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IN VITRO BOAR FERTILITY DURING SUMMER AND AUTUMN SEASON

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Abstract: A primary objective of this study was to determine the effect of breed, frequency of utilisation, boar age and season on following sperm characteristics: volume of ejaculate (ml), sperm concentration ($x10^6$ spermatozoa/ml), total count and count of functional spermatozoa in ejaculate ($x10^9$ spermatozoa), motility of spermatozoa in native ejaculate and after dilution (%), number of produced doses, percent of dead and viable spermatozoa. Research included 4 boars of Landrace breed (n=40 ejaculates) and 13 boars of Large White pig (n=89 ejaculates). Ejaculates were analyzed during two seasons (summer and autumn), a dividing line being a calender start of autumn. Interval between two mounts was observed on two levels: ≤7 and ≥ 8 days. The assessment of the effect was done by means of a General Linear Model procedure. Breed and frequency of utilisation did not affect an average expression and variability of sperm characteristics. By increasing boar age by one day the volume of ejaculate increases by 0.142 ml. In ejaculates taken during autumn season a higher concentration of sperm per ml of ejaculate by $53.38x10^6$ spermatozoa ($p=0.033$) was determined. Higher motility ($p<0.001$) of native i.e. diluted semen of 5.7, i.e. 8.2% was determined in ejaculates taken during summer season. A higher percent (+13.47%; $p<0.001$) of dead spermatozoa was determined in ejaculates in autumn season.

Key words: boar, breed, sperm, frequency of utilisation, age, season

Introduction

An artificial insemination is a primary method of reproduction in an intensive pig production *(Lopez Rodruguez et al., 2017)*. Boar fertility exerts a considerably greater impact on reproductive efficiency of breeding stock in comparison with the fertility in sows *(Stančić, 2014).* In selection of breeding animals a great attention was payed to the traits of growth and food conversion. Thus, *Robinson and Buhr (2005)* suggest that major aims of selection of male animals are in the first place the traits that can have greater economic importance, primarily traits of growth.

The characteristics of sperm may vary under the impact of different genetic and non-genetic factors: breed, age, season, intensity of utilisation and other factors (*Kondracki et al., 2009; Smital, 2010; Kunowska-Slósarz and Makowska, 2011; Wilczyńska et al., 2013).*

There are differences between breeds regarding the size of testes, number of spermatozoa per ejaculate, volume of ejaculate, concentration and motility of sperm *(Caisin and Snitco, 2016)*. *Wolf and Smital (2009)* determined in their research that there exists a difference in volume of ejaculates between boar breeds. Boar breed and season of collecting sperm have a significant effect on the volume of ejaculate, as well as on the share of motile spermatozoa *(Okere et al., 2005).*

Frequency of sperm collecting is an important factor in boar reproduction. It happens that some boars are more often used because of their easier manipulation or shorter preparation time for mount, while the intensity of using is ignored and the pauses between mounts are too short what leads to excessive exhaustion of boar and to obtaining ejaculates of poor fertile quality *(Savić, 2014).*

High summer temperatures have a direct negative impact on the process of spermatogenesis in testes. In their research *Savić et al. (2013)* determined that ejaculate quality parameters are significantly higher during a cooler season of the year in relation to a warm season. *Argenti et al. (2018)* state that although there are differences in microclimatic parameters between seasons which are manifested primarily through higher temperature-humidity index during summer in relation to other periods of the year, the impact of season on sperm characteristics is low.

Volume of ejaculate increases up to the age of about two years and remains more or less constant *(Wolf and Smital, 2009*). From a biological aspect one of the reasons of increase in the production of sperm with boar age can be explained by the increase in the number of Sertoli cells in testes (*Kanokwan, 2011)*. The highest values of volume of ejaculates, total number and number of functional spermatozoa were determined in the age of 2.5-3 years *(Wang et al., 2017)*.

The aim of this research was to study the effect of the most important factors (breed, interval between two mounts, season and age of boar) on average manifestation and variability of sperm characteristics.

Materials and Methods

The research was conducted on a pig farm with its own reproductive and commercial breeding stock in the period from August to October. Boars were placed in a separate pen, in boxes of dimensions 2x4 m, with partially latticed and concrete flooring. Housing microclimatic conditions were manually regulated by a vertical and horizontal ventilation. Nutrition was based on balanced feed mixtures while fresh water was available ad libitum.

The trial included 4 boars of the Landrace breed (n=40 ejaculates) and 13 boars of Large White breed (n=89 ejaculates). In order to be included in the analysis a boar was to realize a minimum of three successful mounts during a trial period. The ejaculates were analysed in the course of the two seasons (summer and autumn), and the dividing line was a calendar beginning of autumn. The interval between two mounts was observed on two levels: ≤7 and ≥ 8 days.

The research included: volume of ejaculate (VOL, ml), concentration of sperm (CON, $x10^6$ spermatozoa/ml), total count of spermatozoa in ejaculate (NT, $x10^9$ spermatozoa), total count of functional spermatozoa (NF, $x10^9$ spermatozoa), percentage of motility of native semen (MOTN, %), percentage of motility of semen after dilution (MOTD, %), number of produced doses (NPD), percentage of dead (PM, %) and percentage of viable spermatozoa (PZ, %).

Boar sperm collection was done by a standard manual method, by bringing the boar into the box with phantom. Volume of ejaculate was expressed in millilitres with an accuracy of ±2 ml, and it was measured by a graduated cylinder. By means of a photo colorimeter concentration of native sperm was estimated. Total number of spermatozoa in ejaculate was obtained by multiplying VOL by CON. Number of functional spermatozoa was calculated by multiplying NT by MOTN. The estimation of motility of the mass of spermatozoa in native ejaculate and after dilution was carried out by a subjective assessment, by a microscopic examination. A percentage of dead and viable spermatozoa in sperm was determined on a permanent Eosin-Nigrosin stain preparation where living spermatozoa appear on a dark background non-stained while the dead ones are parly or completely stained (*Savić and Petrović, 2019*). Doses for insemination were standardized at the volume of 100 ml.

The estimation of the effect of factors on variation of the sperm characteristics was performed by means of a General Linear Model in SAS 9.1.3 statistical package *(SAS Inst. Inc., 2002-2003)*, by a following statistical model:

$$y_{ijkl} = \mu + B_i + I_j + S_k + BI_{ij} + BS_{ik} + IS_{jk} + BIS_{ijk} + b(x_{ijkl} - \bar{x}) + e_{ijkl},$$

where: $y_{ijk}l$ – is an analysed characteristic of ejaculate; μ – general population average; B_i – effect of boar breed (i= 1,2); Ij – effect of interval between two consecutive mounts (j= 1,2); S_k – effect of season (k= 1,2); BI_{ij}, BS_{ik}, IS_{jk}, BIS_{ijk} – interactions; $b(x_{ijkl}-\bar{x})$ - linear regression effect of boar age and e_{ijkl} – random error.

Comparing the Least Square Means (LSMeans) values of sperm characteristics was done by t-test.

Results and Discussion

The effect of studied factors on variability of sperm characteristics is shown in Table 1. The season of sperm collecting had an effect on qualitative traits of ejaculates (CON, MOTN, MOTD, PM, PZ). The low values of determination coefficients (<30%) obtained by the model applied suggest that studied effects can to a small degree explain variability of studied sperm characteristics. The age of boar during collecting the ejaculate only had an effect on volume, while determined value of regression coefficient shows that with increasing the age of boar by one day the volume of ejaculate increases by 0.142 ml.

The research conducted is parly consistent with the results of the research of *Smital (2010) and Wierzbicki et al. (2010)* who determined the effect of different genetic and non-genetic effects on mean expression and variability of sperm characteristics. *Petrocelli et al. (2015)* determined a significant effect of season on the vitality, total and primary abnormalities, volume and concentration of sperm. The research of *Tereszkiewicz and Pokrywka (2020)* showed a seasonal differences in physical characteristics of semen of studied boar breeds.

Table 1. Effect of factors included in the model on variability of sperm traits

Traits	B	I	S	b±SE	R^2
VOL (ml)	ns	ns	ns	0.142±0.046 **	0.140
CON ($x10^6$/ml)	ns	ns	*	-0.071 ±0.058 ns	0.134
NT ($x10^9$)	ns	ns	ns	0.021±0.025 ns	0.096
NF ($x10^9$)	ns	ns	ns	0.015±0.021 ns	0.128
MOTN (%)	ns	ns	***	-0.003±0.004 ns	0.231
MOTD (%)	ns	ns	***	0.002±0.005 ns	0.232
NPD	ns	ns	ns	0.002±0.002 ns	0.058
PM (%)	ns	ns	***	0.005±0.007 ns	0.294
PZ (%)	ns	ns	***	-0.005±0.007 ns	0.294

VOL – volume of ejaculate, CON – concentration of sperm, NT – total number of spermatozoa, NF – number of functional spermatozoa, MOTN – motility of native semen, MOTD – motility of semen after dilution, NPD – number of produced doses, PM – percentage of dead spermatozoa, PZ – percentage of viable spermatozoa, B – effect of breed, I – interval between two consecutive jumps, S – season; b – coefficient of regression (age of boar), R^2 – coeficient of determination; Statistical significance (p): ns=p>0.05; *=p<0.05; **=p<0.01; ***=p<0.001; ¶Model also included interactions of factors that were not statistically significant.

Sperm concentration varied under the effect of season (Table 2). In the ejaculates collected during autumn season a higher concentration (p=0.033) of sperm per ml of ejaculate by 53.38x10^6 spermatozoa was determined. Lower concentration of sperm during summer period can be a consequence of a negative effect of high summer temperatures. *Savić et al. (2015)* likewise determined that season had an effect on concentration of spermatozoa, the highest concentration (242.16x10^6 spermatozoa/ml) being recorded during autumn months. The effect of season on concentration of sperm was determined also in the research of *Chinchilla-Vargas et al. (2018)* where ejaculates taken during summer had higher concentration compared to the ejaculates collected during other seasons in the year. In our research higher concentrations of sperm were recorded in the ejaculates collected during autumn season what is in contrast to the research mentioned. In spite of decrease in concentration of sperm in spring and summer, majority of semen parameters was constant over an entire year *(Argenti et al., 2018).* These differences between the studies may be a consequence of different experimental designs but also of genetic structures of studied populations.

Contrary to our research, *Apić et al. (2015)* state that the season affected variability of total count of spermatozoa in ejaculate, a higher value being determined during a cold season. Different to our research, *Smital (2010)* determined that number of functional spermatozoa varied under the effect of season, the highest number being observed in winter period while in summer period the lowest number was detected.

A higher motility (p<0.001) of native, i.e. diluted semen of 5.70, i.e. 8.52% was determined in the ejaculates taken during summer season (Table 2). Our results are in contrast with the conclusions of *Savić et al. (2020)* whose research showed that motility of native semen did not vary under the effect of season.

Table 2. Effect of season on variability of sperm traits

Traits	LSMeans±SE		p
	Summer	Autumn	
VOL (ml)	311.13±12.93	303.04±11.90	0.646
CON (x10^6/ml)	329.86±18.59	383.24±16.05	0.033
NT (x10^9)	106.36±8.08	117.67±6.97	0.292
NF (x10^9)	101.11±5.89	85.34±6.83	0.085
MOTN (%)	85.81±0.98	80.11±1.08	<0.001
MOTD (%)	80.94±1.35	72.42±1.76	<0.001
NPD	15.61±0.61	15.51±0.77	0.922
PM (%)	18.61±1.80	32.08±2.27	<0.001
PZ (%)	81.38±1.80	67.92±2.27	<0.001

VOL – volume of ejaculate, CON – concentration of sperm, NT – total count of spermatozoa, NF – number of functional spermatozoa, MOTN – motility of native semen, MOTD – motility of semen after dilution, NPD – number of produced doses, PM – percent of dead spermatozoa, PZ – percent of living spermatozoa; p - statistical significance.

Contrary to the results of our research, *Savić et al. (2015)* determined that the number of doses produced varied under the impact of the interval between the two mounts and the season further stating that the highest number of doses was produced over autumn months. Also, *Chinchilla-Vargas et al. (2018)* determined that number of doses obtained per ejaculate was lower in the ejaculates collected during spring and winter in comparison with the ejaculates from summer period.

Percentage of dead and viable spermatozoa varied under the effect of season (Table 1). Higher percentage ($p<0.001$) of dead spermatozoa by 13.47% was determined in sperm taken in the autumn season (Table 2). *Knecht et al. (2014)* determined the highest percentage of viable spermatozoa in winter period. A higher count of dead and lower count of viable spermatozoa during the autumn season determined in this research could be a consequence of the occurrence of chronic thermal stress during the summer months. High day temperatures occur in our climatic region all up to early autumn and boars were in longer time period exposed to this stress effect. A period of resuming a physiological optimum was too short what might have affected the results of research.

Conclusion

Sperm characteristics did not vary under the effect of boar breed and frequency of utilization. The age of boar had an effect on the variability of the ejaculates volume. The season of collecting ejaculates affected sperm qualitative characteristics. Low values of determination coefficients indicate that studied effects can to a small degree explain variability of examined sperm characteristics. Taking into account that research period included the end of summer and beginning of autumn period the boars were in a longer time period exposed to the effect of high summer temperatures therefore a longer time was needed in order that production of sperm should resume its physiological optimum what probably affected the results obtained.

In vitro plodnost nerasta tokom letnje i jesenje sezone

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Rezime

Osnovni cilj ovog istraživanja bio je da se utvrdi uticaj rase, frekvence korišćenja, starosti nerasta i sezone na osobine sperme: volumen ejakulata (ml), koncentracija sperme ($x10^6$ spermatozoida/ml), ukupan broj i broj funkcionalnih spermatozoida u ejakulatu ($x10^9$ spermatozoida), pokretljivost spermatozoida u nativnom ejakulatu i nakon razređenja (%), broj proizvedenih doza, procenat mrtvih i živih spermatozoida. U istraživanje su bila uključena 4 nerasta rase landras (n=40 ejakulata) i 13 nerasta velikog jorkšira (n=89 ejakulata). Ejakulati su analizirani tokom dve sezone (letnje i jesenje), a granica razdvajanja bila je kalendarski početak jeseni. Interval između dva skoka posmatran je na dva nivoa: ≤ 7 i ≥ 8 dana. Procena uticaja izvršena je primenom procedure opšteg linearnog modela. Rasa i frekvenca korišćenja nisu uticale na prosečnu ispoljenost i varijabilnost osobina sperme. Povećanjem starosti nerasta za jedan dan, volumen ejakulata se povećava za 0,142 ml. U ejakulatima uzetim tokom jesenje sezone utvrđena je veća koncentracija sperme po ml ejakulata za $53,38x10^6$ spermatozoida (p=0,033). Veća pokretljivost (p<0,001) nativnog, odnosno razređenog semena od 5,7 odnosno 8,2% je utvrđena u ejakulatima uzetim tokom letnje sezone. Veći procenat (+13,47%; p<0,001) mrtvih spermatozoida utvrđen je kod ejakulata u jesenjoj sezoni.

