ISSN 1820-9955 UDK 619

Scientific Veterinary Institute „Novi Sad" Novi Sad
Naučni institut za veterinarstvo „Novi Sad" Novi Sad

Archives of Veterinary Medicine
Arhiv veterinarske medicine

Arch. vet. med.	Vol. 8	No. 2	Pg. 1-120	Novi Sad, 2015.

CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

619

Archives of Veterinary Medicine = Arhiv veterinarske medicine / Editor in Chief Miroslav Ćirković. – Vol. 1, br. 1 (2008) –.– Novi Sad : Scientific Veterinary Institute "Novi Sad", 2014 –.– 25 cm

Published twice a year.

ISBN 1820-9955

COBISS.SR-ID 235692807

Original scientific paper UDK 546.3/.5:599.735

THE INCIDENCE OF HEAVY METALS AND OTHER TOXIC ELEMENTS IN ROE DEER (*Capreolus capreolus)* TISSUES

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Abstract

Levels of lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) and copper (Cu) in the liver, kidney and muscle of 11 individual roe deer (*Capreolus capreolus)* were determined. The samples were prepared by microwave wet digestion. Content of investigated elements was determined by the method of coupled plasma with mass spectometry. The lead concentrations ranged from <0.001 (liver) to 8.455 mg/kg (meat), Cd concentrations ranged from 0.004 (muscle) to 0.818 mg/kg (kidney) and As concentrations ranged from 0.002 (liver) to 0.031 mg/kg (kidney). Concentrations of Hg in examined tissues (liver, kidney, muscle) were under limit of detection (<0.001 mg/kg). The concentration of copper in liver ranged from 3.913 to 104.08 mg/kg. The results of this study showed that no samples exceeded maximum allowed levels for Cd, Hg, As and Cu. Pb concentrations in muscle samples ranged from 0.008 to 8.455 mg/kg. High concentrations of Pb in two muscle samples are most likely due to the proximity of hunting wound area, as lead was not detected in organ samples. The presence of some elements in the tissues of roe deer suggests the necessity of further research aimed at identifying the source of contamination in order to preserve the health of both humans and animals.

Keywords*:* lead, cadmium, mercury, arsenic, copper, tissues, roe deer

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TEŠKI METALI I DRUGI TOKSIČNI ELEMENTI U TKIVIMA SRNA (*Capreolus capreolus)*

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Kratak sadržaj

U ovom radu ispitivan je sadržaj olova (Pb), kadmijuma (Cd), arsena (As), žive (Hg) i bakra (Cu) u jetri, bubrezima i mišićnom tkivu, 11 srna. Uzorci su pripremljeni metodom mikrotalasne vlažne digestije. Sadržaj ispitivanih elemenata je određen metodom indukovano kuplovane plazme sa masenom detekcijom. Koncentracija Pb kretala se u intervalu od <0,001 (jetra) do 8,455 mg/kg (mišićno tkivo), dok se koncentracija Cd kretala od 0,004 (mišićno tkivo) do 0,818 mg/kg (bubreg). Koncentracija As je bila u rasponu od 0,002 (jetra) do 0,031 mg/kg (bubreg). Koncentracije Hg u ispitivanih tkivima (jetra, bubreg, mišić) je bila ispod granica detekcije metode (<0,001 mg/kg). Sadržaj bakra u jetri kretao se u intervalu od 3,913 do 104,08 mg/kg. Izmerene vrednosti za sadržaj Cd, Hg, As i Cu u svim ispitivanim tkivima, a sadržaj Pb u jetri i bubrezima ne prelazi propisane maksimalno dozvoljene vrednosti. Koncentracije Pb u uzorcima mišićnog tkiva kretala se u rasponu od 0,008 do 8,455 mg/kg. Visoke koncentracije Pb u dva uzorka mišića, verovatno su posledica blizine mesta odstrelne rane, s obzirom na to da u uzorcima organa istih životinja nije detektovano Pb. Zbog prisustva toksičnih elementa u tkivima i organima srna, neophodna su dalja istraživanje kako bi se identifikovali izvori kontaminacije, a u cilju očuvanja zdravlja ljudi i životinja.
Ključne reči: olovo, kadmijum, živa, arsen, bakar, tkiva, srna

INTRODUCTION

Contamination of the environment with hazardous compounds and elements of anthropogenic origin is of increasing concern because of its effect on the entire biosphere, i.e., the micro-flora and –fauna of soils, plants and higher life, including humans and animals (Selenius et al., 1996). Many wild animals are exposed to diverse toxic substances by consuming contaminated plants and animals, or water, soil and air (Živkov Baloš et al., 2015). The accumulation of toxic heavy metals in plants and soil may increase the risk of transfer to

herbivorous wild mammals and game animals or to livestock (Bilandžić et al., 2010). As animals can move freely and find their own food, the game is a link in the chain that accumulates pollutants from the environment. Monitors have recently been defined as organisms in which changes in known characteristics can be measured to assess the extent of environmental contamination, so that conclusions on the health implication for other species of the environment as a whole can be drawn. Monitors may provide information about environmental concentrations of essential and toxic metals of importance for life, displaying deficiency and toxicity, respectively (Selinus et al., 1996).

In order to fully understand the exposure of animals to many pollutants originating from the environment and to assess the harmful effect and estimate the risk, it is necessary to carry out a systematic study and gather data on degree and type of pollution, as well as distribution of hazardous chemicals in nature. Nowadays, a number of studies have been based on the determination of chemical contaminants in animal tissues and organs. As the result of these findings, it is possible to estimate the level of human exposure to negative effects of these pollutants. The monitoring and control of game meat safety should include control measures for live animals, control measures during hunting and after shooting, guidelines for official meat inspection, control measures for carcass processing and surveillance of chemical residues (Petrović et al., 2014).

Since it fulfils numerous criteria (e.g. widely geographic distribution, relatively small home range, territorial living and browsing nutrition strategy, huge availability of basic data, relatively simple sampling procedure) roe deer has been often mentioned in the literature as a good or even excellent monitor of toxic elements burdens on the environment (Pokorny, 2000).

The purpose of this study was to evaluate the concentration of environmental contaminants lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd) and copper (Cu) in tissues of free-living roe deer in Serbia, as important information in performing assessments of the risk for both wildlife and humans. The obtained results were compared with relevant data reported from other countries.

MATERIAL AND METHODS

Samples of liver, kidney and meat of roe deer *(Capreolus capreolus)* shot by hunters were collected from hunting ground of Begeč settlement, municipality Novi Sad. Sampling of wild animal organs was performed during the 2013/2014 hunting season. Animals were selected according to neither sex nor age. Thus, liver and kidney samples were collected from each animal (total of

11 animals). Upon collection, all samples were placed into labeled plastic bags and stored at -18° C to avoid tissue degradation prior to analysis.

The samples (1g) were prepared applying the microwave (Ethos, Labstation Microwave, Milestone), digestion method (14) with the use of the mixture H_2O_2/HNO_3 (1:4, v/v). After this process, the samples were transferred to 50 mL volumetric flasks and diluted with deionized water. Analyses of Pb (NoG-M, IT 0.1 s/P), Cd (NoG-M, IT 1 s/P), As (He-M, IT 1 s/P), Hg (NoG-M, IT 1 s/P) and Cu (He-M, IT 0.1 s/P) were conducted by ICP-MS 7700 mass spectrometer (Agilent Technologies). Solutions used for calibration were prepared from commercial stock standard solutions with 1000 mg/l of each element (Accustandard). To calculate the recovery percentage, 6 samples of meat have been spiked with known amounts of Cd, As, Hg, Pb and Cu analytical standards. The obtained results are presented in Table 1.

Table 1: Isotopes, limit of detection (LOD) and recovery rates for monitored elements

Element	Isotope	LOD (mg/kg)	Recovery (%)
Cd	^{111}Cd	0.001	96.1
As	^{75}As	0.001	100.4
Hg	^{201}Hg	0.001	83.7
Pb	^{208}Pb	0.001	88.1
Cu	^{63}Cu	0.001	102.9

Statistical analysis was performed by the STATISTICA 12 software package, version 16.0. Data were grouped according to tissue and presented as mean ± standard error, minimum and maximum values.

