting grounds, while loss of domestic animals is of minor significance (Marinović, 2008). Summer nutrition of lynx consists primarily of rodents, while winter nutrition consists of wild even-toed ungulates (roe and red deer, chamois, wild boar, mouflon). Lynx may occasionally also prey on birds, foxes and small domestic animals (sheep and goats). According to Stahl et al. (2001) sheep losses by lynxes in France in period 1984-1998 amounted to 0.14-0.59% of total number of sheep, wherein lambs and young sheep were most common victims. Lynx may down prey four times its size.

## DAMAGE PREVENTION MEASURES

Different averting methods may prove to be uncertain particularly in the case of carnivores. Most important methods by all means are population control and considered hunting (Popović and Đorđević, 2009). "Službeni glasnik Republike Srbije" (Official Journal of Republic of Serbia) (1994) recommends „Continuous monitoring of numbers and spread of both periodically and permanently open season predators and regulation of their population through shooting." Lesser importance may be attributed to additional nurture provided by carrion baits (Đurđević, 2004), while construction of feeders is recommended for bears. However, this additional food source may incite bears, thus accustomed to new scents, to enter human habitats and scavenge for food in garbage containers and landfills, as is the case in the US and Canada (Halter, 1972).

Beside these, additional nourishment of wild carnivores is also used for population control, considered hunting, research of nourishment quality, estimation of trophy value, prevention of migration, scientific research, reduction of poaching... Construction of feeding grounds for carnivores must account for a series of factors in order to ensure peace for these animals and simultaneously keep them as far from human habitats as possible (Dečak et al. 2005).

## CONCLUSION

Foxes and, as of recent, jackals, may cause most damage in hunting grounds primarily thanks to their large numbers. Damage is generally inflicted through destruction of game offspring. Wolves and lynxes are present in lesser numbers but may pose a serious problem for both game and livestock. As an omnivore, brown bear may cause most damage on farming crops, orchards and beehives, while only some specimens may pose a threat for domestic animals and even people. Particular form of damage is spreading of diseases. Beneficial influence of carnivores, such as reduction of rodent vermin and sick, old and out-of-shape animals is also to be considered. Most important measures focused on reduction of damage caused by carnivorous wildlife Population control are considered hunting and additional nourishment.

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# ISHRANA DIVLJAČI IZ REDA ZVERI – ŠTETE I KORISTI ZA LOVNU PRIVREDU, ŠUMARSTVO I POLJOPRIVREDU

ZORAN POPOVIĆ, NENAD ĐORĐEVIĆ, MILOŠ BEUKOVIĆ

**Izvod**

U radu je dat pregled različitih oblika šteta koju pravi divljač iz roda zveri, pre svega u lovištima, ali i u samoj poljoprivredi. Jedan od najvećih problema za lovišta predstavljaju predatori, koji u potrazi za hranom uništavaju pre svega podmladak i jaja divljači. Jednu od najvećih šteta na podmladku divljači čini šakal, koji je zadnjih godina u ekspanziji. Nasuprot tome, lisica se hrani pre svega mišolikim glodarima, a u manjem procentu i podmladak divljači. Štete od vuka mogu biti na divljači, ali i na svim vrstama domaćih životinja. Divlja svinja, u ograđenim lovištima, takođe, može da napravi velike štete uništavajući podmladak druge divljači. U pogledu šteta koje čini, jeste medved. Ova vrsta zveri je pre svega biljojed, pa pri ishrani pravi štete u usevima kukuruza i drugih žita, voćnjacima i pčelinjacima. U odnosu na sve pomenute vrste zveri, koje u ishrani rado koriste i lešine, ris se hrani isključivo živim plenom. Selektivni odstrel je jedna od mera za kontrolu brojnosti predatora. Planiranje i izgradnja mrciništa u lovištima omogućava dodatnu ishranu zveri, čime se smanjuju štete na lovnim vrstama divljači.