Ključne reči: nerast, rasa, sperma, frekvenca korišćenja, starost, sezona

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References

APIĆ J., VAKANJAC S., RADOVIĆ I., JOTANOVIĆ S., STANKOVIĆ B., KANAČKI Z. (2015): Effect of season on boar semen quality. Contemporary Agriculture, 64, 1-2, 9-13.

ARGENTI L.E., PARMEGGIANI B.S., LEIPNITZ G., WEBER A., PEREIRA G.R., BUSTAMANTE-FILHO I.C. (2018): Effects of season on boar semen parameters and antioxidant enzymes in the south subtropical region in Brazil. Andrologia. 50, 4,e12951. doi: 10.1111/and.12951

CAISIN L., SNITCO T. (2016): The influence of the seasonality on boars sperm production - breeder of different species. Bulg. J. Agric. Sci., 22, Suppl. 1, 118–122.
CHINCHILLA-VARGAS J., KERNS K., ROTHSCHILD F. M. (2018): Lunar and climatic effect on boar ejaculate traits. Animal Reproduction Science, 193, 117-125.
KANOKWAN K. (2011): Association and expression study of CD9, PLCz and COX-2 as candidate genes to improve boar sperm quality and fertility traits. Institut für Tierwissensechaften, Abt. Tierzucht und Tierhaltung der Rheinischen Friedrich-Wilhelms- Universität Bonn. Inaugural-Dissertation.
KNECHT D., ŚRODOŃ S., DUZIŃSKI K. (2014): The influence of boar breed and season on semen parameters. South African Journal of Animal Science, 44, 1, 1-9.
KONDRACKI S., WYSOKIŃSKA A., KOWALEWSKI D., MUCZYŃSKA E., ADAMIAK A. (2009): Season's influence on the properties of male domestic pig semen. Rozprawy naukowe Pope John Paul II State School of Higher Vocational Education in Biała Podlaska, III**,** 177-187.
KUNOWSKA-SLÓSARZ M., MAKOWSKA A. (2011): Effect of breed and season on the boar's semen characteristics. Annals of Warsaw University of life sciences- SGGW. Animal Science, 49, 77-86.
LOPEZ RODRIGUEZ A., VAN SOOM A., ARSENAKIS I., MAES D. (2017): Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Management, 3, 1, 1-12.
OKERE C., JOSEPH A., EZEKWE M. (2005): Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. Journal of Animal and Veterinary Advances, 4, 10, 885-888.
PETROCELLI H., BATISTA C., GOSÁLVEZ J. (2015): Seasonal variation in sperm characteristics of boars in southern Uruguay. Revista Brasileira de Zootecnia, 44, 1, 1–7.
ROBINSON J.A.B., BUHR M.M. (2005): Impact of genetic selection on managment of boar replacement. Theriogenology, 63, 668-678.
SAS INST. INC. (2002-2003): The SAS System for Windows. Cary. NC.
SAVIĆ R. (2014): Fenotipska i genetska varijabilnost plodnosti nerasta. Doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu.
SAVIĆ R., PETROVIĆ M. (2019): Praktikum iz svinjarstva. Univerzitet u Beogradu, Poljoprivredni fakultet, 1-182.
SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N. (2013): The effect of breed, boar and season on some properties of sperm. Biotechnology in Animal Husbandry 29, 2, 299-310.

SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N., POPOVAC M., GOGIĆ M. (2015): Ejaculate properties and reproductive efficiency of large white boars during exploitation. Biotechnology in Animal Husbandry 31, 3, 397-405.
SAVIĆ R., RADOJKOVIĆ D., STOJILJKOVIĆ N., PARUNOVIĆ N., GOGIĆ M., RADOVIĆ Č. (2020): Effect of breed of performance tested boars on ejaculate traits. Biotechnology in Animal Husbandry 36, 3, 309-316.
SMITAL J. (2010): Comparasion of environmental variations in boar semen characteristics of six breeds and their crossbreds over an eight-year period. Research in Pig Breeding, 4, 1, 26-32.
STANČIĆ I. (2014): Reprodukcija domaćih životinja. Poljoprivredni fakultet, Novi Sad.
TERESZKIEWICZ K., POKRYWKA K. (2020): Results of breeding use of insemination boars of selected breeds in the seasons of the reproductive cycle of the European wild boar (*Sus scrofa* L.). Acta Sci. Pol. Zootechnica, 19, 2, 37–46.
WANG C., LI J.L., WEI H.K., ZHOU Y.F., TAN J.J., SUN H.Q., JIANG S.W., PENG J. (2017): Linear growth model analysis of factors affecting boar semen characteristics in Southern China1. Journal of Animal Science, 95, 12, 5339–5346.
WIERZBICKI H., GÓRSKA I., MACIERZYŃSKA A., KMIEĆ M. (2010): Variability of semen traits of boars used in artificial insemination. Medycyna Weterynaryjna, 66, 11, 765-769.
WILCZYŃSKA E., KONDRACKI S., WYSOKIŃSKA A., KOWALEWSKI D., GAJOWNIK K. (2013): The quality of boar semen of Polish Large White, Polish Landrace, Duroc and Pietrain breeds in different months of the year. Scientific Annals of Polish Society of Animal Production, 9, 1, 49-56.
WOLF J., SMITAL J. (2009): Effect in genetic evaluation for semen traits in Czech Large White and Czech Landrace boars. Czech J. Anim. Sci., 54, 8, 349-358.

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VARIABILITY OF THE NUMBER OF LIVE-BORN PIGLETS UNDER THE INFLUENCE OF FEMALE GENOTYPE, YEAR OF FARROWING AND PARITY

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Abstract: The aim of this study was to determine the influence of sow genotype, year of farrowing and parity on the number of live-born piglets. Fertility traits of sows were tested during fourteen years (from 2007 to 2020) in one pig herd/population. The study included 10159 sows, 4 genotypes: Landrace, large White, Landrace x large White and large White Yorkshire x Landrace. The sow fertility data set contained data on 36189 parities. Based on the obtained results, it was determined that the genotype of sows, year of farrowing and parity had a statistically highly significant (P <0.01) influence on the number of live - born piglets.

Key words: fertility, sows, genotype, parity, year.

Introduction

The achieved production results in pig breeding directly depend on a large number of economically important groups of traits. Two important traits, which affect the economic efficiency of pig production, are the reproductive and productive capacity of sows (*Wähner and Brüssow, 2009; Škorput et al. 2020*). The annual productivity of sows is determined by the size of the litter and the number of parities per sow per year. Increasing one parameter affects the increase in sow productivity (*Kosovac et al., 2005; Radojković et al., 2005; Popovac et al., 2012; Živković et al., 2018*).

The size of a sow litter can be described as the number of live-born, stillborn, total-born and weaned piglets. The number of weaned piglets is even more commercially important than the size of the litter at birth. However, due to the widespread technological procedure of uniforming-equalization of litters of different sows that were farrowed at approximately the same time, this trait is second in importance, right after the number of live-born piglets. (*Luković, 2006; Radojković, 2007)*.

The number of live-born piglets is influenced by numerous external and genetic factors, as well as their interactions. There are reported data on farms on many effects that can be included in the models. Data on pig fertility, which are recorded on modern industrial farms, give a satisfactory description of the effects on the number of live-born piglets. The effects that can be included in the models are: parity, year of farrowing, mating or farrowing season, genotype, sire of the litter or sire breed, age at farrowing and different reproductive cycle intervals that affect the number of live piglets, and therefore the overall efficiency of pig production. Research in the direction of analysis of variability of fertility traits of sows was performed by: *Luković (2006); Luković et al. (2006, 2007); Radojković (2007); Radojković et al. (2005, 2014, 2018); Popovac et al. (2012); Luković and Radojković (2013); Škorput et al. (2016); Živković et al. (2018); Freyer (2018).*

Given the above, the aim of this study was to determine the influence of sow genotype, age and parity on the variation of fertility traits.

Material and Methods

Fertility traits of sows were tested during fourteen years (from 2007 to 2020) in one pig herd/population. The study included 10159 sows, 4 genotypes of Landrace, Large White and F1 crossbreeds of these breeds. The data set contained 36189 records on sow fertility and the following variables: animal identification number, genotype of the litter sire and dam, date of mating, date of farrowing, parity, and number of live-born piglets.

The values of statistical indicators for phenotypic expression and variability of the tested trait were calculated by the method of least squares using the GLM procedure of the software package SAS (*SAS Inst., Inc., Cary, NC*), using the following model:

Model:

$$Y_{ijkl}=\mu+S_i+P_k+G_l+e_{ijkl}$$

where:

Y_{ijkl} - observation vector for litter size,
S_i - fixed influence of the year of farrowing,
P_k - fixed influence of parity,
G_l - fixed influence of litter genotype,
e_{ijkl}- random error.

The choice of systemic influences in the model is based on the significance of the influences, the coefficient of determination and the degrees of freedom. The results are presented as mean values obtained by the method of least squares (LSMEAN) or as deviations of LSMEAN values from the population average in the form of a graph.

Results and Discussion

Based on the obtained research results (Table 1), a high number of live-born piglets is observed, which indicates a high average fertility of sows in the analysed population. The high coefficient of variation for the analysed trait is a consequence of large differences in litter size of different sows. The presented result of the average value of the number of live-born piglets (16.16) is higher compared to the results presented by *Luković (2006) and Radojković (2007).*

Table 1. Descriptive statistics of the number of liveborn piglets in the analysed population

N	$\bar{x}$	SD	Min	Max	CV
36189	16.16	3.59	0.00	31.00	22.20

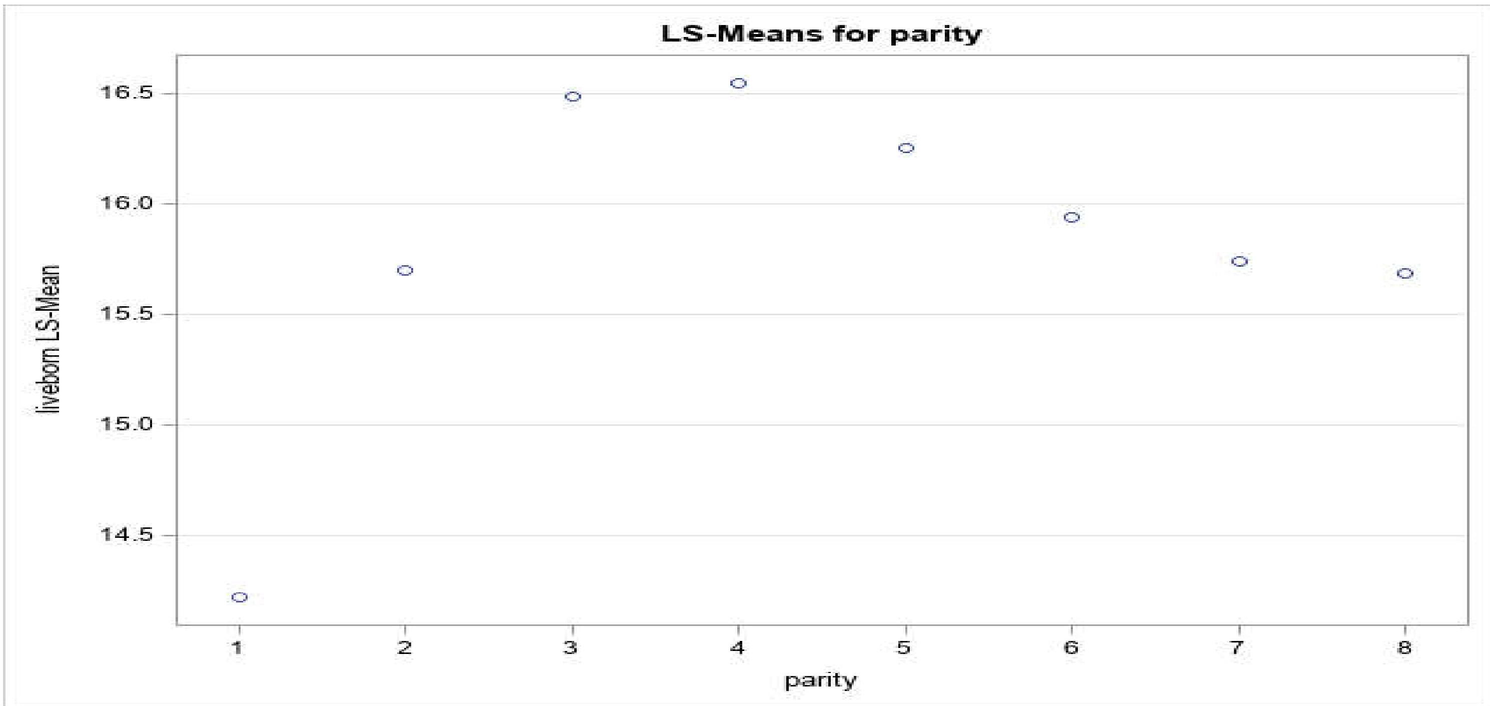
N- Number of litters, $\bar{x}$- mean value, ***SD***-standard deviation, ***Min***- minimum, ***Max***-maximum, ***CV***-coefficient of variation

The influence of genotype on the number of live-born piglets was highly statistically significant (Graph 2 and Table 2). Also, the parity and the year of farrowing had a highly statistically significant ($P<0.01$) influence on the size of the sow litter.