RESULTS AND DISCUSSION

Average values of toxic and trace elements obtained in this study for the livers, kidneys and meat of all investigated roe deer are summarized in Table 2. The obtained values were compared with highest permissible hygienic limits for risk elements according to the maximum allowed levels (MAL) of particular contaminant in food in the Republic of Serbia (Official Gazette, 2011).

The results were compared with the results reported by other authors from our and other countries. Beside that comparison, a collation with some other countries could be interesting as well. An overview of some previous articles addressing toxic elements levels in tissues of free living roe deer *(Capreolus*

capreolus) and red deer (*Cervus elaphus)* is presented in Table 3.

Table 2. Toxic elements concentrations (mg/kg) in different tissues of roe deer

Material	n	**Cd**	**As**	**Hg**	**Pb**	**Cu**
		Mean±S.E. *Range*				
Liver	11	0.0527 ± 0.043 0.005-0.110	0.008±0.006 *0.002-0.017*	< 0.001	0.077 ± 0.092 *<0.001-0.222*	28.071±34.026 *3.913 -104.08*
Kidney	11	0.465±0.224 *0.166-0.818*	0.014±0.008 *0.005-0.031*	<.0.001	0.094±0.107 *<0.001-0.2900*	15.871±6.698 *0.6890 -28.390*
Muscle	4	0.005±0.008 *0.004-0.008*	0.014 ± 0.004 *0.01-0.02*	<.0.001	*0.008-**8.455***	4.2305±0.9411 *3.393-5.568*
MAL	mg/kg	liver 0.50 kidney 1.0 meat 0.05	liver 0.50 kidney 0.50 meat 0.10	liver 0.10 kidney 0.10 meat 0..03	liver 0.50 kidney 0.50 meat 0.10	liver 80.0

MAL -Maximum allowed level (Official Gazette, Republic of Serbia, 2011)

Concentrations of As, Cd and Hg in liver, kidney and muscle samples of roe deer did not exceed the MAL in either of the examined samples (Table 2).

The highest Cd-contamination (average value 0.465 mg/kg) was recorded in the kidneys of roe deer. Somewhat lower Cd levels were found in the liver (0.0527 mg/kg), while muscles were the least contaminated (0.005 mg/kg). The lowest and highest average concentrations of As were measured in liver (average value 0.008 mg/kg) and kidney samples (0.031 mg/kg), respectively. Mercury (Hg) was not detected in any of the investigated samples. Kidneys of roe deer revealed higher Pb-contamination than liver (average values 0.094 mg/kg in kidney and 0.077 mg/kg in liver). The Pb content in two muscle samples of roe deer was very high. High Pb concentrations measured in muscle samples are probably due to the proximity of gunshot wound, especially since such enormously high level of Pb in the examined muscle sample *show* a *strong discrepancy with* respect to Pb values in the liver and kidney (lead was not detected in samples of these organs). The maximum permissible level for Cu is prescribed only for liver (80 mg/kg). The average Cu level detected in the liver was 28.071±34.026 mg/kg, while the value exceeding MAL (104.08 mg/kg) was recorded in only one sample.

Table 3. Content of toxic elements (mg/kg) in different tissues of roe deer and red deer according to various authors

Material	As	Cd	Hg	Pb	Cu	Source, species and country
	Mean±S.E. Range					
Liver	n.i.	0.568±0.502 0.015-2.306	n.i.	n.i.	n.i.	Pompe –Gotal J. and Prevendar Crnić A. (2002) Capreolus capreolus Croatia
Kidney		4.905±6.395 0.223-27.686				
Muscle		0.018±0.019 0.003-0.065				
Liver	n.i.	1.06±0.77 (yearlings) 3.92±0.88 (2 year and more)	n.i.	0.71±0.65 <0.05-9.3	n.i.	Pokorny B. and Ribarič-Lasnik C. (2000) Capreolus capreolus Slovenia
Kidney		7.13±4.43 (yearlings) 22.73±8.92 (2 year and more)		0.03±0.01 <0.05-0.20		
Muscle		0.03±0.02 (yearlings) 0.04±0.01 (2 year and more)		0.05±0.03 <0.05-0.55		
Liver	n.i.	0.21±0.10	n.i.	1.40±0.01	n.i.	Kottferová J. and Koréneková B. (1998) Capreolus capreolus Slovakia
Kidney		2.63±2.24		0.25±0.18		
Muscle		0.02±0.03		0.12±0.03		
Liver	n.i.	0.70±0.39*	n.i.	0.17±0.11*	59±41*	Jarzyńsla G. and Falandysz J. (2011) Cervus elaphus Poland
Kidney		12±8 *		0.30±0.26*	21±4*	
Muscle		0.22±0.13*		0.18±0.34*	11±4*	
Kidney	n.i.	2.071±0.216 0.010-22.076	n.i.	n.i.	n.i.	Beiglböck C. et al. (2002) Capreolus capreolus Austria
Liver	n.i.	0.005-0.50	n.i.	0.077-0.108	n.i.	Bilandžić et al. (2009) Cervus elaphus Croatia
Kidney		2.28-5.91		0.058-3.77		
Muscle		0.005-0.80		0.04-6.69		
Liver	n.i.	0.08-0.79	0.01-0.03	n.i.	n.i.	Petrović et al. (2013) Cervus elaphus Serbia
Kidney		0.03-4.99	0.01-0.10			
Muscle		n.i.	n.i.			
Liver	n.i.	n.i.	n.i.	<0.05-9.30	n.i.	Pokorny (2000) Capreolus capreolus Slovenia
Kidney	< 0.02-0.43		<0.01-0.69	n.i.		
Muscle	n.i.		n.i.	n.i.		

Legend: n.i.- not investigated; **(dry weight)*

Similar results for Cd and Pb distribution were obtained by other authors, but our results for Cd concentrations are markedly lower as compared with other investigations (Table 3). Since the age was not taken into account in data interpretation in the majority of cited studies (Table 3), the levels of toxic element could not be directly compared with our research. Secondly, the bioindicative approach was emphasized in our investigation, but many of cited studies were chosen in the vicinity of local pollution sources (smelters, industrial and mining areas). Finally, some differences between species must be outlined. Red deer (*Cervus elaphus)* whose anatomy of the digestive tract is adjusted to the intake of wide spectrum of feed, receives more wood species (35% of the feed in summer, 30% of the feed amount in winter). Roe deer whose digestive tract is only poorly adjusted to the digestion of cellulose, prefers young summer plants, herbs, fruits (they have narrow leaves and retain less falling particles) but more wide-leaved feed (herbs, summer plants which retain more deposited pollutants) than the red deer. This fact is likely to be the main reason of the inter-species differences (Kottferová and Koréneková B., 1998).

CONLUSIONS

Average as well as maximum concentrations of toxic elements in the majority tissue samples examined during our investigation did not exceed the maximum permissible levels and the levels measured in tissues of European free-living roe deer. The levels of investigated toxic elements are well below the concentrations considered dangerous for animal's health. However, high concentrations of some toxic elements still confirm the imperative of imposing relevant control program that will include veterinary officials as well as hunters and other subjects involved in game meat chain.

AKNOWLEDGMETS

The presented work is part of the research done in the project **TR31084** granted by the Serbian Ministry of Education, Science and Technological Development

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Primljeno: 20.11.2015.
Odobreno: 15.01.2016.