**Ključne reči:** divljač, zveri, štete, kontrola.

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# AGROECONOMICAL ANALYSIS AND ORGANIC AGRICULTURAL PRODUCTION*

RADOVAN PEJANOVIĆ, ANKA POPOVIĆ-VRANJEŠ, GORAN KRAJINOVIĆ, MIRELA TOMAŠ, DANIJEL PETROVIĆ[1]

*SUMMARY: The authors are discussing agroeconomical analysis as a methodological procedure of monitoring organic agricultural production. The starting hypothesis in this paper is that along with biological, technological, agronomic aspects, agroeconomical aspect is very important aspect of this important and promising production.*

***Keywords:*** *organic agricultural production, agroeconomical analysis, business plans, profitability, production costs, market prices.*

## INTRODUCTION

**Agroeconomical analysis** provides a wide range of instruments and methods for monitoring the production. Among many methods of agroeconomical analysis we can isolate the following: analysis of business profitability, cost analysis and cost analysis of the market and the price of organic agricultural products.

## IMPORTANCE OF AGROECONOMICAL ANALYSIS

**Agroeconomical analysis** gives us an answer to a range of issues that are crucial to the commencement of this production, as well as for the maintenance and development (improvement) of this production.

**Business plan** is “the alpha and omega” of agroeconomical analysis. It contains an integral set of goals, policies, strategies and a set of individual program activities for the

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Review paper / *Pregledni rad*

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implementation of selected concepts of business farms (companies). It is a conceptual framework for designing and properly connecting the vision, mission, business objectives, policies, directions, methods and pace of current development and behavior of farms (enterprises). Based on that, the key areas such as marketing, production, personnel, finance, procurement, research and development are programmed. Besides management, this plan is facing creditors, internal public and all other whose assistance and support is necessary for implementation of the planned business venture (organic agriculture).

In addition to the business plan as a key economic documents **other** agroeconomical analysis are necessary: analysis of business profitability, cost price analysis, cost analysis, analysis of the economy, analysis of credit conditions, the analysis of financial results, analysis of the organization's operations, analysis of production, demand analysis, competition analysis, market analysis, marketing cost analysis, etc.

One of the basic questions of agroeconomical analysis is the definition of **farm model.** For example, elements of a model agricultural farm crop – cattle breeding direction of organic production are available soil area, buildings, machinery and equipment, labor, production line in cattle production and established structural units, lines of production in crop production, prices of inputs and outputs . Elements of a model farm for organic pig production are: production facilities - machines, buildings, etc; number of sows and the index of farrowing – for the production of pigs, the number of fattening pigs (by conditional animal) - for fattening pigs; workforce, cost of reproduction material and products.

Economic input-output model parameters are further transferred to the appropriate mathematical simplex method. Before the procedure of optimization we have to format the starting simplex matrix, which contains the basic solution based on the assumption that if you do not use all available factors of production on the farm there is a loss in the amount of fixed costs, which have already made the procurement of means of production, workforce engagement, etc.

## ANALYSIS OF ENTERPRISE PROFITABILITY

Analysis of farm business profitability of organic production involves testing and evaluating financial performance in the final results of operations carried out in the sphere of production and trade. Starting from such an understanding, analysis of profitability of operations is reduced to the analysis of the level of profitability achieved in the total volume of completed sales. Operate profitably in the outcome means that the entire business has to **achieve a surplus of revenues over expenditures**, or a **positive financial result**. Opposite, non-profitably business ends with negative financial results or loss. Knowledge about what will be the financial result is gained only during the encounter with the market, e.g. when selling.

Depending on the adopted method of measuring profitability, **the subject of the analysis of profitability** is determined. If we use the following formula to measure profitability:

Profitability = Completed surplus products / Earnings Realization

then the analysis of profitability is focused to examine the following factors that influence profitability: the impact of changes in the cost of reproduction to the level of profitability,

the impact of changes in retail prices, the impact of changes in the range of sales.

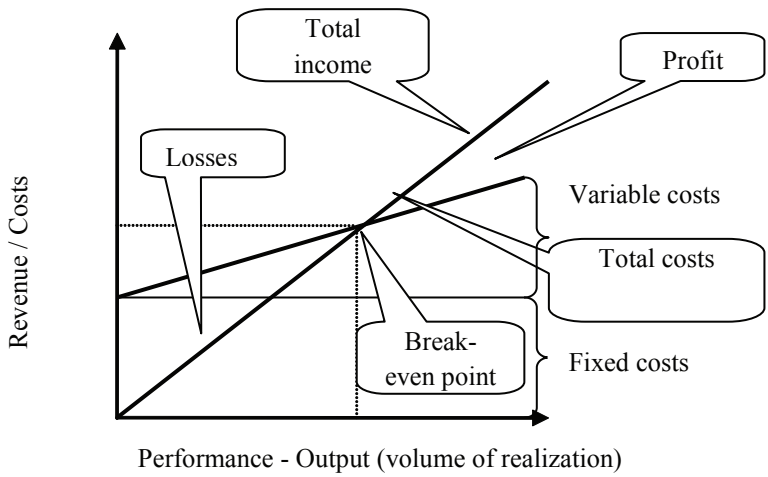
If, however, to measure profitability we use the formula:

Profitability = Completed surplus product / business invested assets

then the analysis of profitability engages in the critique of the investment funds policy, with special emphasis on the effectiveness of investment in fixed and variable capital. In this analysis and critique of the effectiveness of investment is focus on examining the following issues: analysis of investment directions, the intensity of investment analysis, and analysis of suitability of investment, e.g. optimal relationship between the size of investments and realized effects.

In studding and monitoring of enterprise profitability important issue is the **threshold (turning point) of profitability.** This analysis is conducted to investigate the relationship between the cost - income - results. Threshold of profitability (break-even point, or B / E point) is the point at which the value of completed sales and total costs are equal. To calculate break-even point farm (companies) managers need to know the price of one product units sold (P), variable cost per unit (VC) and total fixed costs (TFC): BE = TFC / (P - VC).

Relations that are examined can be presented graphically:




Picture 1. Analisys of break even point (graph of rentability)
Slika 1- Analiza prelomne tačke (grafikon rentabilnosti)

Break-even point of profitability is the point of intersection of the total income and expenses. This is the point at which arises or ceases the positive business. It is such a combination of factors of production that it allows to a farm only minimal condition of existence.

The importance of turning points of profitability for the current sale is that it may affect the orientation of management regarding the return on assets and risk, as well as in the direction of reducing operating costs of farms (enterprises).

## ANALYSIS OF COSTS AND COST PRICE

**Cost analysis** means examining the dynamics and structure of the total costs of reproduction and their relation to other categories, or results of operations. Analysis of the cost is basic and integral part of the analysis of economy.

Cost analysis starts from the analysis of the total cost of reproduction, and then access various forms of parsing, which can be done in three ways: by type of costs, the place of cost, by the carriers of the costs. There are combined parsing as: types of costs by areas (e.g. transmission calculation sheet); cost carriers by place (total cost of the product in stages of production); types of costs by the carriers and places (phase calculation of the product).

The most important form of dividing the costs by type is the separation of fixed and variable (proportional, progressive, and declining balance) so that the costs can be analyzed according to the volume of production. Frequently all variable costs are treated as proportional to the volume of production.

A special importance is the observation and analysis of the types of costs sectioned in stages of the production process: the cost of sales and operations, the costs of procurement and material supplies, costs of preparing production, production costs (by technological stages); costs driving direction; administration costs.

The purpose of cost analysis is to discover the places and types of expenses that can be reduced or avoided, and thus achieve better results in the following business period.

Analysis of the **cost price** involves examining changes in the cost and impact on the capabilities to reduce costs. Therefore, the objective of price cost analysis is possibility to reduce the price cost. To achieve this it is necessary to analyze the causes of the increase or decrease in cost.

Of great importance for the analysis of the cost is the analysis of its elements, because if they are properly placed, if absolutely fixed costs and absolutely proportional costs are clearly separated from other costs, which have mixed character, then in the analysis of the cost can be clearly seen the causes that are related to the digressive and progressive nature of some costs.

When it comes to organic agricultural production, cost analysis and cost price analysis must take into account **the costs of adequate measures of agricultural engineering.**

One of the essential conditions for the economical organic production is possibility to provide nutritional elements in the soil. Because of the relatively cheap and effective supplying of necessary nutrients using fertilizers, application of organic fertilizers in intensive plant production in the country was usually ignored. Use of nutrients from the manure was very modest, especially on large farms. Because of the small number of livestock keeping and modern conditions, they obtained a very small amount of manure. According to some estimates, instead of every fourth or fifth year, lots were been fertilized with manure every 18-20 years. In terms of organic methods of production, if the fertilization is done with manure, which is supplied at the farm, it is necessary to determine:

- amount of solid and liquid wastes in livestock production by the animal;
- total annual amount of manure for all animals on farms;

- content of the main nutrient elements in the total estimated amount of manure;
- the needs of individual crops to the major nutrient elements;
- bribed the needs of crops for nutrient elements

When applying fertilizer in terms of organic methods of production, the attention goes to some problems, such as: cost of soil fertility control, usage of fertilizer and control of environmental factors in plant production, with the aim of achieving stable high yield, good quality, minimum investment of material, energy and labor - economic optimization and protection of agro-system, environment and biosphere from the pollution.