Table 2. Analysis of the significance of the influence in the model for the number of liveborn piglets in the analysed population

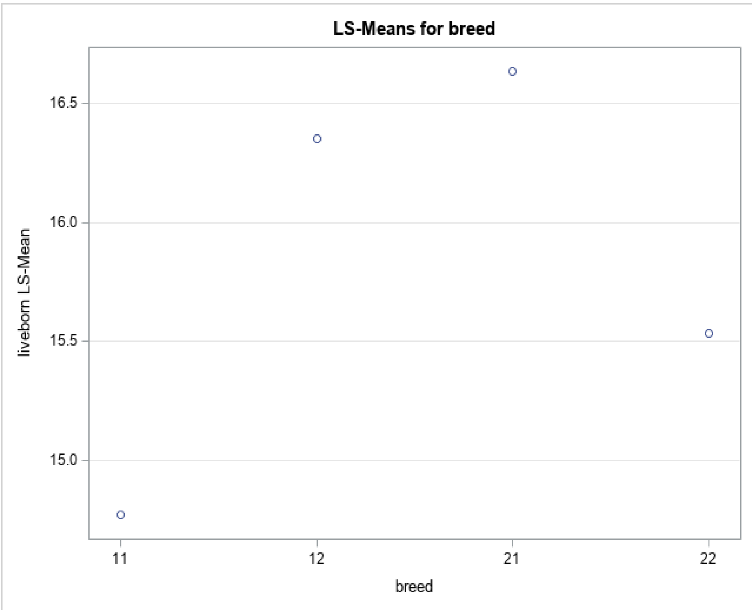
Source of variation	d.f.	MS	F	Pr>F
Genotype	3	778.23	79.89	<0.0001
Year	13	4905.91	503.60	<0.0001
Parity	7	3945.29	404.99	<0.0001

The highest number of live-born piglets in sow litters is observed in the third and fourth parity, after which a slight decline is observed (Graph 1). This is in accordance with reports by number of authors (*Kosovac et al., 2005; Luković and Radojković, 2013; Popovac et al., 2012; Škorput et al., 2016; Freyer, 2018)* who state that the highest numbers live-born piglets are achieved in the interval between the third and sixth farrowing, and after reaching maximum fertility in subsequent parities the value of this parameter slowly decreases. Such a result is based on the influence of sow age on litter size. Females grow until the end of the second year, when they reach their final size, and the size of their reproductive organs increases in that period, which leads to an increase in fertility.



Graph 1. Influence of parity on the number of live-born piglets

In the examined litter, the average number of live - born piglets varied between sow genotypes from 14.77 to 16.63 (Table 3). Differences in the average number of live-born piglets were highly statistically significant between all genotypes included in the analysis. The number of live-born piglets in a sow's litter can also be affected by whether the sows are purebred or are the result of crossbreeding (*Radojković, 2007*). When crossing different breeds, a heterosis effect can be manifested in a larger number of live-born piglets, which is confirmed in this analysis. Purebred sows had a lower number of live-born piglets compared to crossbreeds (Graph 2).



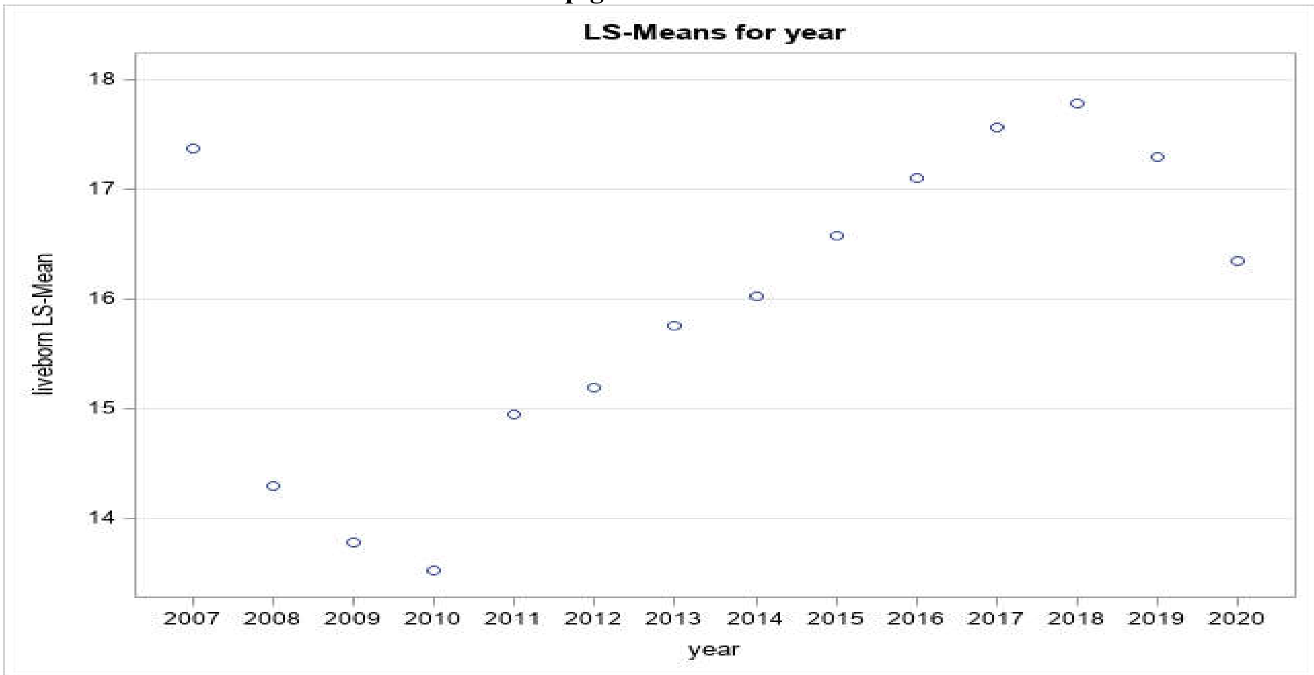
Breed	Live born	Pr > |t|
11	14.77±0.14	<0.0001
12	16.35±0.04	<0.0001
21	16.63±0.05	<0.0001
22	15.53±0.10	<0.0001

Graph 2. Influence of genotype on the number of live born piglets
11-Large White, **22**-Landrace, **12**- Large White x Landrace,
21- Large White x Landrace

Table 3. LSM ± S.E.value for the number of liveborn piglets

The influence of the year on the number of live-born piglets of sows and gilts is shown in Graph 2. Great variability in the number of live-born piglets between years was observed, which is contrary to *Savić et al. (2011*). The mentioned authors have not determined a statistically significant influence of the year of farrowing on the number of liveborn piglets. The influence of the year of farming usually explains the effect of environmental factors that prevailed in certain years, such as nutrition and various technological procedures during the rearing of females (*Radojković, 2007; Popovac, 2016)*. Also, this influence can include the effects of selection in successive overlapping generations, producing at the same time. This usually happens when the study is conducted for many years.

Graph 3. Influence of the year on the number of liveborn piglets



Conclusion

Based on the results obtained in this study, we can conclude that the genotype of sows, age and parity have a statistically highly significant (P<0.01) influence on the number of live-born piglets.

In the analysed population, the number of live-born piglets of large White sows was 14.77, Landrace 15.53, Landrace x Large White 16.63 and Large White x Landrace 16.35. Purebred sows had a smaller number of live-born piglets compared to crossbreeds. The highest number of live-born piglets in sow litters is observed in the third and fourth parity, after which a slight decline is observed. The variability in the number of live-born piglets between years is a consequence of the action of external factors and partly of the selection effect that is carried out over many years.

Varijabilnost broja živorođene prasadi pod uticajem genotpa plotkinja, godine prašenja i pariteta

Nenad Stojiljković, Dragan Radojković, Čedomir Radović, Marija Gogić, Vladimir Živković, Zoran Luković, Dubravko Škorput

Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj genotipa krmače, godine prašenja i pariteta - rednog broja prašenja na broj živorođene prasadi. Osobine plodnosti krmača ispitivane su tokom četrnaest godina (od 2007. do 2020. godine) u jednom zapatu svinja. Istraživanjem je obuhvaćeno 10159 krmača, 4 genotipa: Landras, Veliki Jorkšir, Landras x Veliki Jorkšir i Veliki Jorkšir x Landras. Set podataka o plodnosti krmača sadržao je podatke o 36189 prašenja. Na osnovu dobijenih rezultata utvrđeno je da genotip krmača, godina prašenja i paritet statistički visoko značajno (P<0,01) utiču na broj živorođene prasadi.

Ključne reči: plodnost , krmače, genotip, paritet, godina.

Acknowledgment

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References

FRAJER G. (2018): Maximum number of total born piglets in a parity and individual ranges in litter size expressed as specific characteristics of sows. Journal of Animal Science and Technology 60:13.

KOSOVAC O., PETROVIĆ M., ŽIVKOVIĆ B., FABJAN M., RADOVIĆ Č. (2005): Uticaj genotipa i prašenja po redu na variranje osobina plodnosti svinja. Biotechnology in Animal Husbandry, Institut za stočarstvo, Zemun 21 (3-4): 61-68.

LUKOVIĆ Z., RADOJKOVIĆ D. (2013): Dispersion parameters for litter size in pigs. International Symposium Modern Trends in Livestock Production, October 2013., 129-139.

LUKOVIĆ Z. (2006): Covariance functions for litter size in pigs using a random regression model. Doctoral Dissertation. University of Ljubljana, Biotechnical Faculty Ljubljana, Zootechnical Department, pp 1–93.

POPOVAC M. (2016): Fenotipska i genetska varijabilnost proizvodnih i osobina dugovečnosti krmača. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet Beograd, 1-178.

POPOVAC M., RADOJKOVIĆ D., PETROVIĆ M., MIJATOVIĆ M., GOGIĆ M., STANOJEVIĆ D., STANIŠIĆ N. (2012): Heritability and connections of sow fertility traits. Biotechnology in Animal Husbandry, 28 (3): 469-476.

RADOJKOVIĆ D. (2007): Varijabilnost osobina plodnosti i procena priplodne vrednosti svinja. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet Beograd, 1-208.

RADOJKOVIĆ D., PETROVIĆ M., MIJATOVIĆ M. (2005): Estimation of genetic variability of fertility traits of pigs. Biotechnology in Animal Husbandry, 21 (3-4): 93-97.

SAS Inst. Inc. (2011): The SAS System for Windows, Release 9.4. Cary, NC.

SAVIĆ R., PETROVIĆ M., RADOVI Č. (2011): Estimation of heritability coefficients of number of born alive piglets in the first three farrowings Swedish Landrace sows. Biotechnology in Animal Husbandry, 7 (1): 85- 92.

ŠKORPUT D., TORČEK I., MENČIK S., MAHNET Ž., KLIŠANIĆ V., KAROLYI D., LUKOVIĆ Z., SALAJPAL K. (2020): Čimbenici plodnosti krmača banijske šare svinje. 55th Croatian & 15th International Symposium on Agriculture, February 16-21, 2020, Vodice, Croatia, 389-393.

ŠKORPUT D., SMETKO A., KLIŠANIĆ V., ŠPEHAR M., MAHNET Ž., LUKOVIĆ Z. (2016): Mogućnosti selekcije na veličinu legla u crne slavonske svinje. Proceedings of 51st Croatian and 11th International Symposium on Agriculture, Opatija, Croatia, February 2016, 368-370.

WÄHNER M., BRÜSSOW K. P. (2009): Biological potential of fecundity of sows. Biotechnology in Animal Husbandry, 25 (5-6): 523-533.

EFFECTS OF INVESTMENTS IN CAPITAL CROP PRODUCTION - A COMPARATIVE ANALYSIS OF THE REPUBLIC OF CROATIA AND THE EUROPEAN UNION

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Abstract: Creating preconditions for economic development nowadays is unthinkable without investments. From the economic aspect, investments affect the turnover of capital, which creates material and technical preconditions for local development. It is noticeable that in practice, investments are given importance only in the theoretical sense, in other words, they are talked about a lot and speculated in the media. Therefore, it is necessary to improve business in terms of modernization of tangible assets, all to create new values, as a necessary prerequisite for further survival. The paper is thematically focused on the importance of investments and their direction in the improvement of the production process, particularly the introduction of innovations and the importance of modernization of production. The increase in the amount of investment on the one hand is determined by the increase in capital equipment, but on the other hand it must be accompanied by investment spending. That is why the paper aims to show the economic importance of investing in technologies that modernize the production process, which in the long run contributes to a stronger competitive position.

Key words: investments, crop production, capital intensive production, tangible assets, production volume

Introduction

The production process is the basis of any industrial production and includes all activities and actions that result in the conversion of input materials (raw materials, semi-finished products) into a finished product. It also includes all assets and personnel on which and with which activities are performed from the warehouse of input material to the warehouse of finished products. It consists of: technological process, transport process, organization process, and information process, so it is an indivisible whole of technology, technology, organization, and economy *(Mikac and Blažević, 2007)*. The concept of capital-intensive agricultural production implies the elaboration of the following elements *(Selaković, 1994)*:

• Production is a spatially and temporally determined process of conscious action of several factors, the result of which is the creation of material goods and services.

• The production process is the basis of any production and implies the process of converting input materials (raw materials, semi-finished products) into a finished product.

• Production goals imply the realization of the planned quantities of products from the production program.

• Technological process refers to the change of appearance, shape, dimensions, and properties of materials, change of physical and chemical properties.

• Production system is defined as an organizational form that integrates a group of different functions as subsystems necessary for the realization of the production process.

• The business system includes all the resources and activities necessary to achieve a particular business goal. Business success is reflected in the ratio of income and expenses.

• Information is a unit of measure of an organization, it represents everything that acts as an entrance to an organizational node, subsystem, or system, and its manifestations are always related to the material or energy that are its carriers, but not its content.

• The information system aims to collect and process information that is put into the function of production.

• Production organization is any connection and harmonization of an activity and solving the problems that arise from it, with the purpose of achieving common goals, for example raising performance. Organization implies both the process of organization and the formal structure that results from that process. The task of the organization of production is the temporal and spatial harmonization of the elements of production: human labour, available resources and means of labour.

Thus, the process of capital-intensive agricultural production does not consist only of simple physical activities, but also involves the application of teamwork and modern systems *(Bell and Senge, 1980)*. Capital-intensive production is inconceivable without investment. Investments have an important place in the application of this concept. The importance of investment is reflected primarily in the following *(Jones, 2007)*:

• ensure the continuity of production, and thus economic growth,

• provide extended reproduction,

• create material and technical conditions for the improvement of living standards,

• contribute to maintaining economic stability at the local level.

Investment management includes a set of all activities undertaken to effectively bring the investment process to a final conclusion. Investing as a process contains two very important and interrelated dimensions. These are time and uncertainty *(Hirt and Block, 2005)*. The time period that elapses from the appearance of the idea of need for investment to the realization of the investment, such as putting the investment into operation is usually very long. The time period of investment and uncertainty are directly correlated *(Pike, 2009)*. Longer investment times cause more uncertainty. Given its complexity, investing is a process that can cause negative effects due to wrong decisions *(Litterman, 2003)*. Therefore, investing cannot and must not be a spontaneous process, because it necessarily produces a situation in which wrong decisions are completely certain *(Sisek, 2005)*. The damage that can occur due to a spontaneous investment process or even mismanagement of investments is not limited to the company that leads the investment process, but can be extended to partners and the wider community.