Original scientific paper UDK 639.215(497.11)

INFESTATION OF *LARNAEA CYPRINACEA* (CRUSTACEA: CEPOPODA) IN DIFFERENT CATEGORIES OF COMMON CARP (*CYPRINUS CARPIO*) REARED IN SERBIA

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Abstract

Lernaea cyprinacea in common carp and other warm water fishes is one of the major problems of aquaculture industry in Serbia. Infestations with *Lernaea* are most prevalent in the summer months and occur more commonly in stagnant or slow-moving water bodies. The optimal temperature range for *Lernaea* development is 26–28°C. In the present study infection rate of *L. cyprinacea* in two different categories of fish pond cultured common carp was done. Pathological effects and control of the disease of infected fish were also followed. Fish sampling was done during the summer months of 2014, in 3 common carp fish ponds. One hundred twenty fish samples were collected from all carp ponds (n=120). After fish sampling collected *Lernaea* were transferred to the laboratory for parasite study. Prevalence of the infection was calculated for two age populations of carps. According to the obtained results, the parasite prevalence were significantly higher in carp fingerlings than in older carps ($P<0.05$). The highest numbers of parasites were found on fins and skin. Carps fry infestation of *Lernaea* caused intense inflammation and ulcers what leading to secondary bacterial and fungal infections. These secondary infections sometimes worsen and kill the fish. In carps for consumption *L. cyprinacea* infestation reduces the meat quality and lessening marketability of fish, because infected carps cause disgust of consumers and cannot be recommended for human consumption. Technological measures such as improvement of ambient conditions, adequate feed, optimum stock density, reduction of stress, good water quality and lime addition twice a week in quantity of 50 kg/ha are the most efficacy in combatting this disease.

Key words: *Lernaea cyprinacea,* common carp, fingerlings, infestation

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INFESTACIJA SA *LARNAEA CYPRINACEA* (CRUSTACEA: CEPOPODA) KOD RAZLIČITIH KATEGORIJA RIBNJAČKOG ŠARANA (*CYPRINUS CARPIO*) GAJENOG U SRBIJI

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Kratak sadržaj

Lernaea cyprinacea kod šarana i drugih toplovodnih riba jedan je od važnih problema ribarske industrije u Srbiji. Infestacija sa parazitima iz roda *Lernaea* najčešće se javlja tokom letnjih meseci i to uglavnom u stajaćim i sporo tekućim vodama. Optimalna temperature za razvoj *Lernaea* kreće se od 26–28°C. U ovom radu utvrđena je prevalenca lerneoze kod dve različite kategorije ribnjačkog šarana. Patološki efekti i kontrola bolesti kod zaraženih riba takođe su ispraćeni. Uzorkovanje riba rađeno je tokom letnjih meseci 2014 godine na tri šaranska ribnjaka. Ukupno je sakupljeno sto dvadeset uzoraka sa svih ribnjka (n=120). Nakon uzorkovanja sakupljeni paraziti *Lernaea* prebačeni su u laboratoriju radi identifikacije. Prevalenca infestacije urađena je za dve različite starosne kategorije šarana. U skladu sa dobijenim rezultatima prevalence je bila značajno veća kod mladunaca šarana nego kod konzumnog šarana ($P<0.05$). Najveći broj parazita nađen je na perajima i koži. Kod infestacije šaranske mlađi sa *L. cyprinacea* dolazi do inflamacija i stvaranja ranica što pospešuje pojavu sekundarnih bakterijskih i gljivičnih infekcija, koje mogu da dovedu do uginuća mlađi. Kod konzumnog šarana infestacija sa *L. cyprinacea* smanjuje kvalitet mesa i sbosobnost ribe da se plasira na tržište, jer takva riba izaziva gđenje potrošača što je čini neupotrebljivom za ishranu ljudi. U kontroli ovog obolenja najbolje je primenjivati tehnološke mere poput, adekvatne ishrane, optimalne gustine nasada, smanjenja stresa, dobrog kvaliteta vode i dodavanja kreča u količini od 50 kg/ha, dva puta nedeljno.

Ključne reči: *Lernaea cyprinacea,* šaran, mlađ, infestacija

INTRODUCTION

Lernaea cyprinacea Linnaeus, 1758 (Crustacea: Copepoda), commonly known as “anchor worm”, is an important crustacean parasite of freshwater

fish that has a wide geographic range. Parasite is widely distributed in Africa, Asia, North America and Europe, and there are reports about incidence of it in fish ponds and natural aquatic ecosystems (Demaree, 1967; Dorovskikh, 1993; Hoffman, 1999; Silva-Souza et al., 2000; Nagasawa et al., 2007; Hassan et al., 2008; Ćirković and Novakov, 2013). This parasite reproduces continuously in warm season and its adult form sets on the skin of common carp, grass carp, bighead carp and silver carp. *L. cyprinacea* have nine stages in the life cycle, including three free-living naupliar stages, five copepodid stages and one adult stage. After mating on the fish host, the males die and females metamorphose and insert their anterior body into the host tissue and then produce eggs (Nagasawa et al., 2007; Barson et al., 2008). Also, the parasite does not need an intermediate host for growth and development. The highest incidence of *Lerneaea* infestation is in warm season when the temperature is suitable for growth and reproduction of this parasite, i.e., 22-30 °C. At this temperature the life cycle completes within 17-22 days (Nagasawa et al., 2007; Barson et al., 2008; Madanire Moyo and Barson, 2010). If temperatures fall below 20°C, juvenile *Lernaea* are unable to complete their development and at 14°C, females will not reproduce. However, adult females can overwinter on the fish host, producing eggs when water temperatures warm up in the spring. *Lernaea cyprinacea* have serious deleterious effects on their freshwater fish hosts (Hoffman, 1999). *Lernaea* creates skin damages, reduces growth and osmoregulation ability, and also influences behavior and decreases fish resistance against stressful condition. Infection by the *Lernaea* can decrease the marketability of older fish intended for consumption. Lerneosis occurs in different age categories of common carp. The relationship between the size of cyprinid fishes and *L. cyprinacea* infections has been studied by Amin et al., (1973), Dorovskikh, (1993) and Pérez-Bote, (2000).

The main goal of the present study was to determine infection rate of *L. cyprinacea* in two different categories of fish pond cultured common carp, i.e., carp fry and carp for consumption. We also aimed to investigate its pathological effects, control of the disease and marketability of infected fish.

MATERIALS AND METHODS

The investigation was carried out during 2014, on 3 common carp fish farms in Serbia, Vojvodina province. Fish material included 1- to 4 yr-old pond-cultured common carp (*Cyprinus carpio*). Sampling at the fish farms was conducted out between June and August within the intervals of 7-14 days. One hundred twenty fish samples were collected from all carp ponds (n=120).

From each pond were collected 20 samples of young (fingerlings) and 20 samples of older (for consumption) fish.

The sampling program was done using nets. The external body surfaces as well as the gill chamber and mouth cavities of each fish were examined for the presence of adult parasite and penetration sites for anchored cepopods were localized. A method used by Barson et al., (2008) was used for *L. cyprinacea* parasite identification. All live and healthy fish were returned to the fish pond.

Data analysis was performed to determine significant differences in the two age categories of carps. Statistica 12 software and Excel (Microsoft Excel, 2007) were used for data analysis. Post-hoc Duncan tests were used for statistical analysis of differences. $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The infection parameters (the number of parasitized fish and prevalence) obtained *of L. cyprinacea* infestation on the 3 cyprinid fish ponds are given in Table 1. *L. cyprinacea* prevalence (Tabble 1, Graph 1) were significantly higher in carp fingerlings than in older carps which are ready for consumption ($P<0.05$). The highest numbers of parasites were found on fins (Figure 1), and skin. *L. cyprinacea* was not found in the nasal and oral cavities.

Table 1. Prevalence, number of positive and negative carps infected with *L. cyprinacea*

Age categories	Fish ponds	Number of sampled fish	Result		Prevalence (%)
			Positive	Negative	
Carp fingerlings	I	20	3	17	16,6 ± 3.75[a]
	II	20	4	16	
	III	20	3	17	
Carps for consumption	I	20	0	20	5 ± 2.21[b]
	II	20	2	18	
	III	20	1	19	

Prevalence values are mean ± SD (n=60). [a,b] Means with different letter indexes are significantly different ($P \leq 0.05$)