## MARKET ANALYSIS AND PRICE OF ORGANIC AGRICULUTURAL PRODUCTS

**Market analysis** involves identifying and measuring factors that define the size of potential markets (domestic and foreign). Dimensions of the market can be understood as the number of customers in a particular area in a certain time for which a product is intended. The potential of the market is usually less than the number of buyers and it is defined by the number of potential customers who show some interest for the organic product. Qualified market is the one that has economic assumptions to buy a given product. It is important to establish target (desired) participation, as a percentage of potential and qualified markets.

To achieve the same economic result as the conventional mode of production, farmers in organic production needs to establish higher selling prices. The lowest **selling prices** of certain agricultural products, with which is possible to achieve the same amount of margin as in conventional mode of production, can be calculated with following equation (Sredojević, 2000)

$P_1c_1 - VT_1 = MP_1$

$P_2c_2 - VT_2 = MP_1$

or

$$C_2 = P_1c_1 - (VT_1 - VT_2) / P_2$$

$$c_2 = (P_1c_1 - \Delta VT) / P_2$$

or simply expressed through the **cover margin**, the product price in alternative methods of production, can be obtained as follows:

$c_2 = (MP_1 - VT_2) / P_2$

where:

$P_1$ - the amount of product (yield) in terms of conventional methods of production;

$P_2$ - the amount of product (yield) in terms of organic methods of production;

$C_1$ - the price of the product in terms of conventional methods of production;

$VT_1$ - direct external variable costs in terms of conventional methods of production;

$VT_2$ - direct external variable costs in terms of organic methods of production;

$MP_1$ - margin coverage in terms of conventional methods of production.

To determine the prices of certain products according to the shown methods, it is necessary to calculate the amounts of listed indicators for each product. These indicators

are determined with establishing **the differential calculation by individual lines of production.** In a similar way the minimal prices of final products at the farm are determine.

In the countries of Economic Union there has been a radical **change in agricultural policy** during the past decade. Since 1992 the EU has supported agricultural production methods that take into account environment, natural resources and the diversity of plant life. Rural development policy for the period after 2000 confirms the essential role that farmers play in providing the functions of maintaining the environment that goes beyond the desirable way of keeping agricultural production and the basic legal standards. According to above mentioned, farmers who take into respect standards and technology, agricultural production, related to environmental protection, are being **supported by the state** for a period of at least five years. It is envisaged that for certain types of obligations a longer period is approved, depending on their effects on the environment. Help is granted annually and is calculated according to the loss of revenue and additional costs that occur because of commitments. Reform of EU Common Agricultural Policy from 2003, introduce, as a new element, single farm payment (SFP), independent of production volume. These payments are linked to environmental protection, respect of good manufacturing practices, food safety, protection of plants and animals and applying appropriate production standards, and maintaining agricultural areas and the environment in good production conditions (Bogdanov, 2004).

## CONCLUSIONS

Successful development and economic power of organic farms in agricultural production depends on many **economic factors, external and internal nature.**

It is necessary to **systematically and continuously monitor** the impact of macroeconomic and microeconomic factors to the business entities in organic agricultural production. For that there are competent agroeconomists that have been studying for decades at the Faculty of Agriculture in Novi Sad and Zemun.

The basic and essential question that must be constantly analyzed and monitored is a question of economic feasibility of organic agriculture. To achieve this it is necessary to organize **a unique (by the methodology) statistical-accounting documentation on all farms.** This would enable real insight into the actual costs of production, cost price and comparison, as, in addition to quality, important factors of competitiveness.

In addition, the appropiate methodological instruments for scientific analysis of production should be chosen, such as farm modeling, production optimization, cost-benefit analysis, the threshold analysis (turning point) of profitability, audits, market analysis and price, etc.