In capital-intensive crop production, there is no need to plan to increase the area and intensity of resource use. On the contrary, we should insist on development in terms of increasing sustainability, productivity, and quality to optimally use and protect available resources *(Vukadinović and Jović, 2012)*. In the continuation of the research, the volume of investments into tangible assets will be considered. Investments into tangible assets can include, among other things, investments in the regulation of irrigation and land reclamation systems of agricultural land as an important factor in crop production. In addition to irrigation itself, it is necessary to take into account agro-technical measures which significantly increase the amount of humus in depleted soils, and thus the absorption capacity of the soil. Furthermore, energy efficiency and long-term competitiveness of agricultural producers can significantly support the diversification of energy sources, i.e. the use of biodiesel from their production capacities. And finally, investing in fixed assets, facilities, work equipment, and sophisticated equipment is very important because in this way a better production performance is achieved. All listed investments are statistically determined as tangible assets.

The aim of the paper was to show the economic importance of investing in technologies that modernize the production process, which in the long run contributes to higher production with a tendency to reduce total costs, while creating the preconditions for more competitive production.

Materials and Methods

The following table 1 shows the values of total production and investment into tangible assets. The data refer to the Republic of Croatia and the European Union (EU) and are expressed in millions of euros.

Table 1. Volume of production and investments into tangible assets in the Republic of Croatia and the EU (Prepared by the author based on data from the Central Bureau of Statistics of the Republic of Croatia; *DZS, 2020, Eurostat, 2020*)

Year	Republic of Croatia		European Union	
	Production volume	Investments	Production volume	Investments
2016	2.165.997.833	371.343.521	55.448.697.787	10.189.096.313
2017	2.311.802.514	299.566.871	57.862.355.002	11.996.353.245
2018	2.356.531.661	314.387.844	58.402.996.404	12.105.347.012
2019	2.278.756.551	347.554.226	59.640.428.902	12.401.994.338
2020	2.543.046.357	336.887.417	59.814.695.714	12.922.600.004

The data from the table above show the realized volume of field (plant) production in the Republic of Croatia and the EU, and investments directed to field production. In the Republic of Croatia, on average, 14.32% of the realized production volume is invested in new fixed assets (working stands), construction facilities, irrigation systems, agro-technical measures and renewable energy sources, while the value of investments for this purpose in the EU is 20, 87%.

Results and Discussion

The values expressed in Table 1 show that in the EU the volume of crop production and investments have a continuous growth, i.e. a higher volume of production for each subsequent year, is encouraged by a larger volume of investments, while in the Republic of Croatia the situation is diverse. On average, EU farmers invest 6.15% more of their earnings in new capital values compared to agricultural producers from the Republic of Croatia, which results in an increase in the total mass produced. Based on the correlation coefficient, it is possible to express the strength of the connection between the production concept in the Republic of Croatia and the EU. The ratio of production and investment in the Republic of Croatia is negative, i.e. the correlation is -0.39, which indicates a weak and negative relationship. In the Republic of Croatia, the concept of labour-intensive production is more represented, i.e. the labour force is significantly represented in the process of field production. The situation is quite the opposite in the EU, the data from Table 1 show a moderate positive relationship of 0.97, which indicates the conclusion that new capital is continuously invested, which creates new production values in the following cycles of reproduction. For large economic organizations in which capital-intensive production dominates, cost reimbursement is of great importance due to the structure of their total costs, because in such companies fixed costs have a significant share in total costs. "Fixed costs increase with increasing depreciation, i.e. increasing the capital intensity of the company, while variable costs decrease. Hence the division into labour-intensive companies and capital-intensive companies." *(Belak, 1995)*. The dependence of investments on the volume of production is calculated by the linear trend equation:

$$y = a + bx$$

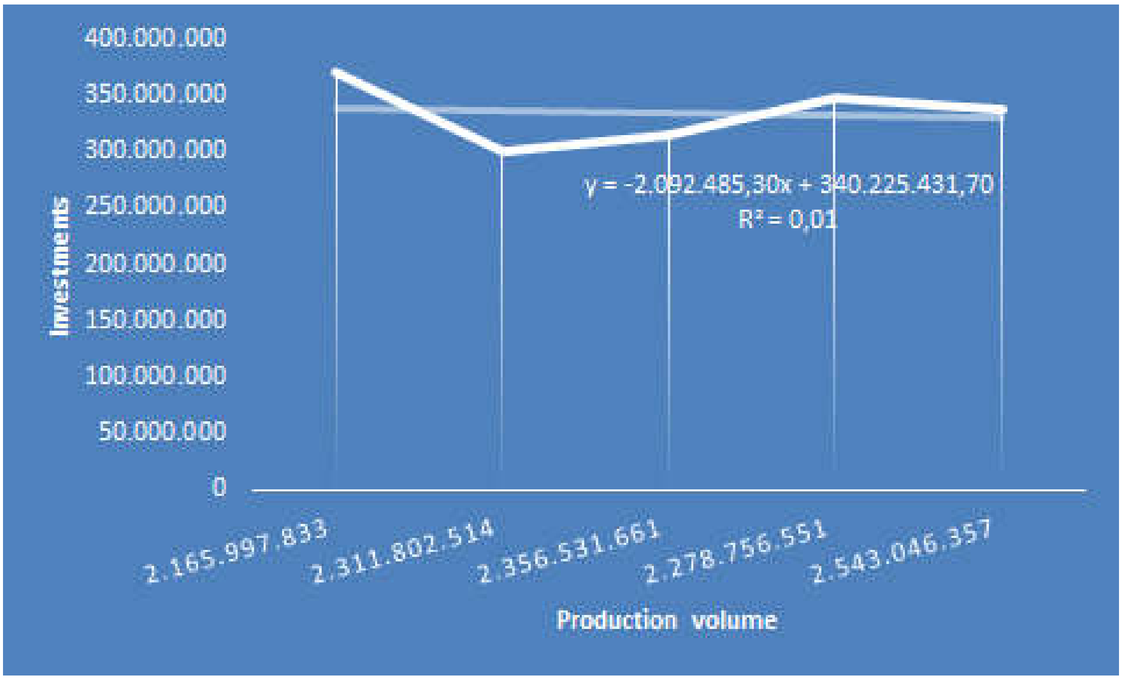

Figure 1. Linear trend of investment dependence on production volume in the Republic of Croatia (developed by the author based on the data from Table 1)

Based on the Figure 1 it could be observed that if the volume of production increased by 1, we can expect a decrease in investment by - 2,092,485.30. Also, 10% of the relationship between production volume and investment is explained by projected linear model.

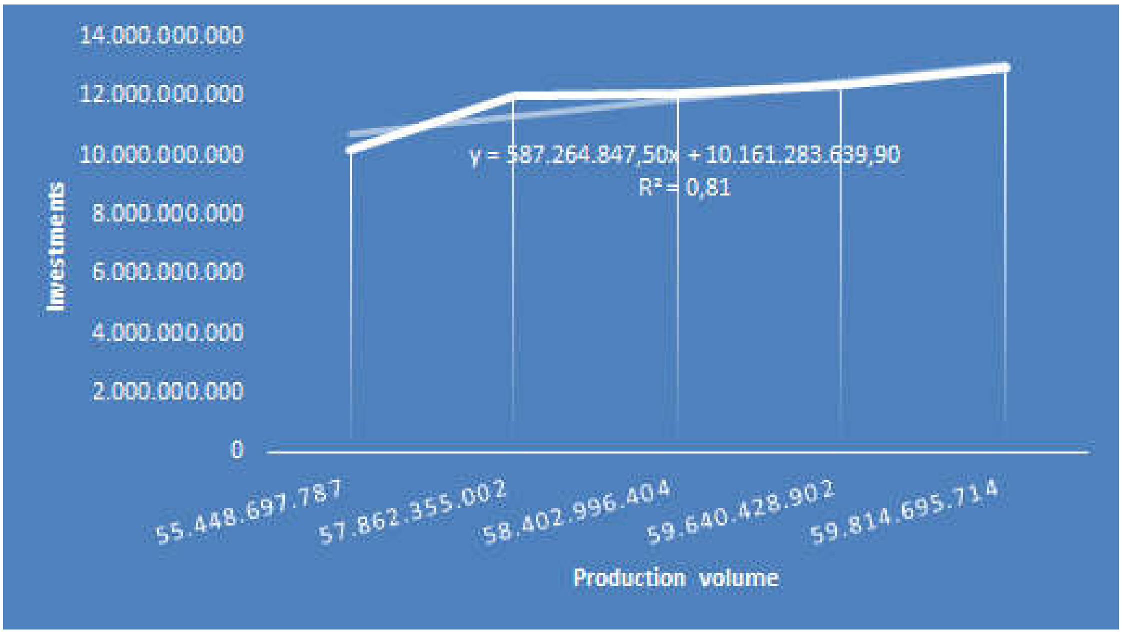

Figure 2. Linear trend of investment dependence on the volume of production in the EU (developed by the author based on the data from Table 1)

Based on the trend presented at the Figure 2 it could be observed that if the production volume increased by 1, we can expect an increase in investment by 587,264,847.50. Also, 81% of the relationship between production volume and investment is explained by used linear model.

Graphic results show that agricultural producers in the EU, unlike agricultural producers in the Republic of Croatia, maintain a positive proportion according to the current concept of the ratio of the volume of field production and investment in tangible assets. Any additional increase in production volume will result in an increase in investment. The trend of the ratio of the volume of field production and investment in tangible assets in the Republic of Croatia shows a discrepancy, because although the volume of production increases, the volume of investment decreases. The reasons can be multiple, but in general such a situation can be attributed to a large fiscal burden in relation to disposable income.

Conclusion

Investments are a generator of economic growth. However, if there is no favourable market climate, ie if monetary and fiscal policies are not implemented in the function of creating stimulating investment conditions, investments do not have sufficient strength to cause positive market shifts. In the conducted research, this is shown by the results in the Republic of Croatia. Capital-intensive production requires the engagement of machines, equipment and sophisticated technological production systems in the production process, on a larger scale than simple work. Capital-intensive production requires a higher level of investment, a higher amount of funds and financial resources. The capital-intensive production process is mostly automated and as such is able to generate a continuous increase in production volume. Since capital-intensive production relies mainly on machinery and equipment, such a concept requires long-term investments that pay off over the years. This statement is also supported by the results of the research, which is visible for the EU.

Efekti ulaganja u proizvodnju kapitalnih useva - uporedna analiza Republike Hrvatske i Evropske Unije

Dragan Dokić, Maja Gregić, Mirna Gavran, Vesna Gantner

Rezime

Stvaranje preduslova za ekonomski razvoj danas je nezamislivo bez ulaganja. Sa ekonomskog aspekta, investicije utiču na promet kapitala, što stvara materijalno-tehničke preduslove za lokalni razvoj. Uočljivo je da se u praksi ulaganjima daje značaj samo u teorijskom smislu, drugim rečima, o njima se mnogo priča i spekuliše u medijima. Zbog toga je potrebno unaprediti poslovanje u smislu modernizacije materijalne imovine, a sve radi stvaranja novih vrednosti, kao neophodnog preduslova za dalji opstanak. Rad je tematski fokusiran na značaj ulaganja i njihov smer u poboljšanju proizvodnog procesa, posebno na uvođenje inovacija i značaj modernizacije proizvodnje. Povećanje iznosa ulaganja, s jedne strane, određeno je povećanjem kapitalne opreme, ali s druge strane mora biti popraćeno investicionom potrošnjom. Zato rad ima za cilj da prikaže ekonomski značaj ulaganja u tehnologije koje modernizuju proizvodni proces, što dugoročno doprinosi jačanju konkurentske pozicije.

Ključne reči: investicije, ratarska proizvodnja, kapitalno intenzivna proizvodnja, materijalna sredstva, obim proizvodnje

References

BELAK V. (1995): Menadžersko računovodstvo. RRiF Zagreb, pp. 207.
BELL J., SENGE P. (1980): System dynamics and scientific method. In: Randers J. Waltham editor. Elements of the system dynamics method Williston, VT: Pegasus Communications, 141-145.
DRŽAVNI ZAVOD ZA STATISTIKU, DZS (2020): https://www.dzs.hr
EUROSTAT (2020): https://ec.europa.eu
HIRT G., BLOCK S. (2005): Managing Investments, McGraw-Hill, New York, pp. 91.
JONES C.P. (2007): Investments. John Wiley & Sons, Hoboken, pp. 88.
JOVANOVIĆ P. (2003): Upravljanje investicijama. Grafoslog Beograd, Beograd, pp. 15.
LITTERMAN B. (2003): Modern Investment Management. John Wiley & Sons, Hoboken, New York, pp. 61.
MIKAC T., BLAŽEVIĆ D. (2007): Planiranje i upravljanje proizvodnjom. Tehnički fakultet Rijeka, pp. 13.
PIKE R. (2009): Corporate finance and investment. Pearson Education, Harlow, pp. 148.
SELAKOVIĆ M. (1994): Organizacija poslovnih sistema. Tehnički fakultet Rijeka, Rijeka, 135- 149.
SISEK B. (2005): Strane izravne investicije u Hrvatskoj – uzroci neuspjeh. Zbornik Ekonomskog fakulteta u Zagrebu, Zagreb, 93-97.
VUKADINOVIĆ P., JOVIĆ P. (2012): Investicije. Univerzitet Singidunum, Beograd, 5-27.

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MYCOPOPULATION OF ALFALFA AND RED CLOVER HAY IN SERBIA

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Abstract: Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) belong to the family *Fabaceae* (*Papilionaceae*). Alfalfa and red clover have a special place in the crop rotation. After plowing the areas under alfalfa and red clover, large amounts of nitrogen and organic matter remain in the soil, whose decomposition and mineralization improve the physical, chemical and microbiological properties of the soil. So far, there have been no more detailed researches of the mycoflora of alfalfa hay and red clover in Serbia. In this paper, we present the results of preliminary research on the mycopopulation of 200 samples of alfalfa and red clover hay. A total of 4 genera of fungi were isolated from alfalfa and red clover hay, namely: *Fusarium, Phoma, Rhizoctonia and Verticilium.*

Key words: alfalfa, red clover, mycopopulation

Introduction

Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) belong to the order *Fabales* (*Leguminosae*), the family *Fabaceae* (*Papilionaceae*) and the genus *Medicago* and *Trifolium*, respectively (*Kojić, 1984; Yli-Mattila et al., 2010*).

Considering field crops used as livestock feed, alfalfa has the most important role so it is refered as the "queen of fodder plants". As one of the oldest and the most important perennial fodder plants, alfalfa gives a high yield of fodder of excellent quality.