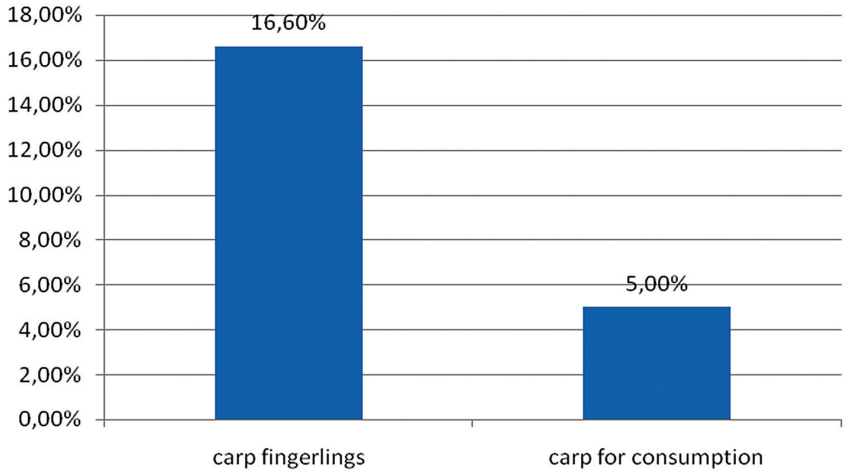

Chart 1. Comparison of *L. cyprinacea* percent prevalence between carp fingerlings and carps ready for consumption

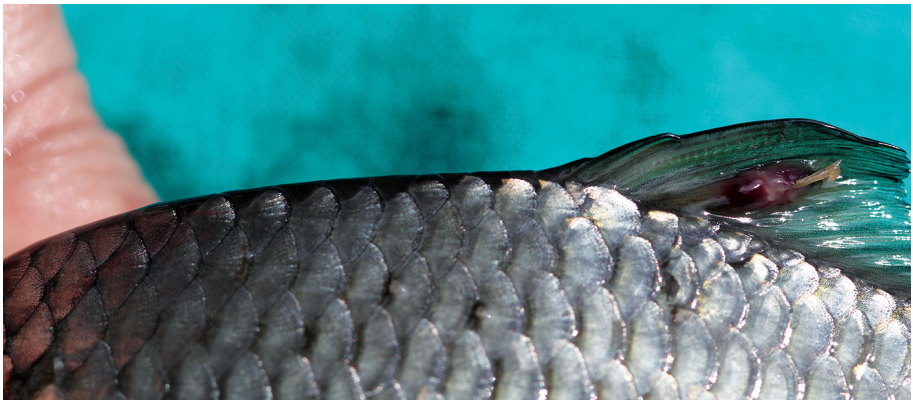

Figure 1. *L. cyprinacea* anchored to the dorsal fin of the carp fingerling.

Controlling many important parasitic diseases is still far from satisfactory condition, and more supervision is needed. Aquaculture development over recent decades and concerns about the prevalence of parasites have caused experts' more attention to this problem created by parasites and their importance in decreasing aquatic animals reproduction power. In general, the most important common crustacean parasites of fresh water fishes in Serbia is *L. cyprinacea*. *Lernaea* infestations are particularly common in cyprinids, especially in common carp, but also in koi, goldfish, and other related carp, altho-

ugh numerous other freshwater species are susceptible (Lester and Hayward, 2006). It is often present in all categories and species of freshwater fishes. So, it is necessary to pay attention to the incidence, pathological effects and control of this parasite, what is essential for carp aquaculture enhancing. In the present study prevalence was 16.6 % in carp fingerling, respectively 5 % in carps for consumption. This is because the parasites usually occur more frequently in younger than in older category of common carp, especially when fry are cultured in high stock density with poor body condition (Ćirković and Novakov, 2013). The parasite in cyprinid species seems to be mainly located in the fins. In the present study, the preferred site was the fins what is in accordance with the studies of Amin et al., (1973); Saraiva and Valente, (1988); Sterling et al., (1995); Dorovskikh (1996); Pérez-Bote (2000). The pathogenic effect comes down to the inflammation of the affected tissue. At the attachment places there is inflammation and ulcers with narrow white brim. These are the places which may become inhabited by pathogenic bacteria and fungi. Infected fingerlings are weak and anaemic (Ćirković and Novakov, 2013). In older caterogy the major problem is marketability of fish. Since this parasite is the arthropod that are found on the skin it is easily visible, should be removed before harvesting of fish. Otherwise, such carps cause disgust of consumers and cannot be recommended for human consumption. The most important is control of disease. In order to prevent the disease it is necessary to rear young fish separately from other fish categories and prevent weed fishes which can be source of the infection from entering fish pond (Ćirković et l., 2015). Several therapies are available for control of lernaeids; however, options are very limited for food fish and pond production (Francis-Floyd and Reed, 2011). The most efficacy measure is application of lime twice a week in concentration of 50 kg/ha. The improvement of ambient conditions, adequate feed and optimum stock density deliver best results.

CONCLUSION

Lerneosis is one of the most common diseases in freshwater fishes. *L. cyprinacea* prevalence was 16.6 % in carp fingerling, respectively 5 % in carps for consumption. *Lernea* in carp fry cause inflammation and ulcers at the attachement place which result in offten pathogenic bacteria and fungi infections. The major problem of *L. cyprinacea* infestation in older cytegory is reducing the quality and lessening marketability of fish. Technological measures (optimal density, feeding, reduction of stress, good water quality) and lime addition are key factor in combatting this disease.

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Primljeno: 20.12.2015.
Odobreno: 15.01.2016.

Original scientific paper UDK 619:612.017:636.1

IMMUNITY TO *RHODOCOCUS EQUI* INFECTION IN HORSES

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Abstract

Rhodococcus equi is an opportunistic bacterium that commonly infects foals and immunocompromised patients. Due to the large economic losses that it can cause in the field of horse breeding, the microorganism has been studied in details, including its immunological aspect. Within the humoral immunity, the most important immunoglobulins are those of the class G (IgG), produced as a response to the surface antigen associated with virulence (virulence associated protein A, VapA). IgG antibodies provide resistance to pneumonia in foals and have a dose dependent protective effect. In addition to them, the protective role of plasma is achieved through various cytokines. Cellular mechanisms are important for killing bacteria within the macrophage. Virulent strains which carry a plasmid with the gene for VapA stimulate the production of interferon gamma (IFN-γ), a key cytokine to kill these bacteria. The presence of IFN-γ is crucial for the removal of microorganisms from the lungs and prevention of formation of pulmonary granulomas. For the complete removal of bacteria cooperation of the humoral and cellular immunity is necessary. Particularly significant is opsonization, which increases phagolysosomal fusion. Vaccination and

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application of hyperimmune plasma play a vital role in the treatment of disease. Only alive and virulent bacterium is capable of producing protective immunity in horses. The use of hyperimmune plasma in foals results in a lower percentage of sick animals and less severe clinical progression of the disease. Further research is needed in order to create a safe and effective vaccine.

Key words: *Rhodococcus equi*, immunity, horses, vaccine

IMUNITET KOD KONJA NA *RHODOCOCCUS EQUI* INFEKCIJU

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Kratki sadržaj

Rhodococcus equi je uslovno patogena bakterija koja najčešće inficira ždrebad i imunokompromitovane pacijente. Zbog velikih ekonomskih gubitaka koje može da izazove u uzgoju konja, mikroorganizam je detaljno proučen, između ostalog sa imunološkog aspekta. U okviru humoralnog imuniteta, najznačajniji su imunoglobulini klase G (IgG), nastali na površinski antigen udružen sa virulencijom (virulence associated protein A, VapA). IgG povećavaju otpornost ždrebadi na pneumonije, a imaju dozno zavisno zaštitno delovanje. Pored antitela, zaštitna uloga plazme ostvaruje se preko različitih citokina. Celularni mehanizmi ubijanja su značajni zbog ubijanja bakterije unutar makrofaga. Virulentni sojevi koji su nosioci plazmida sa genom za VapA stimulišu produkciju gama interferona (IFN-γ),

ključnog citokina za ubijanje bakterija. Prisustvo IFN-γ je presudno za uklanjanje mikroorganizma iz pluća i sprečavanje nastanka plućnih granuloma. Za potpuno odstranjenje bakterija, neophodna je kooperacija humoralnog i celularnog imuniteta. Posebno je značajna opsonizacija, koja povećava fagolizozomalnu fuziju. Vakcinacija i primena hiperimune plazme zauzimaju značajno mesto u terapiji bolesti. Samo živa i virulentna bakterija je sposobna da izazove zaštitan imunitet kod konja. Primena hiperimune plazme kod ždrebadi rezultuje manjim procentom obolelih jedinki i manje ozbiljnim kliničkim tokom bolesti. Neophodna su dalja istraživanja u cilju kreiranja bezbedne i efikasne vakcine.