**Agroeconomical analysis** gives an answer to a range of issues that are crucial for the commencement of this production, as well as for its maintenance and development (improvement). Rational usage of all factors of production is one of the important conditions of economy of organic agricultural production. It is particularly important, necessary and indispensable (permanent) analysis of: analysis of business profitability, cost analysis and cost price, analysis of the market and the price of organic agricultural products and analysis of gross margins. Each of these analysis is in the function of

productivity, economy, profitability and environmental sustainability as key principles of business in the modern economy. Cost analysis from this study confirms the hypothesis that the operating costs of production (especially in our conditions) are very high. This fact must be known to all social entities (especially the state) that are interested in the development of organic agricultural production.

When it comes to economics and business organization of organic farms it is necessary to constantly monitor and analyze the **experience of the European Union,** and some developed countries. This experience shows, in short, that it is necessary with measures of agricultural policy and other methods, always to encourage and support this production (lead price policy). Only in that way, in the conjunction with **internal and external efficiency of state support,** it is possible to maintain and develop this important and promising agricultural production.

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# AGROEKONOMSKA ANALIZA I ORGANSKA POLJOPRIVREDNA PROIZVODNJA

RADOVAN PEJANOVIĆ, ANKA POPOVIĆ-VRANJEŠ, GORAN KRAJINOVIĆ, MIRELA TOMAŠ, DANIJEL PETROVIĆ

**Izvod**

Autori razmatraju agroekonomsku analizu kao metodološki postupak praćenja organske poljoprivredne proizvodnje. Polazna hipoteza u radu je da je uporedo sa biološkim, tehnološkim, agronomskim aspektima, veoma važan agroekonomski aspekt ove važne i perspektivne proizvodnje.

**Ključne reči:** organska poljoprivredna proizvodnja, agroekonomska analiza, biznis plan, rentabilnost, troškovi proizvodnje, tržište i cene.

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# INSTRUCTIONS FOR AUTHORS

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.

**THE PAPER TITLE** is written in bold letters, Font Size 11 , centred. The title should be lowered below the upper margin clicking enter 4 times and writing should be commenced in the fifth line.

THE NAME AND SURNAME of the authors are written in normal letters, Font Size 10, centred, with a single space below the paper title. With mark 1, in superscript, ( click Insert Footnote) above the name of the last author, the Footnote is marked, stating the title, the name and surname, the rank and the institution in which the respective authors are employed.

*SUMMARY*: ( italic), Font Size 10 (Justify) with a single space below the name and surname of the author of the paper. The summary presents the basic objective, the material and method of the study, the significant results and the conclusion (500 characters maximum).

***Key words***: minimum 3 and maximum 6 words. Below the summary, Font Size 10.

Footnote (Insert Footnote):

[1]Titula, first name and surname, address for the all of autors.

Corresponding author: first name and surname, address, tel., fax., e-mail.

**INTRODUCTION** (bold) , centred, Font Size 10. Text normal, Justify, with a single space below the title.

**MATERIAL AND METHOD OF THE STUDY** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the title.

**RESULTS** (bold), centred, Font Size 10. Text normal, Font Size 10, Justify with a single space below the table.

**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.

The order of papers is arranged according to the alphabetical order of the initial letter of the surname of the first author, without numbering. Literature text Font Size 9.

After the literature a short table of contents should be written in English as follows:

**TITLE**, in capital letters (bold), centred, Font Size 10.

NAME AND SURNAME OF AUTHORS , in capital letters (normal), centred, Font Size 10.

**Summary**, in small letters (bold), centred, Font Size 10.

Text, Font Size 10 (normal), Justify.

**Key words:** in small letters.

The tables should be clear, as simple and neat as possible. The titles, headings (text) and subtext in tables, should be in Serbian and English ( Serbian – normal, English –italic). Font Size 9. The tables are set in a specific place in the text.

The photographs, drawings, graphs and schemes, should be given in a separate supplement (an original file format –TIF, JPG, with 300 dpi, or a vector format with letters turned into curves – CDR, AI).and the place where they are to be printed should be marked in the text, by writing in the text the caption of the photograph, drawing or scheme. For instance:

Graf. 1. Koncentracije spermatozoida u ejakulatu nerasta, zavisno od godisnje sezone (Font Size 9, normal).

*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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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«*

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