Alfalfa and red clover have a special place in the crop rotation. After plowing of areas under alfalfa and red clover, large amounts of nitrogen and organic matter remain in the soil. The decomposition and mineralization of this organic matter improve the physical, chemical and microbiological properties of the soil (*Tapia et al., 2005; Yli-Mattila et al., 2010*).

Alfalfa and red clover are usually grown independently, although they can also be used as components of grass-legume mixtures. Alfalfa and red clover hays are rich in proteins, with excellent amino acid composition and high digestibility (*Tapia et al., 2005; Yli-Mattila et al., 2010*). Alfalfa hay proteins are the cheapest source of protein in animal feed (*Ocokoljić et al., 1983*). In Serbia, alfalfa is the most important fodder perennial legume and is grown on about 190,000 ha. In the years of full exploitation, alfalfa can achieve high yields of green mass (70-90 tha^{-1}) and about 20 t ha^{-1} of dry matter in four to five cuts per year (*Lugić and Dinić, 2010*). Important regions for its cultivation in our country are: Vojvodina, especially Banat (Vršac, Kikinda, Zrenjanin), Bačka (Bečaj, Subotica, Sombor, Senta, Kula, Odžaci) and Srem (Sremska Mitrovica, Šid, Ruma, Stara Pazova). In central Serbia, the most significant alfalfa production areas are in Posavina: Mačva, Pomoravlje, Stig, Šumadija and Timočka Krajina (*Lukić, 2000*).

Red clover (*Trifolium pratense* L.) is a very important fodder crop. It is especially grown where the soil is not suitable for growing alfalfa. Red clover is considered a short-term fodder crop due to it lasting 2 to 4 years. It tolerates moist and acidic soils better than alfalfa. It is also important to note that red clover has less resistance to various diseases compared to other forage crops (*Yli-Mattila et al., 2010*).

Alfalfa and red clover are very important and productive fodder crops in agriculture, but like all crops, they are susceptible to infectious diseases that can limit their production. Disease control of these crops is very important in order to achieve higher economical production of protein-rich livestock feed.

Alfalfa and red clover diseases can reduce yields and quality. Also, diseases can reduce lifespan of composition of these crops. Sometimes, due to the disease, there can be a sudden deterioration of alfalfa and clover, which causes irreparable damage in productions to farmers.

Relatively low yields of alfalfa and red clover in Serbia are certainly a consequence of the impact of inadequate agricultural techniques and, sometimes, utilization of inadequate soil, but also of the occurrence of pests and plant pathogens (*Mijušković, 1993; Yli-Mattila et al., 2010*). Many diseases are characterized by plant death, which reduces the yield and quality of these crops. Disease pathogens attack individual parts or the whole plant in various developmental phenophases (*Vučković, 1999; Yli-Mattila et al., 2010*). For many years, the reduced longevity of alfalfa and red clover crops has been one of the main problems in the production of these fodder crops around the world. Frost damage, root mycosis, and bacterial wilt are all common factors that cause this problem (*O'Rourke and Millear, 1966; Yli-Mattila et al., 2010*).

The aim of this research was to determine phytopathogenic fungi on alfalfa and red clover hay, with the task to clarify causes of reduced quality and yield of these forage crops, as well as premature decay of their composition.

Material and Methods

Alfalfa and red clover hay was sampled on the territory of the Republic of Serbia in Rasina district (Globoder, Gaglovo, Selo Varvarin, Kobilje, Veliki Šiljegovac) and Pomoravlje district (Bobovo, Gložane, Tropanje) in the period of 2017 and 2018. Samples were taken by cutting off parts of alfalfa hay and red clover. The samples obtained in that way were carefully washed under running water. After rinsing, stems were carefully cut into 0.5 to 1 cm long pieces. The prepared stem sections were disinfected with 96% ethanol solution for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 min and then washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. Five pieces of the plant parts (roots and stems) were placed in individual Petri dishes in four replications. They were kept in a thermostat at 25 °C in 12 h light / 12 h night regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial growth, the peak part of the mycelium was reseeded on PDA again.

Microscopic examination was performed using microscope Olympus CX31. Morphological identification of fungi to the genus was carried out using a standard key. The frequency of isolation in % was calculated according to the formula by *Vrandečić et. al. (2011)*:

$$\text{(\%) Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results

In this study, mycopolation on alfalfa and red clover hay was monitored in the period from 2017 to 2018. On the territory of the Republic of Serbia, a total of 200 samples of alfalfa and red clover hay were collected in two districts: Rasina district (Globoder, Gaglovo, Selo Varvarin, Kobilje, Veliki Šiljegovac) and Pomoravlje district (Bobovo, Gložane, Tropanje). Out of a total of 200 samples, 160 samples of alfalfa and red clover hay developed a fungal colony, while 40 samples did not develop any fungal colonies (Table 1). A total of 120 alfalfa samples were tested, of which 92 samples developed fungal colonies and 28 alfalfa hay samples did not. Out of 80 tested samples of red clover hay, 68 samples developed fungal colonies and 12 samples did not.

Fungi from the genera *Fusarium, Phoma, Rhizoctonia* and *Verticilium* were isolated from alfalfa and red clover hay (Table 1).

Table 1. Frequency of fungal isolation on alfalfa and red clover on the hay

District	Year of sampling	Number of samples Plant part - stem	Fungal species - stem	(%) Isolation frequency
Red clover – Globoder	2017	20	*Fusarium* sp. *Phoma* sp.	60 35
Red clover – Gaglovo	2017	20	*Fusarium* sp. *Rhizoctonia* sp. *Phoma* sp	15 40 20
Red clover – Selo Varvarin	2018	20	*Fusarium* sp. *Verticillium* sp. *Phoma* sp.	20 60 15
Red clover – Kobilje	2018	20	*Fusarium* sp. *Verticillium* sp. *Phoma* sp.	15 40 20
Alfalfa - Gložane	2017	20	*Fusarium* sp. *Rhizoctonia* sp.	65 20
Alfalfa - Kobilje	2017	20	*Fusarium* sp. *Phoma* sp.	55 20
Alfalfa - Globoder	2017	20	*Fusarium* sp. *Phoma* sp.	45 40
Alfalfa - Bobovo	2018	20	*Verticillium* sp. *Rhizoctonia* sp.	45 40
Alfalfa - Veliki Šiljegovac	2018	20	*Fusarium* sp.	60
Alfalfa - Tropanje	2018	20	*Fusarium* sp.	70

A difference in the frequency of isolations of individual genera of fungi was noticed in these researches, depending on the site from which alfalfa and red clover hay originates.

Table 1 clearly shows that fungi from the genus *Fusarium* were present in all examined site, except in the site Bobovo - Pomoravlje district. Namely, representatives of the genus *Fusarium* were not isolated on alfalfa hay from this site, but only isolates belonging to the genera *Verticilium*. and *Rhizoctonia* were found. It can be concluded that the representatives of the genus *Fusarium* are most often isolated from alfalfa and red clover hay in almost all examined sites on the territory of the two districts of Rasina and Pomoravlje (Table 1). Fungi of the genus *Rhizoctonia* were isolated from the hay of red clover from the site Gaglovo - Rasina district, as well as from the hay of alfalfa at the location Gložane and Bobovo - both belong to the Pomoravlje district. Out of 10 examined sites, fungi of the genus *Phoma* were isolated from alfalfa and red clover hay samples at 6 localities (Table 1).

Discussion

Genera *Fusarium*, *Rhizoctonia*, *Phoma* are dominant in annual and perennial legumes worldwide (*Tivoli et al., 2006; Villegas-Fernández and Rubiales, 2011; Salam et al., 2011; Sillero and Rubiales, 2014, Vasić et al., 2015, Vasić et al., 2017*). *Al-Jaradi et al. (2018)* in Oman detected *Fusarium equiseti* on *Phaseolus vulgaris*.

Lukezić (1973), in his research, did not isolate *Colletotrichum trifolii* from alfalfa stem 90 days after harvest, most likely due to high humidity which favors the development of other pathogens. He isolated a large number of *Fusaurium* sp. from the same alfalfa hay samples, which is in accordance with the results of these studies.

Krnjaja et al. (2004) in their research divided the mycopopulation on seeds of 3 varieties of red clover K9, K17 and NS Kolubara, and two seed treatments - rinsing and surface disinfection (T1) and surface disinfection of seeds (T2). They found that the frequency of species of the genus *Fusarium* sp. varied from 0 to 13%, considering the presence of *Fusarium* sp. on the seeds of any of the tested varieties. Using T2 treatment, the incidence of *Fusarium* species varied from 1 to 13%, depending on the variety.

Vasić et al. (2011) studied mycopopulation on alfalfa seed. In the pathogenicity test, treated *P. vulgaris* showed yellowing symptoms with presence of lesions and root rot on the taproot. *F. equiseti* is one of the causal agents of foot and root rot disease which infects *Phaseolus vulgaris*, *Pisum sativum* and other crops.

Miličević et al. (2013), in Croatia, determined two *Fusarium* species, *F. verticillioides* and *F. proliferatum* on bean seeds. So, for these reasons, it is recommended to utilize crop rotation of four years, when it comes to the sowing of faba bean and pea (*Salam et al., 2011*). *Salam et al. (2011*) also cited *Phoma medicaginis* var. *pinodella* as significant pathogen in pea. *Rhizoctonia solani* Kühn is soil parasite that can cause serious problems in many legumes, especially on faba bean (*Assunção et al., 2011*). In Canada, 304 faba bean genotypes were tested on the resistance to *R. solani* and only five of them were identified with high resistance (*Rashid and Bernier, 1993*). *Al-Jaradi et al. (2018*) also isolated *Rhizoctonia solani* from *Vigna unguiculata* in Oman.

Ligoxigakis et al. (2002) determined *V. dahliae* in Greece as a parasite on beans and other legumes.

Rhizoctonia solani Kühn is a soil parasite that can cause serious problems on many legumes especially on beans (*Assunção, 2011*). In Canada, 304 bean genotypes were tested for resistance to *R. solani* and only five were identified with high resistance (*Rashid and Bernier, 1993*). *Ligoxigakis et al. (2002*) determined *V. dahliae* in Greece as a parasite on beans and other legumes.

According to *Tegegn, (2017)* taproot*,* chocolate spot reduces yield by up to 61%, with the presence of the probability of complete crop failure due to the disease. *Tegegn, (2017)* reported that rust can incur a maximum yield loss of up to 21%. So far, the control of these diseases had been attempted through the use of improved varieties, cultural practices, chemical fungicides and integration of two or more of the above options in Integrated Disease Management (IDM) scheme.

In these researches, a difference in the frequency of isolation of individual genera of fungi was noticed, depending on the locality from which alfalfa and red clover hay originates.

Conclusion

This paper presents the preliminary results considering mycopopulations on alfalfa and red clover hay, originating from two districts in Serbia - Rasina and Pomoravlje. Alfalfa and red clover are very important fodder crops for the production of hay and silage for livestock feed in our country. These researches are just the beginning of the research of phytopathogenic fungi on alfalfa and red clover hay.

Alfalfa and red clover are important forage crops and their importance as a livestock feed is growing within our country. This research is the beginning of a more comprehensive study of phytopathogenic fungi on alfalfa and red clover hay. So far, there were no significant researches in this direction in Serbia, so the future researches related to the hay of alfalfa and red clover will go in the direction of selection of genotypes with increased tolerance to fungal diseases.

Mikopopulacija na senu lucerke i crvene deteline u Srbiji

Sanja Živković, Tanja Vasić

Rezime

Lucerka (*Medicago sativa* L.) i crvena detelina (*Trifolium pratense* L.) pripadaju familiji *Fabaceae* (*Papilionaceae*). Lucerka i crvena detelina imaju posebno mesto u plodoredu. Nakon razoravanja površina pod lucerkom i crvenom detelinom u zemljištu ostaju velike količine azota i organskih materija, čijim se razlaganjem i mineralizacijom popravljaju fizičke, hemijske i mikrobiološke osobine zemljišta. Detaljnijih istraživanja mikoflore sena lucerke i crvene deteline u Srbiji do sada nije bilo. U ovome radu iznosimo rezultate preliminarnih istraživanja mikopopulacije 200 uzoraka sena lucerke i crvene deteline. Iz sena lucerke i crvene deteline ukupno je izolovano 4 roda gljiva, i to: *Fusarium*, *Phoma, Rhizoctonia* i *Verticilium*.

Ključne reči: lucerka, crvena detelina, mikopopulacija

References

ASSUNÇĂO I. P., NASCIMENTO L.D., FERREIRA M.F.; OOLIVIRA F.J., MICHEREFF S.J., LIMA G.S.A. (2011): Reaction of faba bean genotypes to *Rhizoctonia solani* and resistance stability. Horticultura Brasileira, 29, 492-497.
AL-JARADI A., AL-MAHMOOLI I., JANKE R., MAHARACHCHIKUMBURA S., AI-SAADY N., AL-SADI A.M. (2018): Isolation and identification of pathogenic fungi and oomycetes associated with beans and cowpea root diseases in Oman. PeerJ 6:e6064. http://doi.org/10.7717/peerj.6064
KOJIĆ M. (1984): Botanika. Naučna knjiga, Beograd. pp 519.
KRNJAJA V., LEVIĆ J., IVANOVIĆ M., TOMIĆ Z., MRFAT-VUKELIĆ S. (2004): Incidence of *Fusarium* species on red clover seed. Biotechnology in Animal Husbandry, 20, 1-2, 101-108.
LIGOXIGAKIS E. K., VAKALOUNAKIS D. J., THANASSOULOPOULOS C. C. (2002): Host range of *Verticillium dahliae* in cultivated species in Crete. Phytoparasitica, 30, 2, 141-146.