Ključne reči: *Rhodococcus equi*, imunitet, konji, vakcine

INTRODUCTION

Rhodococcus equi (*R. equi*) is an intracellular bacterium that survives mechanisms of phagolysosomal fusion, which compromises the effectiveness of antibiotic therapy (von Bargen and Haas, 2009). The microorganism is present in the soil, on all continents except Antarctica (Prescott, 1991).

It causes diseases in various animal species (Takai *et al*., 1996; Suvajdžić, 2000; Suvajdžić *et al*., 2001; Flynn *et al*., 2001; Takai *et al*., 2003; von Bargen and Haas, 2009) but predominantly in horses (Prescott, 1991; Muscatello, 2012a; Suvajdžić *et al*., 2015), which is why this bacterium is called "equinocentric" (Vázquez-Boland *et al*., 2013).

It can be found in the soil of 50-95% households which breed horses, as *R. equi* is present in horses' feces in very high concentrations. In the feces of mares, *R. equi* is present in concentration from 10^2 to 10^3 colony forming units (CFU) per gram. It is isolated from the feces of foals, starting from the first week of life. In the feces of four weeks old foals it is present in the amount from 10^4 to 10^5 CFU. Such high concentration stays up to 8 and 10 weeks of age, when it starts to decrease and, as foals are maturing, settles to the concentration characteristic for mares (Takai, 1997). This is in accordance with the research of optimal doses for the foal experimental challenge. The dose of 10^2 CFU gave mild clinical presentation, mimicking natural infection (Sanz *et al*., 2013). The highest excretion by feces, 10^6 to10^8 CFU per gram, appears in the period until 8 weeks of life, precisely at the time when the foals are most receptive to infections (Takai, 1997).

In human population, infections by *R. equi* often affect immunocompromised patients (Weinstock and Brown, 2002; Suvajdžić and Považan, 2006; Tuon *et al*., 2007). Ten percent of infected patients were already being treated with

immunosuppressive drugs, which are an integral part of the therapy during organ transplantation and in autoimmune diseases (La Rocca *et al*., 1998). It is believed that about two-thirds of patients infected with *R. equi* suffer from HIV (Harvey and Sunstrum, 1991). However, it can also cause diseases in immunocompetent persons (Kedlaya *et al*., 2001; Suvajdžić, 2004; Suvajdžić *et al*., 2015).

Managing control and prevention of this disease in veterinary medicine represents an economic category for the single reason that losses which may occur in the case of an epidemic in stables, especially of thoroughbred horses, would be enormous. In addition to general hygiene and sanitation, specific active and passive prophylaxis should occupy the place they deserve in the system of overseeing animal health (Muscatello, 2012a).

IMMUNITY IN *RHODOCOCCUS EQUI* INFECTIONS

Rhodococcus equi usually infects foals and very rarely adult horses (Vázquez-Boland *et al*., 2013). Infections occur sporadically, except in foals, where they can have enzootic character. The immune system has a key role in development of infection. In foals, the infection is associated with the period of vanishing of maternal antibodies present in colostrum. In adult animals, accommodation, food and environmental conditions have a significant role in the course of infection, mainly due to their effect on the general immunological conditions (Muscatello, 2012b).

Infection of foals occurs within the first week of life. The onset of clinical signs occurs approximately in 50 days. This also indicates that the incubation period is longer in natural infections, when compared to extremely high inocula sizes used in experiments. (Horowitz *et al*., 2001).

There are implications that some mares give birth to foals that are particularly sensitive to *R. equi* infections. Such sensitivity can be explained by low levels of colostrum antibodies, functional immaturity of neutrophils in some foals, or genetic predisposition to infections (Wilks *et al*., 1982).

Due to the big impact on the health of horses, studies of immune response to infection with *R. equi* were mostly conducted on horses, in addition to murine models. Many aspects of immunological reactivity of horses were investigated, including both cellular and humoral mechanisms.

HUMORAL IMMUNITY IN HORSES

The digestive tract is probably the main source of antigenic stimulation of *R. equi* in horses. In foals, the level of maternal antibodies decline to the

lowest level in the period of eight weeks of age, after which foals are actively producing antibodies. Foals with low levels of colostral antibodies, detected by an ELISA test, are particularly vulnerable to rhodococcal pneumonia (Hietala and Ardans, 1987). Those with higher levels of antibodies show a milder form of the disease (Martens *et al*., 1989). Accordingly, the maximum sensitivity of foals occurs in the period between 2 and 6 months of age (Hines *et al*., 1997).

The protective role of antibodies that are produced as the response to the surface antigen virulence-associated protein A (VapA) was shown. The G class of immunoglobulins (IgG), created against this antigen was present in bigger concentration when compared to the concentration obtained by immunization with intact but killed *R. equi*. Also, the increase in the mare serum opsonizing activity is more pronounced in the group that received the VapA antigen compared to the group that received the whole bacteria (Cauchard *et al*., 2004).

Immunoglobulins created to VapA can be considered a diagnostic parameter in horses' infection, (Sanz *et al*., 2016), bearing in mind that this antigen is unique to equin *R. equi* - human or other animal's isolated strains did not posses this antigen (Occampo- Sosa *et al*., 2007)

In the study of IgG to VapA and IgG subclasses, it is shown that VapA- specific IgG(T) is the first antibody with the increase of titar. Also, this immunoglobulin is potentially revised as a prognostic parameter in detection of naturally occurring rhodococcal pneumonia (Sanz *et al*., 2015).

After infection, subclasses of detected antibodies include IgGa, IgGb, IgGc and IgM, with significantly higher levels of IgGa and IgGb subclasses in foals than in horses. (Jacks *et al*., 2007a) Isotype IgGa is the most important in the prevention of pneumonia in foals (Hooper-McGrevy *et al*., 2003).

Antibodies against the VapA antigen administered to mice showed a dose-dependent protective effect. After exposure to *R. equi,* the control group without applied antibodies died. The group that received a lower dose of the antibodies was partially protected, although the pathohistological examination showed bacteria in the spleen or lungs. In the mice that received the full dose, there were no deaths or signs of the disease, nor was the microorganism found in their tissues (Fernandez *et al*., 1997).

However, in a study that examined the passive immunization, in a colt with very low titer of antibodies, there have been no clinical manifestations of the disease, while another colt with a high titer developed serious illness with *infausto* prognosis. Levels of antibodies are not always in correlation with the opsonizing activity. Therefore, it is assumed that non-specific plasma factors, such as lymphokines and interferons, can also impact the protective effect of the immune plasma (Martens *et al*., 1989).

CELLULAR IMMUNITY IN HORSES

Bearing in mind the intracellular nature of *R. equi*, it is believed that cell-mediated mechanisms are the most effective against this disease (Vázquez-Boland *et al*., 2013). Aside from the disappearance of maternal antibodies, the decreased killing capacity of macrophages in foals contributes to their susceptibility in age of 1-5 months. (Berghaus *et al*., 2014)

The study which compared bronchoalveolar and monocyte- derived macrophages, revealed that the replication of *R. equi* is greater in bronchoalveolar macrophages, with 3- 100 fold increase during 48 hours (Berghaus *et al*., 2014). This is logical, having in mind that predominant route of infection is aerosol inhalation of *R. equi* (Giguère *et al*., 2011a).

Rhodococcus is able to survive intracellularly and to replicate in macrophages, both in murine peritoneal, as well as equine alveolar. The number of bacteria increases with time after the initial phase lag period of the first 6-12 hours of infection, during which the number of intracellular bacteria remains unchanged. During the subsequent 36 hours, the number increases five fold or more, to the inability of quantification, while preserving cell viability. A similar phenomenon was observed with mycobacteria, with the fact that *R. equi* more rapidly divides. Replication within macrophages is characteristic of *R. equi* strains that possess VapA (Hondalus and Mosser, 1994).

The first step in intracellular killing of pathogens is macrophage activation by interferon gamma. The CD4+ and CD8+ T lymphocytes from the lungs are able to produce interferon gamma (INF-γ) (Hines *et al*., 2003). Pulmonary clearance of the pathogen depends precisely of the increase in the number of pulmonary T cells and IFN-γ production (Kanaly *et al*., 1995; Hines *et al*., 2001; Hines *et al*., 2003).

In studies conducted on mice, it has been shown that there are two phenotypes of CD4+ T lymphocytes, which are referred to as Th1 and Th2. The classification was based on the cytokines they produce: IFN-γ is characteristic of the Th1 phenotype, IL-4 of the Th2. The transfer of adequate cell lines, demonstrated that Th1 phenotype led to the complete removal from the lungs (zero CFU), while in the Th2 lines led to the development of pulmonary granuloma. Natural killer (NK) cells can also produce INF-γ, but transferring them alone does not lead to clearance of bacteria from the lungs (Kanaly *et al*., 1996). The production of IFN-γ in horses is triggered by VapA. It causes the same later response in horses as well as in mice (Hines *et al*., 2001; Lopez *et al*., 2002). Since the protein VapA is encoded by the *vap*A gene located on the plasmid (Tan *et al*., 1995), only the virulent strains, carriers of this plasmid, may be removed from the lungs (Hines *et al*., 2003).

Infection of macrophages by *R. equi* leads to a rapid translocation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) from cytoplasm into nucleus, resulting in production of large amounts of inflammatory cytokines. Some of the most important are TNF, interleukin IL-12 and nitric oxide, NO. There is an assumption that these paths of non specific immunity are the main reason why the microorganism rarely causes diseases in immunocompetent adult animals (Darrah *et al*., 2004).

In a study on normal human peripheral blood mononuclear cells it has been demonstrated that they secrete TNF-α, IL-6 and IL-8 when they are stimulated with killed *R. equi*. Levels of TNF-α were increasing during the first 48 hours, wherein the 90% of secreted cytokines were detected after 36 hours. For IL-6, this level was reached after only 12 hours. Production of IL-8 was at its maximum at the beginning, and leveled off between 12 and 48 hours (Pece *et al*., 1997).

The effect of foal's age on cytokine production is also revised. The expression of production signals in bronchoalveolar macrophages for IL-1β, IL-10, IL-12 p40 and IL-8, as well as IL-1β and IL-12 p40 production signals in bronchoalveolar lavage macrophages in 1-3 days old foals is higher than in foals of other age. This also indicates exposure to infection with *R. equi* in very early period of life (Berghaus *et al*., 2014).

It has been shown that the lack of galectin-3 receptor increases resistance to *R. equi* infections. Mice with and without this receptor were compared. Macrophages lacking this receptor are less susceptible to *R. equi*, its reproduction and survival. This receptor normally provides a balanced response to nonspecific immunity, affecting the production of IL-1β by macrophages (Ferraz *et al*., 2008).

COOPERATION OF HUMORAL AND CELLULAR IMMUNITY

The best descriptions of important interactions between cell-mediated immunity and humoral immunity come from *in vitro* studies that have investigated the mechanisms of killing *R. equi* by macrophages (Hietala and Ardans, 1987).

Alveolar macrophages in foals experimentally exposed to *R. equi* phagocyte and kill both non opsonized and opsonized microorganisms more efficiently than alveolar macrophages in foals that were not exposed to the bacteria. Macrophage activity in foals which were already in contact with *R. equi*, was similar to the macrophage activity in adult horses. However, the rate of killing of opsonized *R. equi* was slightly lower in foals than in horses. In

all tested alveolar macrophages, opsonization of *R. equi* significantly increases phagolysosomal fusion when compared to nonopsonized bacteria (Hietala and Ardans, 1987; Giguère *et al*., 2011).

The use of plasma with antibodies also decreased bacterial viability in the extracellular matrix, but not in the macrophages (Dawson *et al*., 2011).

IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY IN HORSES

Conventional therapy with antibiotics, with some being more effective than others (Giguère *et al*., 2015), is practically impossible without immunological support, with nature taking its course (Berghaus *et al*., 2011). Scientific attempts, with various approaches to increasing antibiotics in target sites included streptolysin O (Horohov *et al*., 2011; Gurel *et al*., 2013) and liposomal forms of antibiotic (Burton *et al*., 2015). In this way, we can "help nature" with immunomodulating support.

Field research conducted by Magnusson in 1923 and 1924 failed to justify vaccination with an inactivated culture of *R. equi*. Vaccination of foals with isolates of *R. equi,* which were inactivated with formalin, failed to protect animals from a strong intratracheal exposure to the bacteria (Magnusson, 1923).

Lopez *et al*. (2008) tested a live attenuated strain of *R. equi* which was a riboflavin auxotroph on a mouse and a colt. This strain has kept the virulence plasmid. However, in a vaccine it is important to balance immunogenicity and attenuation. It is shown that such a vaccine must increase immunogenicity to demonstrate its success in neonatal foals. In order to make disease prevention more effective on endemic farms, a vaccine should be able to induce an immune response very early in life (Lopez *et al*., 2008). Currently, there is no vaccine, but new candidates are being tested (Cauchard *et al*., 2013).

Other attempts with immunostimulans containing *Propionibacterium acnes* showed no effect as imunomodulators in therapy in foals (Sturgill *et al*., 2011).

Pneumonia in foals can be prevented by vaccination of pregnant mares. These vaccines contained VapA and exoenzim. The result of this research was the decline of mortality rates in cases of rhodococcal pneumonia on farms that have vaccinated mares from 3% to 1.2% (Becú *et al*., 1997).

Studies on a few endemic farms in Japan, United States and Argentina have shown that administration of hyperimmune plasma in foals led to protection in the earliest period of life. Some of the recommendations for prevention of pneumonia are: separation of foals in clean and ventilated paddocks, serological testing on 30th and 45th days after birth and immunization with plasma

immediately after birth (Higuchi *et al*., 1999), no later than second day of life (Giguère et al., 2011a; Vázquez-Boland *et al*., 2013)

Unspecific measures, such as feces removal from paddocks, avoiding spreading manure on pastures, irrigation of holding pens, foaling at pastures- gave unsatisfying results (Giguère *et al*., 2011b)

CONCLUSION

Only alive and virulent *R. equi* bacteria can cause immunity in mice. Avirulent live strains and the strains that were killed by heat did not show this activity (Takai *et al*., 1999). Effective immunization against intracellular bacterial pathogens requires the use of living microorganisms, rather than inactivated, in order to initiate a cellular immune response (Collins, 1988).

In passive immunization, the quality of donor's plasma is crucial: immunized donor's plasma protects significantly from severe illnesses when compared to non immunized donor's plasma (Martens *et al*., 1989). *In vitro* reaction of passively immunized foals was similar to actively immunized mares (Becú *et al*., 1997).

In our country there is no immunoprophylaxis or immunotherapy for *R. equi*. Would it be reasonable to take into consideration collecting indigenous isolates, determining antigens and creating a vaccine that covers the antigenic determinants (including VapA) present in our area? Serum production on the same isolates could be professionally, scientifically and economically justified.

AKNOWLEDGMENTS

This work has been financially supported by a grant from Ministry of Education, Science and Technological Development, Republic of Serbia, Project numbers III 46012 and III 41012.

The authors wish to thank Milica Radovanović for her revision of the English language.

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Primljeno: 25.11.2015.
Odobreno: 15.01.2016.