LUGIĆ Z., DINIĆ B**.** (2010): Proizvodnja, konzervisanje i iskorišćavanje kabaste stočne hrane kao osnovni uslov održivog razvoja stočarstva. Zbornik radova simpozijuma "Tradicija i budućnost stočarstva u brdsko planinskom području sa posebnim osvrtom na sjeničko peštersku visoravan", 22-24. jun, Sjenica, 20-49.
LUKEZIĆ F.L. (1973): Dissemination and survival of *Colletotrichum trifolii* under field conditions. Phytopathology, 64, 57-59.
LUKIĆ D. (2000): Lucerka. Naučni institut za ratarstvo i povrtarstvo, Novi Sad.
MILIČEVIĆ T., KALITERNA J., IVIĆ D., STRIČAK A. (2013): Identification and occurrence of *Fusarium* species on seeds of common vetch, whithe Lupine and some wild legumes. Agriculture, 19, 25-32.
MIJUŠKOVIĆ M. (1993): Najčešće mikoze lucerke u Crnoj Gori. Poljoprivreda i šumarstvo, XXXIX, 3-4, 55-64.
OCOKOLJIĆ S., MIJATOVIĆ M., ČOLIĆ D., BOŠNJAK D., MILOŠEVIĆ P. (1983): Višegodišnje leptirnjače. Prirodni i sejani travnjaci. Nolit, 118-141.
O'ROURKE C.J., MILLEAR R.L**.** (1966): Root rot and root microflora of alfalfa as affected bz potassium nutrition. Frequencz of cutting, and leaf infection. Phytopathology, 56, 1040-1046.
RASHID K. Y., BERNIER C. C. (1993): Genetic diversity among isolates of *Rhizoctonia solani* and sources of resistance in *Vicia faba*. Canadian Journal of Plant Pathology, 15, 23-28. https://doi.org/10.1080/07060669309500845
SALAM M.U., DAVIDSON J.A., THOMAS G.J., FORD R., JONES R.A.C., LINDBECK K. D., MACLEOD W. J., KIMBER R.B.E., GALLOWAY J., MANTRI N., van LEUR J.A. G., COUTTS B.A., FREEMAN A.J., RICHARDSON H., AFTAB M., MOORE K.J., KNIGHTS E.J., SILLERO J.C., ROJAS-MOLINA M.M., EMERAN A.A., RUBIALES D. (2011): Rust resistance in faba bean. Grain Legumes, 56, 27-28.
SILLERO J.C., RUBIALES D. (2014): Response of *Vicia* species to *Ascochyta fabae* and *Uromyces viciae-fabae*. Czech J. Genet. Plant Breed., 50, 109-115.
TAPIA S., PARDO F., PERICH F., QUIROZ A. (2005): Clover root borer *Hylastinus obscurus* (Marsham) (Coleoptera: Scolytidae): has no preference for volatiles from root extracts of disease infected red clover. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 55, 158-160.
TEGEGN ADDISU (2017): Effect of aerated and non-aerated compost steepages on the severity and incidence of major fungal diseases of faba bean; *Botrytis fabae*, *Uromyces vicia fabae* and *Ascochyta fabae*. Plant, 5, 6, 85-92. doi: 10.11648/j.plant.20170506.11
TIVOLI B., BARANGER A., AVILA C.M., BANNIZA S., BARBETTI M., CHEN W.D., DAVIDSON J., LINDECK K., KHARRAT M., RUBIALES D., SADIKI M., SILLERO J.C., SWEETINGHAM M., MUEHLEAUER F.J. (2006):

Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica, 147, 223-253.

VASIĆ T., ANĐELKOVIĆ S., ŽIVKOVIĆ S., ANĐELKOVIĆ B., TERZIĆ D., MILENKOVIČ J. (2011): Appearence and frequence of fungi on alfalfa seed in Serbia. Biotechnology in Animal Husbandry, 27, 4, 1579-1584.

VASIĆ T., MILENKOVIĆ J., LUGIĆ Z., TERZIĆ D., STANISAVLJEVIĆ R., BLAGOJEVIĆ M., DJOKIĆ D. (2015): Mycopopulation of different vetch genotypes in Serbia. Plant Protection, Belgrade, 66, 1, 291, 45-50.

VASIĆ T., MILENKOVIĆ J., ANDJELKOVIĆ S., TERZIĆ D., MARKOVIĆ J., DJOKIĆ D., SOKOLOVIĆ D. (2017): Fungal pathogens of vetch genotypes in Serbia. Journal of Mountain Agriculture on the Balkans, 20 2, 192-200.

VILLEGAS-FERNÁNDEZ A. M., RUBIALES D. (2011): Chocolate spot resistance in faba bean. Grain Legumes, 56, 29-30.

VRANDEČIĆ K., ĆOSIĆ J., JURKOVIĆ D., POČTIĆ J. (2011): Mycopopulation of medicinal plants in Croatia . Agriculture, 17,2, 18-21.

VUČKOVIĆ S. (1999): Krmno bilje. Institut za istraživanja u poljoprivredi “Srbija”, Beograd, Bonart, Nova Pazova. pp 1-553.

YLI-MATTILA T., G. KALKO G., HANNUKKALA A., PAAVANEN-HUHTALA S., HAKALA K. (2010): Prevalence, species composition, genetic variation and pathogenicity of clover rot (*Sclerotinia trifoliorum*) and *Fusarium* spp. in red clover in Finland. Eur J. Plant Pathol., 126, 13-27. doi 10.1007/s10658-009-9516-1

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AN INSIGHT INTO THE MYCOTOXICOLOGICAL SITUATION – RECENT EXPERIENCE AND CLOSE PREDICTION

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Abstract:Mycotoxins are secondary metabolites of various fungi, primarily of *Aspergillus*, *Penicillium* and *Fusarium* genera. Fungal species commonly enter the food chain through contaminated food and feed, mainly cereals, which get infested prior to and at the harvest, or during (improper) storage. Although there are over 300 mycotoxins that have been isolated and chemically characterized, worldwide research has focused on those which significantly impact humans and animals. This paper presents test results of 340 samples of feedingstuffs and complete feed mixtures for different species and categories of farm animals randomly analyzed during 2019 and 2020 (120 and 220 respectively) for the presence of aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin and T-2/HT-2 toxin. Deoxynivalenol was the most frequently detected, while 8.82% of results in 2019 and 7.46% in 2020 were above the permitted levels. Pigs, as the most sensitive species of animals, are particularly affected by this. One sample of mixture for piglets, which contained deoxynivalenol 1.76 mg/kg, was the most contaminated, while the overall maximal value was determined in barley (4.73 mg/kg). The aim of this paper was to give a brief insight into the recent mycotoxicological situation with animal feed on the Serbian market. The results indicate the need for regular and comprehensive monitoring of fungal contaminants and their detrimental impacts on animal and human health, as well as the implementation of predicting models in the prevention strategies.

Key words: aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin, T-2/HT-2 toxin, feed

Introduction

Mycotoxins are produced by various fungal species, belonging primarily to genera *Aspergillus*, *Penicillium* and *Fusarium,* as their secondary metabolites. There are also other fungal genera, including *Alternaria*, *Cladosporium*, *Chaetomium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Phoma*, *Phomopsis*, *Pithomyces* and *Stachybotrys,* that contain mycotoxigenic fungi. Under favorable environmental conditions, when temperature and moisture are suitable, fungi proliferate and may produce these toxic substances. The functions of mycotoxins have not been clearly established, but they are believed to play a role in eliminating other microorganisms competing in the same environment. They are also believed to help parasitic fungi invade host tissues. Toxigenic fungi (molds) are known to produce one or more secondary metabolites, but not all fungal species are toxigenic and not all secondary metabolites are toxic (*Brase et al., 2009*). Fungi commonly enter the food chain through contaminated food and feed, mainly cereals, which get infested prior to and during harvest, or during (improper) storage (*Nesic et al., 2021*). Although there are over 300 mycotoxins that have been isolated and chemically characterized, worldwide research has focused on those types causing significant harm to humans and animals (*Nesic et al., 2014*).

Even though there are geographic and climatic differences in the production and occurrence of mycotoxins, exposure to these substances is worldwide (*Eskola et al., 2020*). The accumulation of mycotoxins is known to reflect weather conditions. The two most important factors that affect the life cycle of all microorganisms, including mycotoxigenic fungi, are water availability and temperature. Given such a strong influence of meteorological situation, it is clear that climate change is very much reflected on mycotoxins (*Battilani et al., 2016; Nesic, 2018; Perrone et al., 2020).* Rising temperatures, changes in precipitation, rising sea levels, floods and droughts are widely present. As a result of the increase in temperature, more insects will appear on the crops, which are an important factor in the contamination with toxigenic fungi. The number of birds consuming these insects can also increase, which also contribute to crop damage and greater synthesis of mycotoxins. Changes in crop phenology are also predicted. Therefore, climate change is expected to strongly affect the geographical distribution of crops, as well as their mycoflora. So, predictive modeling is a very promising approach, with necessity to consider as many combinations of mycotoxins and crops at one locality as possible (*Van der Fels-Klerx, 2016).*

The aim of this paper was to evaluate the mycotoxicological situation of feed samples on the Serbian market collected during 2019 and 2020, as well as to point out the need to implement preventive measures and predictive models against the presence of toxigenic fungi and their metabolites in the food chain.

Materials and Methods

Analysis of 340 samples of feedingstuffs and complete feed for different species and categories of farm animals were carried out in the Institute of Veterinary Medicine of Serbia during 2019 and 2020. Quantification of aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin and T-2/HT-2 toxin was done using commercial ELISA (enzyme-linked immunosorbent assay) kit according to the manufacturer's instructions (Neogen Veratox), with the detection range 1-8 µg/kg , 0,25-2 mg/kg, 25-500 µg/kg, 2-25 µg/kg and 25-250 µg/kg, respectively. The absorbance was determined at a wavelength of 650 nm on an ELISA plate reader (Tecan Sunrise, Switzerland). Results were interpreted in relation to the levels permitted by the Serbian *Regulation on the quality of animal feed.*

Results and Discussion

Results of mycotoxicological examination of 340 feed samples for different species and categories of farm animals during 2019 and 2020 showed the presence of mycotoxins, as presented in Table 1. The most frequent presence and the highest concentrations were determined for deoxynivalenol. In 2019 8.82% of results and in 2020 7.46% were above the permitted levels. This is especially important for raising pigs, which are the most susceptible species. Therefore, the highest level of contamination, which was determined in the mixture for piglets, and was 1.76 mg/kg, poses a serious threat. The overall maximal value of deoxynivalenol was determined in barley (4.73 mg/kg).

Table 1. Mycotoxin content (mg/kg) in feed analyzed in 2019 and 2020

Mycotoxin	2019		2020	
	Maximum	Mean	Maximum	mean
Aflatoxin B1	0.089	0.012	0.013	0.002
Zearalenone	0.239	0.063	0.081	0.073
Ochratoxin	0.008	0.007	0.050	0.037
T-2/HT-2 toxin	0.078	0.042	0.093	0.057
Deoxynivalenol	3.6 (wheat)	1.255	4.73 (barley)	0.748

Deoxynivalenol (vomitoxin) is produced by *F. graminearum, F. culmorum, F. crookwellense, F. sporotrichioides, F. poae, F. tricinctum, and F. acuminatum (Nesic et al., 2014).* Intoxication with this metabolite is manifested by a decrease in food intake or its refusal, vomiting, and digestive disorders with subsequent losses of weight gain. From a practical viewpoint deoxynivalenol is of outstanding importance among the B type trichothecenes because of its frequent occurrence at levels high enough to cause adverse effects, especially in pigs *(EFSA, 2013)*. Other animals are regarded to be less sensitive, but they still suffer from negative consequences, primarily in terms of performance *(Santos et al., 2021)*.

Considering results for aflatoxin presence the situation was different from that in 2013 when most of the samples, about 75%, were highly contaminated and as many as 35% of them exceeded the maximum permitted levels, so did not correspond to the Serbian regulation at that moment *(Nesic and Pavlovic, 2013)*. It also differed from the results of *Krnjaja et al. (2019)* obtained for aflatoxin B_1 in poultry feed in 2016 when 14.29% were above the regulation limits. Tests performed in 2019, and especially in 2020, for aflatoxin B_1 detection revealed low and mostly undetectable concentrations. For other mycotoxins, as shown in Table 1, average content was slightly above limit of detection (LOD) for the applied methods (LOD: 0.002 mg/kg for ochratoxin, 0.025 mg/kg for T-2/HT-2 and zearalenone), while most of the values were below the LOD of the used ELISA protocols.

Zearalenone was the second most frequently detected mycotoxin, after deoxynivalenol. In practice, the co-occurrence of deoxynivalenol and zearalenone, or even additional mycotoxins in contaminated cereals exacerbates the management of affected animals *(Döll and Dänicke 2011)*. A group of Italian researchers *(Palumbo et al., 2020)* collected and published data on the content of mycotoxins in cereals in Europe in the period from 2010 to 2018. They concluded that mycotoxins fumonisin, deoxynivalenol, aflatoxins and zearalenone were mainly found in wheat and maize. An important fact is that in 54.9% of the samples, the simultaneous appearance of two or more mycotoxins was proven. This, due to the synergistic effect, drastically complicates the situation, as well as the methods of struggle.

One of the newer approaches in prevention of mycotoxicological problems includes the possibility of predicting their occurrence and the use of so-called “predictive models”. These are the scenarios of the effects of climate change on mycotoxins, i.e. studies of the effects of changes in precipitation, temperature, etc. on mycotoxin contamination. They combine data on meteorological conditions, pathogens (fungi and mycotoxins) and hosts (plant species). Thus, the predictions are highly specific to one locality, plant species and even strain, as well as type of fungi and their metabolites *(Nesic, 2018)*.

Climate change forecasts for Europe by the end of this century have been published in a document called the Green Paper *(European Commision, 2007)*. The temperature will rise drastically. In the very south, locally up to 5.5^{0}C, while in Serbia approximately 3.5–4.5^{0}C. Precipitation will be reduced in the south, in some localities even up to 40%, while towards the north of the continent it will increase to almost drastic proportions. In general, the so-called desertification will occur in

Southern and Southeastern Europe which will contribute to the synthesis of aflatoxins, while in Central Europe, due to the higher moisture, and even more frequent floods, contamination with *Fusarium* species and their metabolites will increase.

Conclusion

Based on the results of the mycotoxicology tests in 2019 and 2020, as well as previous years of analytical experience, the continuous presence of mycotoxins (one or more), with occasional specific outbursts, is evident. Mycotoxins remain an unavoidable problem that needs to be fought in the best possible way, while a constant good management system for these natural hazards is still required. Due to climate change, the risk to human and animal health is transforming and growing, and strategies to mitigate adverse effects are becoming more complex. This also has economic consequences. Therefore, the multidisciplinary scientific community has recently been given the task of enabling the prediction of the occurrence of mycotoxins and the preparation of targeted prevention.