Original scientific paper UDK 619:616.988:636.2

THE PROPOSAL OF A PROGRAM FOR CONTROL, SUPPRESSION AND ERADICATION OF BOVINE VIRAL DIARRHEA VIRUS INFECTION IN CATTLE HERDS IN THE REPUBLIC OF SERBIA

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Abstract

Bovine Viral Diarrhoea (BVD) is a viral infection of cattle caused by Bovine Viral Diarrhoea Virus (BVDV). The most important source of infection is persistently infected and sick cattle, but also other susceptible species. BVDV infection, except for cattle, occurs in sheep, pigs, goats and wild ruminants. These animals can be reservoirs of the virus, and thus the source of infection. The greatest economic losses caused by BVDV infection in cattle farming are the direct consequences of transplacental infection, as a result of foetal deaths, congenital malformations, neonatal and postnatal mortality, including mucosal diseases and slow growth and poor performance results of the surviving animals, as well as acute infections of respiratory and alimentary infections. Economic losses due to BVDV infection can be seen in a cattle herd even several years after the infection. Moreover, BVDV infection is considered as the third most important disease to cattle farming industry, immediately after rinderpest and the foot-and-mouth disease. The primary task of BVDV infection control is to prevent prenatal infection. This procedure includes the determination and elimination of persistently infected (PI) animals from the herd. After the removal of these animals, great attention must be paid to introducing new animals into the herd and preventing the occurrence of transplacental infection. On the basis of existing data and real suppositions about the distribution of BVDV infection in the territory of Serbia, the great economic damages it causes, as

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well as the general tendency to resolve problems in European states, and for the competitiveness of our farmers and food industries in the production of milk, meat or breeding material after joining the EU market, it is necessary to draft legal regulations in the control of this bovine viral disease in Serbia. The mentioned control could be based on the voluntary BVDV eradication program on herd's level in the beginning and on establishing herds with BVDV free status. Certification of BVDV free status of herd will be done by Veterinary Directorate, and the register of herds with BVDV free status could be "on line" available and maintained by the Veterinary Directorate. The essence of this proposal, a volunteer program of establishing herds with BVDV free status, is based on several steps or phases, which includes frequent diagnostic testing, removal of PI animals from the herd and the introduction of strict biosecurity measures.

Key words: BVD, control program proposal, Serbia

PREDLOG PROGRAMA KONTROLE, SUZBIJANJA I ISKORENJIVANJA INFEKCIJE IZAZVANE VIRUSOM GOVEĐE VIRUSNE DIJAREJE U ZAPATIMA GOVEDA U REPUBLICI SRBIJI

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Kratak sadržaj

Goveđa virusna dijareja (Bovine Viral Diarhea – BVD) je virusna infekcija goveda čiji je uzročnik virus goveđe virusne dijareje (Bovine Viral Diarhea Virus – BVDV). Najznačajniji izvor infekcije su perzistentno inficirana i bolesna goveda, a mogu biti i druge prijemčive vrste. Infekcija BVD virusom, osim kod goveda, javlja se kod ovaca, svinja, koza i divljih preživara. Ove životinje mogu biti i rezervoari virusa, a samim tim i izvor infekcije.

Najveće štete koje BVDV infekcija nanosi govedarstvu su direktne posledice transplacentarne infekcije, kao rezultat fetalnih uginuća, kongeni-

talnih malformacija, neonatalnog i postnatalnog mortaliteta uključujući i bolest sluznica, slab rast i performanse preživelih životinja, kao i infekcije respiratornog i digestivnog trakta. Ekonomski gubici prouzrokovani BVDV infekcijom mogu se ispoljavati u zapatu goveda i nekoliko godina nakon infekcije, naime BVDV infekcija se smatra trećom bolesti po ekonomskom značaju u govedarstvu odmah iza kuge goveda i slinavke i šapa. Primarni zadatak kontrole BVDV infekcije je prevencija prenatalne infekcije. Ovaj postupak obuhvata i ustanovljavanje i otklanjanje perzistentno inficiranih (PI) jedinki iz zapata. Nakon otklanjanja PI životinja veliku pažnju treba posvetiti unošenju novih grla u zapat i sprečavanju pojave transplacentarne infekcije. Bovina virusna dijareja je značajno raširena u celom svetu. U razvijenim zemljama, procenat serološki pozitivnih životinja se kreće između 60-90%. Ispitivanja raširenosti ove infekcije na području Srbije koja se sprovedena u poslednjih 15 godina ukazuju na njenu značajnu raširenost na području cele države i to kako u malim tako i u velikim zapatima goveda. Na osnovu ustanovljenih podataka i realnih pretpostavki o raširenosti BVDV infekcije na području Republike Srbije, velikih ekonomskih šteta do kojih ona dovodi, kao i na tendenciju rešavanja problema u evropskim državama i konkurentnosti stočara i prehrambene industrije iz Srbije u proizvodnji mleka, mesa ali i priplodnog materijala nakon pridruživanja tržištu EU, postoji potreba pokretanja regulative u kontroli ove virusne bolesti goveda u Republici Srbiji. Pomenuta kontrola bi se u početku mogla bazirati na dobrovoljnom programu eradikacije BVDV infekcije na nivoima zapata i uspostavljanju zapata slobodnih od BVDV infekcije. Sertifikaciju zapata bi vršila Uprava za veterinu i vodila „*on line*“ dostupan registar takvih zapata sa svim podacima o zapatu i datumom važenja sertifikacije zapata. Suština ovog predloga volonterskog programa uspostavljanja zapata slobodnih od BVDV infekcije se zasniva na nekoliko koraka odnosno faza, koje obuhvataju učestala dijagnostička ispitivanja, uklanjanje PI jedinki iz zapata i uvođenje strogih biosigurnosnih mera.

Ključne reči: BVD, predlog programa kontrole, Srbija

INTRODUCTION

Bovine viral diarrhoea (BVD) is virus infection of cattle, which still remains a mystery for both cattle owners and clinician veterinarians and scientists even if it was first described decades ago by Olafson et al., in 1946. The causal agent is one of the smallest RNA viruses (Bovine Viral Diarrhoea Virus – BVDV) from the genus *Pestivirus,* family *Flaviviridae.* The most common

source of infection are persistently infected and sick cattle, but also some other susceptible species such as sheep, swine, goat and a range of wild ruminants. These animal species could be also potential virus reservoirs in nature (Nettleton and Entrican, 1995).

The pathogenesis of BVDV in cattle is highly unique and manifests distinct features that are not observed in other infections of viral aetiology (Houe, 1999). Intrauterine infection of the foetus before 125 gestation day, because of the immaturity of foetal immune system, results in *recognizing* viral antigens as the own *antigens, which in turn induces immune tolerance and persistent infection after they are born* (Bronwlie et al., 1984; Bronwlie, 1991). *Such persistently infected (PI) animals are usually born as clinically healthy, yet harbouring the virus that continues to replicate in their bodies and shedding it into the environment for life. In PI animals (mostly aged 6-24 months) mutation* of the existing persistent noncytopathic (*ncp)* biotype of the *virus* to the cytopathic (*cp)* biotype occurs and consequently produces clinical form of mucosal disease (MD) with rapid fatal outcome. In view of that, the consequences of foetal infection including persistently infected (PI) animals and subsequent development of fatal mucosal disease are unique feature of BVDV infection (Bronwlie, 1991; Houe, 1999). Existence of noncytopathic and cytopathic viral strains and their complex relation toward susceptible animals, especially pregnant cows and their foetuses, as well as potential transmission to other susceptible species such as domestic and feral ruminants and pigs strongly emphasize the importance of better understanding of this viral infection of cattle.

BVD infection of cattle is characterized by a wide spectrum of clinical manifestations including transient fever, viral diarrhoea of cattle, respiratory disturbance, lower conception rate in breeding females, abortions, foetal mummification, congenital malformations, immune tolerance and persistent infection progressing to MD. Moreover, subclinical infection associated with leucopoenia and immunosuppression can occur (Baker, 1987).

BVD virus infection, both acute and subacute, is commonly associated with rather silent clinical manifestation or has a subclinical course. Thus, the infection often remains unnoticed in cattle herds or is neglected (Roeder and Harkness, 1986; Paton et al., 1999). The situation changes when the infection occurs in pregnant animals causing transplacental infection of the foetus. Substantial health and economic losses are due to frequent failed insemination, stillborn calves with congenital malformations, absence of conception, prolonged service periods, early embryonic mortality, abortions or delivery of PI offspring (Baker, 1987). Such persistently infected progeny are apparently clinically healthy yet remain viraemic for life and shed huge amounts of the

virus into the environment via their *secreta* and excreta. Consequently, those animals are the most important reservoirs of the virus in the herd throughout their life (Baker, 1987; Bronwlie, 1991). The outcome of persistent infection is always fatal and due to clinical form of mucosal disease. In addition to the aforementioned direct losses, indirect damage associated with BVDV infection is far too broader and immeasurable for both individual herds and entire cattle production in the country.