Uvid u mikotoksikološku situaciju – nedavna iskustva i bliska predviđanja

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Rezime

U ovom radu su predstavljeni rezultati ispitivanja 340 uzoraka komponenti i kompletnih smeša za ishranu životinja koji su analizirani tokom 2019. (120 uzoraka) i 2020. godine (220 uzoraka) na prisustvo aflatoksina B_1, deoksinivalenola, zearalenona, ohratoksina i T-2/HT-2 toksina, sa ciljem da se stekne uvid u mikotoksikološku situaciju u hrani za životinje koja se koristi u Srbiji. Mikotoksini su sekundarni metaboliti različitih plesni, pre svega vrsta iz rodova *Aspergillus, Penicillium* i *Fusarium*. U lanac hrane oni obično dospevaju preko kontaminiranih žitarica, koje se infestiraju pre i tokom žetve ili u uslovima nepravilnog skladištenja. Iako postoji više stotina različitih mikotoksina koji su izolovani i hemijski identifikovani, praktični značaj imaju oni sa najvećim uticajem na zdravlje ljudi i životinja. U hrani za životinje u navedenom periodu najčešće je bio prisutan deoksinivalenol, i to u nedozvoljenim koncentracijama u 8,82%

uzoraka iz 2019. godine i 7,46% iz 2020. godine. Ovakva situacija je naročito nepovoljna za svinjarstvo, s obzirom da su na deksinivalenol svinje najosetljivija vrsta životinja. Maksimalna koncentracija od 1,76 mg/kg je bila utvrđena u uzorku smeše za prasad, dok je ukupna maksimalna vrednost ustanovljena u uzorku ječma (4,73 mg/kg). Svi ostali mikotoksini su bili u okviru propisima dozvoljenih granica, čak uglavnom ispod limita detekcije primenjenih metoda, dok je drugi po učestalosti bio zearalenon. Dobijeni rezultati ukazuju na potrebu za redovnim i sveobuhvatnim nadzorom usmerenim na kontrolu sadržaja ovih prirodnih kontaminenata koji imaju veliki globalni uticaj na bezbednost hrane za životinje i ljude, odnosno njihovo zdravlje. Takođe je poželjno da se primeni i mogućnost predviđanja i prognoze prisustva mikotoksina, pri čemu bi prediktivni modeli postali obavezan deo preventivnih strategija.

Key words*:* aflatoksin B1, deoksinivalenol, zearalenon, ohratoxin, T-2/HT-2 toksin, hrana za životinje

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References

BATTILANI P., TOSCANO P., VAN DER FELS-KLERX H.J., MORETTI A., CAMARDO LEGGIERI M., BRERA C., RORTAIS A., GOUMPERIS T., ROBINSON T. (2016): Aflatoxin B1 contamination in maize in Europe increases due to climate change. Scientific Reports, 6, 24328.

BRASE S., ENCINAS A., KECK J., NISING, C.F. (2009): Chemistry and biology of mycotoxins and related fungal metabolites. Chemical Reviews, 109, 9, 3903–3990.

DÖLL S., DÄNICKE S. (2011): The Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON) in animal feeding. Preventive Veterinary Medicine, 102, 132–145.

EFSA (2013): Deoxynivalenol in food and feed: occurrence and exposure. EFSA Journal, 11, 10, 3379,

ESKOLA M., KOS G., ELLIOTT C.T., HAJŠLOVÁ J., MAYAR S., KRSKA, R. (2020): Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited ‘FAO estimate’ of 25%. Critical Reviews in Food Science and Nutrition, 60, 16, 2773-2789.

EUROPEAN COMMISION (2007): Green Paper from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions - Adapting to climate change in Europe – options for EU action. 1-27.

KRNJAJA V., PETROVIĆ T., STANKOVIĆ S., LUKIĆ M., ŠKRBIĆ Z., MANDIĆ V., BIJELIĆ Z. (2019): Mycobiota and aflatoxin B1 in poultry feeds. Biotechnology in Animal Husbandry, 35, 1, 61-69.

NEŠIĆ K., HABSCHIED K., MASTANJEVIĆ K. (2021): Possibilities for the Biological Control of Mycotoxins in Food and Feed. Toxins, 13, 3, 198.

NEŠIĆ K. (2018): Mycotoxins – climate impact and steps to prevention based on prediction. Acta Veterinaria – Beograd, 68, 1, 1-15.

NEŠIĆ K., IVANOVIĆ S., NEŠIĆ V. (2014): Fusarial Toxins - Secondary Metabolites of *Fusarium* Fungi. Reviews of Environmental Contamination and Toxicology, 228, 101-120.

NESIC K., PAVLOVIC N. (2013): Current mycotoxicological profile of Serbian feed. Proceedings of the International 57th Meat Industry Conference “Meat and meat products – perspectives of sustainable production”; June 10-12, Belgrade, 320-323.

– PALUMBO R., CRISCI A., VENÂNCIO A., CORTIÑAS ABRAHANTES J., DORNE J-L., BATTILANI P., TOSCANO P. (2020): Occurrence and Co-Occurrence of Mycotoxins in Cereal-Based Feed and Food. Microorganisms, 8, 1, 74.

PERRONE G., FERRARA M., MEDINA A., PASCALE M., MAGAN N. (2020): Toxigenic Fungi and Mycotoxins in a Climate Change Scenario: Ecology, Genomics, Distribution, Prediction and Prevention of the Risk. Microorganisms, 8, 10, 1496.

REGULATION ON THE QUALITY OF ANIMAL FEED. Official Gazette of RS, 4/2010, 113/2012, 27/2014, 25/2015, 39/2016 and 54/2017, Belgrade.

SANTOS R.R., OOSTERVEER-VAN DER DOELEN M.A.M., TERSTEEG-ZIJDERVELD M.H.G., MOLIST F., MÉZES M., GEHRING R. (2021): Susceptibility of Broiler Chickens to Deoxynivalenol Exposure via Artificial or Natural Dietary Contamination. Animals, 11, 4, 989.

VAN DER FELS-KLERX H.J., LIU C., BATTILANI P. (2016): Modelling climate change impacts on mycotoxin contamination. World Mycotoxin Journal, 9, 5, 717-726.

FUSARIUM SPP. AND DEOXYNIVALENOL CONTAMINATION OF RYEGRASS SEEDS

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Abstract: The aim of this study was to evaluate fungal infection, with a special focus on *Fusarium* spp. and deoxynivalenol (DON) presence, as a participant in Fusarium head blight (FHB) pathogenesis in two cultivars of Italian ryegrass K-13 and K-29. A total of 24 seed samples were collected during harvest in 2019.

By mycological analyses, *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. and non-sporulating species (Mycelia sterilia) were isolated on the seeds of both cultivars of Italian ryegrass. Among *Fusarium* spp., four species, *F. graminearum*, *F. poae*, *F. proliferatum* and *F. subglutinans* were identified in cultivar K-13 and three species, *F. graminearum*, *F. poae* and *F. subglutinans*, in cultivar K-29. *F. graminearum* and *F. poae* were identified as FHB pathogens, of which *F. graminearum* was dominant in both cultivars with 20.5% (cultivar K-13) and 32% (cultivar K-29) compared to *F. poae* which was present in both cultivars with a frequency of 0.5%. The frequency of DON positive samples was 100%. A statistically significant difference in DON level was found between the two tested cultivars, with a higher DON level in cultivar K-29 (5334.33 $\mu g\ kg^{-1}$) compared to cultivar K-13 (4738.58 $\mu g\ kg^{-1}$).

The obtained results indicate that two *Fusarium* species, *F. graminearum* and *F. poae*, were FHB pathogens, with *F. graminearum* as the predominant species in both cultivars of Italian ryegrass. High DON levels (>3000 $\mu g\ kg^{-1}$) in the tested seed of Italian ryegrass indicate on potentially significant participation of DON in FHB pathogenesis, as well as a potential risk for the quality seed production, feed safety and the food chain in general. In Serbia, this is the first report about *Fusarium* infection and DON presence in ryegrass seed.

Key words: *Fusarium* spp., deoxynivalenol, Italian ryegrass

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is annual and also biennial or short-lived perennial bunchgrass. It is a high-yielding and high-quality fodder crop native to southern Europe. It is an integral part of pasture for hay and silage production. Its high nutrition, digestibility, and palatability, conducives to the diet of ruminants (dairy, sheep) (*Simić et al., 2005; 2012*). This crop grows best between 20 and 25°C and requires a cool climate with high rainfall and well-drained soils. It has the fastest growth and regeneration and produces a lot of forage in a short time (*Delić et al., 2012; Kostrzewska et al., 2020*). It is sensitive to strong frosts and quite sensitive to drought, and longer droughts can bring it very low yields (*Simić et al., 2005*).

In recently year, due to global warming, the occurrence of pathogenic and potentially toxigenic fungi from *Fusarium*, *Aspergillus* and *Penicillium* genera has become more frequent on cereal grains and other grasses (*Silva et al., 2014; Torres et al., 2019*). In addition, agro-ecological and environmental conditions in Serbia are favorable to growth of these fungal species on cereal grains, with *Fusarium* species as predominant (*Lević, 2008).* Members of *Fusarium graminearum* species complex (FGSC) cause Fusarium head blight (FHB) disease of small grains and several grasses, including annual ryegrass (*Machado et al., 2015*). *F. graminearum* is the most common pathogen which cause FHB of small grains. The first symptoms of FHB appear after flowering at anthesis. Spikelets are bleached, and with development of mycelium, the disease spreads throughout the head (spike), causing shrivelled seeds (*Francesconi et al., 2019*). *F. graminearum*-infected seeds have poor germination with slow emergence and can cause seedling blight disease (*Wiewióra, 2012*). *F. graminearum* is a hemibiotroph, with the biotrophic (short last from 24 to 32 h after infection) and necrotrophic phase (the pathogen grows and extends within the head through the vascular bundles and parenchyma) (*Bai et al., 2002; Goswami and Kistler, 2004; Trail, 2009*).

In addition to causing FHB disease, *Fusarium* species produce a wide range of toxic secondary metabolites (mycotoxins), which are serious constraints for feed/food production (*Richardson et al., 1996*). The occurrence of these fungal metabolites in food/feed poses healt risks to the consumers (*Haidukowski et al., 2005*). Deoxynivalenol (DON), primarily produced by *F. graminearum*, is the most associated with FHB. It belongs sesquiterpene epoxides, namely trichothecenes. The most commonly DON found in grain/seed from FHB-diseased cereal and grass heads (*Gunupuru et al., 2017*). Negative effects of DON ingestion by experimental animals are manifested as acute (emesis) and chronic poisoning (anorexia, growth retard, neurotoxicity, immunotoxicity as well as reproduction disorders. Likewise, DON can be associated with human gastroenteritis (*Pestka, 2010*). Reffering to participation of DON in FHB pathogenesis, it also has the ability to be virulent and facilitates the spread of fusariosis within the head. Like FHB, DON may cause premature bleaching of head and leaves. DON-producing *F. graminearum* strains can supress programmed cell death (apoptosis) in the leaves and support biotrophic and necrotrophic phases of *Fusarium* infection (*Diamond et al., 2013*). According to *Bushnell et al. (2010)*, DON-producing strains inhibited the formation of the wall thickenings, allowing the pathogen to spread in the head.

According to European Regulations EC No. 1126/2007 amended the Regulation (EC) No. 1881/2006 and EC No. 576/2006, the maximum limits of DON level are 1250 μg kg^{-1} in unprocessed cereal other than durum wheat, oats and maize and 8000 μg kg^{-1} in unprocessed cereal and cereal products except in maize by-products intended for animal feeding, respectively. Quality and healthy seeds are a prerequisite for high yields of ryegrass as well as for safe feed production. The high yield of seed and fodders is correlated with the genetic potential of the selected varieties (*Velijević et al., 2016*). Grass (cereal) cultivars have different types of resistance to FHB (from I to V resistance types), there is no unsusceptible genotype. Types I and II are the most described. Type I resistance is resistance to initial infection, type II is resistance to spread of pathogen within the head. Other types include resistance to seed infection (type III), tolerance to FHB and DON (type IV), and resistance to DON accumulation (Type V) (*Mesterházy, 1995; Boutigny et al., 2008*). In addition to growing resistant cultivars, crop rotation, fertilization, tillage, and the application of fungicides and biological agents can also significantly reduce *Fusarium* seed infestation (*Shah et al., 2018*).

Since the quality and healthy seed is important for ryegrass, livestock and feed production, the main aim of this work was to identify and quantify *Fusarium* spp. associated with ryegrass seeds, as well as to determine DON levels in seed samples of two ryegrass cultivars and to assess its potential risk in FHB pathogenesis.

Materials and Methods

A total of 24 seed samples of two commercial Italian ryegrass cultivars (12 samples of cultivar K-13 and 12 samples of cultivar K-29) were analyzed by mycological and toxicity tests. About 1 kg of ryegrass intended for seed production were randomly taken during the 2019 harvest season. The area of tested ryegrass cultivars was on about one hectare and located on the experimental fields of Institute for Animal Husbandry, Belgrade-Zemun in Serbia.

The each of 24 samples was divided into two sub-samples, so, it was 12 sub-samples per cultivar. In mycological analyses, per each cultivar, the first 12 seed sub-samples were pooled in one representative sample. To evaluate *Fusarium* spp., 600 seeds per cultivar from representative samples were analyzed. First, the seeds were surface-disinfected for 3 minutes with 1% sodium hypochlorite, washed twice with sterilized distilled water and dried on filter paper. Then, the seeds were plated, 10 seeds per 90 mm Petri-dishes with Potato Dextrose Agar (PDA), and incubated at room temperature during 7-10 days. Based on microscopic observations, *Fusarium* species were identified using fungal keys of *Burgess et al. (1994)* and *Leslie and Summerell (2006)*. Incidence of fungal species was presented as percentage values in pooled seed samples.

For mycotoxicological analyses, a total of 24 samples, 12 sub-samples per cultivar, were ground in an analytical mill (IKA A11, Staufen, Germany). Using the moisture analyzer (OHAUS MB35, USA), the moisture content of tested samples was determined. ELISA assay for determining DON levels was done according to the manufacturer's instructions Celer Tecna® ELISA kits. The limit of detection for DON was 40 μg kg^{-1}.

Data were statistically analyzed using the independent-samples T-test (IBM SPSS Statistic 20). Pearson's correlation coefficients between DON levels and moisture contents were determined.

Results and Discussions

The results of mycobiota isolated from the seed of two Italian ryegrass cultivars, K-13 and K-29, are shown in Table 1. The fungal species isolated were *Acremonium* spp., *Alternaria* spp., *F. graminearum*, *F. poae*, *F. proliferatum*, *F. subglutinans* and non-sporulating fungi (Mycelia sterilia) on the seed of both cultivars, except on seed of cultivar K-29, in which *F. proliferatum* was not identified. Among isolated species, *Alternaria* spp. was the most present with 70% and 61.67% on the seed of K-13 and K-29 cultivar, respectively. Considering incidence of *Fusarium* species, *F. graminearum* was the most common at both cultivars, with higher incidence on the seed of cultivar K-29 (32%) than cultivar K-13 (20.50%). *F. graminearum* is followed by *F. subglutinans* which was isolated in a higher percent on the seed of cultivar K-13 (7.50%) compared to cultivar K-29 (2%). A low incidence of *F. poae* (0.50%) was found on the seed of both cultivars, as well as the incidence of *F. proliferatum* on the seed of cultivar K-13 (0.17%). In a similar study, *Wiewióra (2012)* has established *Alternaria alternata* as the most common species on the seed of perennial ryegrass and identified 10 *Fusarium* species of which *F. avenaceum* and *F. solani* were the most frequent. Further, the same autor stated that the most species such as *Alternaria* spp., *Epicoccum* spp., *Septonema* spp. and *Penicillium* spp. were saprophytes or weak parasites, while *Drechslera* spp., *Fusarium* spp., *Phoma* spp., *Curvularia* spp. and *Bipolaris* spp. were presented as pathogenic species. Similar results were reported by *Varga and*

Fischl (2005) on the ryegrass seeds and *Pathak and Zaidi (2013)* on wheat seeds. According to reported data of *Torres et al. (2019)*, the major FHB pathogens include FGSC members and related species such as *F. avenaceum*, *F. culmorum* and *F. poae*. Similarly, in this study, *F. graminearum* and *F. poae* identified as FHB pathogens from which *F. graminearum* was more presence than *F. poae* on the seed of both tested cultivars. *Machado et al. (2015)* have identified three FGSC species, *F. graminearum*, *F. asiaticum* and *F. cortaderie* from diseased ryegrass spikes and confirmed their pathogenicity to ryegrass. It was the first report of FGSC members as head blight pathogens of ryegrass (*L. multiflorum* L.) in Brazil. The results of this study were also the first report of *Fusarium* spp. incidence on the seed of two Italian ryegrass cultivars (K-13 and K-29) in Serbia.

Table 1. Incidence of fungal species on the seed of two tested ryegrass cultivars

Fungal species	Incidence (%)	
	Cultivar of ryegrass	
	K-13	K-29
Acremonium spp.	0.50	0.83
Alternaria spp.	70	61.67
Fusarium graminearum	20.5	32
Fusarium poae	0.50	0.50
Fusarium proliferatum	0.17	0
Fusarium subglutinans	7.50	2
Mycelia sterilia	0.83	3

By mycotoxicological assays, DON was found in all tested seed samples of two ryegrass cultivars, respectively the incidence of DON positive samples was 100%. It has been established the statistically significant higher mean level of DON on the seed of cultivar K-29 (5334.33 µg kg^{-1}) compared to cultivar K-13 (4738.58 µg kg^{-1}). There were no statistically significant differences between means of moisture contents of seeds in tested cultivars (Table 2). By investigating the participation of DON in FHB pathogenesis, *Bai et al. (2002)* have stated that DON production had a significant role in the spread of FHB within a spike. According to the reports of *Diamond et al. (2013)*, relatively low DON levels might inhibite plant programmed cell death (PCD), while high DON levels might induce cell death. So, DON-producing *F. graminearum* strains with low and 10,000 µg kg^{-1} DON levels might contribute to biotrophic and necrotrophic phases development in *Fusarium* infection leading to FHB disease symptoms (*Gunupuru et al., 2017*). In Brazil, the first report of DON-producing *F. graminearum* isolated from ryegrass spikes, as well as their pathogenicity on annual ryegrass reported by *Machado et al. (2015)*. In this study, high DON levels in all seed samples of both cultivars indicating that there was potentially risk of isolated *F. graminearum* strains in FHB pathogenesis.

Table 2. Means of DON level and moisture content in tested seed samples of two ryegrass cultivars

Item	DON level (μg kg^{-1})		Moisture content (%)
	Mean ± S.D.	Range	Mean ± S.D.
Cultivar K-13	4738.58 ± 244.17[a]	3157 – 5586	11.74 ± 0.32
Cultivar K-29	5334.33 ± 147.26[b]	4382 – 5886	11.30 ± 0.53
Level of significance	*	-	ns

Means followed by the same letter within a column are not significantly different at P ≤ 0.05 level; *, ** - significant at the 0.05 and 0.01 probability levels; ns - not significant.

Temperature, moisture and relative humidity are the most important environmental factors influencing the development of FHB and therefore DON accumulation in cereal grains *(Wegulo, 2012)*. Weak positive correlations were found for levels of DON with moisture contents on seeds of tested cultivars K-13 (r = 0.348) and K-29 (r = 0.380) (data not presented). Similarly, positive correlations between moisture-related variables with biological variables such as DON accumulation in wheat cultivars were established by *Cowger et al. (2009)*, *Kriss et al. (2010)* and *Hernandez Nopsa et al. (2012)*.

Conclusion

This study presents a natural occurrence of fungal infection and DON mycotoxin in the seed of two ryegrass cultivars, K-13 and K-29, as a participant in FHB pathogenesis. *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. and non-sporulating fungal species (Mycelia sterilia) were isolated at both cultivars. Among *Fusarium* spp., *F. graminearum*, *F. poae*, *F. proliferatum,* and *F. subglutinans* and *F. graminearum*, *F. poae,* and *F. subglutinans* were identified on the seed of cultivars K-13 and K-29, respectively. *F. graminearum* and *F. poae* were identified as FHB pathogens, with *F. graminearum* as predominant in both cultivars. All tested seed samples were DON positive. DON level in cultivar K-29 was statistically significant higher (5334.33 μg kg^{-1}) than in K-13 (4738.58 μg kg^{-1}). High DON levels (>3000 μg kg^{-1}) in tested seed samples were indicated on potential significant participation of DON in FHB pathogenesis. Both ryegrass cultivars were susceptible to FHB pathogens. These results indicate on importance of health of seeds, especially for ryegrass seeds production as well as forage production. Ryegrass as the weed may also be potentially a source of inoculum for FHB epidemics in cereal crops.

Fusarium spp. i deoksinivalenol kontaminacija semena italijanskog ljulja

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Rezime

Cilj rada bio je da se oceni gljivična infekcija sa specijalnim fokusom na *Fusarium* spp. i prisustvo deoksinivalenola (DON) kao učesnika u patogenezi fuzarioze klasa (FHB) kod dve sorte italijanskog ljulja, K-13 i K-29. Ukupno 24 uzoraka semena sakupljeno je tokom žetve u 2019. godini. Mikološkim analizama izolovane su *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. i nesporulišuće vrste (Mycelia sterilia) na semenu obe ispitivane sorte italijanskog ljulja. Među *Fusarium* spp., identifikovane su četiri vrste, *F. graminearum*, *F. poae*, *F. proliferatum* i *F. subglutinans*, kod sorte K-13 i tri vrste, *F. graminearum*, *F. poae* i *F. subglutinans*, kod sorte K-29. *F. graminearum* i *F. poae* su identifikovane kao FHB patogeni, od kojih *F. graminearum* je dominantnija kod obe ispitivane sorte sa 20,5% (sorta K-13) i 32% (sorta K-29) u odnosu na *F. poae* koja je bila prisutna kod obe sorte sa učestalošću od 0,5%. Učestalost DON pozitivnih uzoraka bila je 100%. Utvrđena je statistički značajna razlika u koncentraciji DON između dve ispitivane sorte, s tim da je utvrđena veća koncentracija DON kod sorte K-29 (5334,33 μg kg^{-1}) u odnosu na sortu K-13 (4738,58 μg kg^{-1}). Dobijeni rezultati ukazuju da su dve *Fusarium* vrste, *F. graminearum* i *F. poae*, FHB patogeni, s tim da je *F. graminearum* preovladjujuća vrsta kod obe ispitivane sorte italijanskog ljulja. Visoke koncentracije DON mikotoksina (>3000 μg kg^{-1}) u ispitivanim uzorcima semena italijanskog ljulja ukazuju na potencijalno značajno učešće DON u FHB patogenezi, kao i na potencijalni rizik za proizvodnju kvalitetnog semena, bezbednost hrane za životinje i lanca ishrane uopšte. U Srbiji, ovo je prva objava o *Fusarium* infekciji i prisustvu DON u semenu italijanskog ljulja.

Ključne reči: *Fusarium* spp., deoksinivalenol, italijanski ljulj

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References

BAI G.H., DESJARDINS A.E., PLATTNER R.D. (2002): Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia, 153, 91-98.

BOUTIGNY A.L., RICHARD FORGET F., BARREAU C. (2008): Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. European Journal of Plant Pathology, 121, 411-423.

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory manual for *Fusarium* research. Third edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, pp. 133.

BUSHNELL W.R., PERKINS-VEAZIE P., RUSSO, V.M., COLLINS J., SEELAND T.M. (2010): Effects of deoxynivalenol on content of chloroplast pigments in barley leaf tissues. Phytopathology, 100, 33-41.

COWGER C., PATTON-ÖZKURT J., BROWN-GUEDIRA G., PERUGINI L. (2009): Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99, 320-327.

DELIĆ D., STAJKOVIĆ-SRBINOVIĆ O., ŽIVKOVIĆ S., PROTIĆ N., RASULIĆ N., KUZMANOVIĆ DJ., SIMIĆ A. (2012): Growth promotion of Italian ryegrass (*Lolium multiflorum* Lam.) by application of plant growth promoting rhizobacteria. Plant Protection, 63, 280, 93-99.

DIAMOND M., REAPE T.J., ROCHA O., DOYLE S.M., KACPRZYK J., DOOHAN F.M., MCCABE P.F. (2013): The *Fusarium* mycotoxin deoxynivalenol can inhibit plant apoptosis-like programmed cell death. PloS One, 8, 7, e69542.

FRANCESCONI S., MAZZAGLIA A., BALESTRA G.M. (2019): Different inoculation methods affect components of Fusarium head blight resistance in wheat. Phytopathologia Mediterranea, 58, 3, 679-691.

GOSWAMI R.S., KISTLER H.C. (2004): Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology, 5, 515-525.

GUNUPURU L.R., PEROCHON A., DOOHAN F.M. (2017): Deoxynivalenol resistance as a component of FHB resistance. Tropical Plant Pathology, 42, 175-183.

HAIDUKOWSKI M., PASCALE M., PERRONE G., PANCALDI D., CAMPAGNA C., VISCONTI A. (2005): Effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. Journal of the Science of Food and Agriculture, 85, 191-198.

HERNANDEZ NOPSA J., BAENZIGER P.S., ESKRIDGE K.M., PEIRIS K.H.S., DOWELL F.E., HARRIS S.D., WEGULO S.N. (2012): Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Canadian Journal of Plant Pathology, 34, 380-389.

KOSTRZEWSKA M.K., JASTRZĘBSKA M., TREDER K., WANIC M. (2020): Phosphorous in spring barley and Italian rye-grass biomass as an effect of inter-species interactions under water deficit. Agriculture, 10, 329.

KRISS A.B., PAUL P.A., MADDEN L.V. (2010): Relationship between yearly fluctuations in Fusarium head blight intensity and environmental variables: A window-pane analysis. Phytopathology 100, 784-797.

LESLIE J.F., SUMMERELL B.A., (2006): The *Fusari*um Laboratory Manual. Blackwell Publishing Ltd, Oxford, pp. 388.

LEVIĆ J. (2008): Vrste roda *Fusarium* u oblasti poljoprivrede, veterinarske i humane medicine. Cicero, Beograd. pp. 1226.

MACHADO F.J., MÖLLER P.A., NICOLLI C.P., Del PONTE E.M. (2015): First report of *Fusarium graminearum*, *F. asiaticum*, and *F. cortaderiae* as head blight pathogens of annual ryegrass in Brazil. Plant Disease, 99, 12, 1859-1859.

MESTERHÁZY A. (1995): Types and components of resistance to Fusarium head blight of wheat. Plant Breeding, 114, 377-386.

PATHAK N., ZAIDI R. (2013): Fungi associated with wheat seed discolouration and abnormalities in *in-vitro* study. Agricultural Sciences, 4, 9, 516-520.

RICHARDSON M.J. (1996): Seed mycology. Mycological Research 100, 4, 385-392.

SHAH L., ALI A., YAHYA M., ZHU Y., WANG S., SI H., RAHMAN H., MA C. (2018): Integrated control of fusarium head blight and deoxynivalenol mycotoxin in wheat. Plant Pathology, 67, 532-548.

SILVA A.E.L., REIS E.M., TONIN R.F.B., DANELLI A.L.D., AVOZANI A. (2014): Identification and quantification of fungi associated with seeds of ryegrass (*Lolium multiflorum* Lam.). Summa Phytopathologica, 40, 2, 156-162.

SIMIĆ A., VUČKOVIĆ S., SABOVLJEVIĆ R. (2005): Seed yield and quality of Italian ryegrass (*Lolium italicum*) containing different ploidy produced in the first harvest year. Plant Breeding and Seed Production, XI, 1-4, 19-24.

SIMIĆ A., VUČKOVIĆ S., SOKOLOVIĆ D., STANISAVLJEVIĆ R., DURONIĆ G. (2012): Response of Italian ryegrass seed crop to spring nitrogen application in the first harvest year. African Journal of Biotechnology, 11, 26, 6826-6831.

TORRES A.M., PALACIOS S.A., YERKOVICH N., PALAZZINI J.M., BATTILANI P., LESLIE J.F., LOGRIECO A.F., CHULZE S.N. (2019): *Fusarium* head blight and mycotoxins in wheat: prevention and control strategies across the food chain. World Mycotoxin Journal, 12, 4, 333-355.

TRAIL F. (2009): For blighted waves of grains: *Fusarium graminearum* in the postgenomics era. Plant Physiology, 149, 103.110.

VARGA Z., FISCHL G. (2005): Infection rates of perennial ryegrass seeds (*Lolium perenne* L.) with different fungi. Communications in Agricultural and Applied Biological Sciences, 70, 195-198.
VELIJEVIĆ N., SIMIĆ A., VUČKOVIĆ S., ĐUKANOVIĆ L., POŠTIĆ D., ŠTRBANOVIĆ R., STANISAVLJEVIĆ R. (2016): Varijabilnost dormantnosti, klijavosti semena i vigora klijanaca sorti crvene deteline i italijanskog ljulja. XXI Savetovanje o biotehnologiji, 11. – 12. mart 2016. godine, Čačak, Srbija. Zbornik radova, 21, 23, 73-79.
WEGULO S.N. (2012): Influencing deoxynivalenol accumulation in small grain cereals. Toxins, 4, 1157-1180.
WIEWIÓRA B. (2012): The effect of seed health of perennial ryegrass (*Lolium perenne* L.) on germination capacity. Plant Breeding and Seed Science, 65, 51-62.

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