The greatest damage for cattle industry associated with BVD directly implicate transplacental infections and consequent foetal mortality, congenital malformations, neonatal and postnatal mortality as well as mucosal disease, poor growth and performance of survivors (Roeder and Harkness, 1986). Considerable economic losses caused by BVDV infection can endanger the herd even for years after infection. For example, economic damage of BVDV infection on 14 cattle herds in The Netherlands was calculated to some 24-161 US Dollars per cow with an average loss of 77 USD (Houe, 1995). In Great Britain, total annual economic loss from BVDV infection is estimated to some 120 million British Pounds (Bitsch and Ronsholt, 1995). Calculated losses associated with BVDV in Denmark are some 17 million USD per year, whereas economic damage attributed only to mucosal disease in eight Danish cattle herds with about 115 animals ranged between 2,380 and 2,980 USD per farm (Houe, 1995).

These data clearly indicate the importance of BVD in cattle production. From economical point of view, BVDV infection is considered the third most important disease in cattle husbandry, immediately after Rinderpest and Foot-and-Mouth Disease. These are only some aspects of health problems and economic losses associated with BVDV infection. The abovementioned examples clearly suggest the rationale for a research study on occurrence, suppression and eradication of BVDV infection as well as resulting positive production effects. Such positive results also include the trade of breeding material free from BVDV infection and consequent improvement of the quality of cattle production in Europe and worldwide (Petrović et al., 2003/a).

In the Republic of Serbia, the first cases of BVD infection were identified on the basis on clinical picture and serological examination in 1966 and 1968, respectively (Cvetnić, 1983). Initial research on the prevalence of infection in Serbia date back to 1973, when testing for the presence of BVDV-specific antibodies was performed on 224 blood sera of cattle from six farms (four from the region of Banat and two from the regions of Belgrade and Valjevo). The results revealed 166 (74%) seropositive animals (specific antibodies were detected in blood of animals that were in contact with the virus, i.e., infected).

The percentage of seropositive cattle at individual farms ranged between 38.8% and 91% (Belić et al., 1973).

By the beginning of 1990s, the examination of cattle from two farms in the area of Valjevo was performed. Of the 86 examined calves from a beef herd 48 (55.81%) were seropositive, whereas the rate of seropositive animals from a dairy farm with total 178 cows ranged between 30.55% and 52.24% depending on the age (Kurčubić, 1993).

A range of serological surveys conducted during last 15 years revealed infection rates in the range of 0-100%, depending on the farming system, herd size and territory. The percentage of seropositive animals is higher in northern and central parts of Serbia compared to the southern regions. The majority of big cattle herds (formerly public-owned farms) are characterized by high BVDV seroprevalence rate (the prevalence of seropositive animals ranges from 60% to even 100%). However, some of those herds are absolutely free from infection (Petrović et al., 2001; Petrović, 2002; Molnar et al., 2003; Petrović et al., 2003/a; Milošević et al., 2004; Petrović et al., 2004/a; Petrović, 2006).

The percentage of seropositive animals among cattle population in small herds or backyards (few cows in a household) is significantly lower, ranging from settlements that are completely seronegative to some villages with low percentage (up to 20%) of seropositive animals. Still, there are some exceptions – in some areas the seropositivity of even 60% has been observed among cattle population in particular villages or regions (Petrović T. et al., 2002; Petrović M. et al, 2002; Molnar et al., 2003; Petrović et al., 2004/a; Petrović et al., 2005; Petrović, 2006). This is mostly attributed to frequent purchasing of breeding material from big and infected herds or collective grazing of cattle on common land. Considering the wide spread of BVDV infection in our epizootical regions understanding of its economic implications as well as control options is of crucial importance (Petrović et al., 2003/b).

The first extensive researches of isolation and genotyping of BVDV isolates in Serbia were performed in the period 1999-2004 revealing the presence of BVDV genotype 1 and subtypes 1f, 1b and 1g (Petrović et al., 2004/b; Petrović, 2006). At that time, BVDV2 genotype has not been identified in Serbia; however, it was introduced in 2008 through cattle trade (unpublished information).

According to the obtained data and convincing anticipation about prevalence of BVD infection in our country, substantial economic losses as well as the current problem-solving trends in EU countries and having in mind the competitiveness of our cattle owners in meat and dairy industry and in the production of breeding material after accession to EU market, the need for relevant legislation pertaining to management and control of this viral disease in

Republic of Serbia *cannot be emphasized enough. It is proposed that the control of infection should be initially based on a BVDV voluntary eradication program at herd level and establishment of BVDV-free herds. Certification of free herds would be administered by Veterinary Directorate, which would provide an online available database of BVDV-free herds containing relevant data on the herd and validity date of the certificate.*

This article gives an overview of procedures for control and eradication of BVDV infection in the world. Also, a draft of the Program for the control, suppression and eradication of BVDV infection in cattle herds in the Republic of Serbia is presented, that is, preparatory procedures and methods for laboratory and epizootiological testing and monitoring aimed at elimination of PI animals as major infection source as well as obtaining and maintaining of BVDV-free status of the herd.

CURRENT STATUS AND PERSPECTIVES OF BVD IN SERBIA AND WORLDWIDE

BVD infection is widespread among cattle population worldwide. When speaking from an epizootiological point of view, the presence of infection is evident in almost all countries. The rate of infected animals, that is, that were in contact with the virus ranges between 60 and 90% in countries with developed cattle industry. In the territory of former Yugoslavia, the infection has been first described by Đuričković et al., in 1966 on the basis of clinical picture and pathomorphological finding. Serological confirmation was realized by Cvetnić et al., in 1968 (Cvetnić S., 1983). The researches on the incidence of the infection in the territory of Serbia performed during past 20 years strongly indicate its significant prevalence throughout the country.

The strategy for control and eradication of BVDV infection has initially been launched in Scandinavian countries. The program was based on identification and elimination of PI animals from the herd without vaccination. Norway was the first country that understood the actual problem of BVDV infection and initiated the eradication program in 1993. Bulk milk samples originating from 26,430 dairy herds were examined using indirect ELISA. Other Scandinavian countries followed the procedure. Essentially, the program relies on the following steps: 1) identification of BVDV free herds, 2) prevention of the infection in these herds and 3) reduction of the number of infected herds (Waage et al., 1996). Reduction of the number of infected herds was accomplished through identification and removal of PI animals from the herd as well as prevention of acute infection outbreak (Oirshot et al., 1999;

Bitsch and Ronsholt, 1995; Waage et al., 1996). If the repeated laboratory test has confirmed the presence of antibodies in the herd, bulk milk samples from 3-5 first-calf heifers were tested. In case of positive finding, further testing was performed using blood sera of five young animals aged 8-12 months. Herds suspected to have one or more PI animals underwent rigorous ban on trade with livestock or grazing cattle on common land until repeated testing confirms their BVDV-free status. Declaring of a herd as BVDV-free is based on two subsequent repeated serological demonstration of the absence of antibodies in bulk milk sample from the milk tank on the farm, bulk milk sample from first-calf heifers and in bulk sample of blood serum from young animals examined at least four months later (Waage et al., 1996).

National voluntary program for control and eradication of BVDV without vaccination started in Sweden after 10-year experience in BVDV control at local and regional levels. Eradication involved identification and elimination of PI animals resulting in getting BVDV-free population (Alenius et al., 1996). After elimination of PI animals, the primary action of the program was control of virus introduction into the herd by direct contact of cattle with PI animal or via newly purchased animal carrying PI foetuses as well as prevention of infection spread by indirect routes such as transportation vehicles, auctions or visitors. The measures pertaining to infected herds encompassed prevention of contacts with cattle and sheep from herds other than those certified as BVDV-free. All animals intended for trade were provided with individual certificate to be free from BVDV infection and to originate from a BVDV-free herd. Owners of BVDV-free herds were advised to purchase new animals exclusively for certified herds. Pregnant seropositive animals were introduced into other herd only if they were seropositive (confirmed presence of antibodies) before conception. The measures taken in infected herds encompassed prevention of contact with animals from other herds, ban on selling animals and purchasing new animals in the period of program duration. Throughout three years, 11,120 (70%) dairy herds and 3,075 (20%) beef herds participated in the control program. In this period, 7,585 (47%) dairy herds and 1,456 (10%) beef herds were certified as BVDV-free. Vaccination was absolutely excluded (Alenius et al., 1996).

Examination of bulk milk samples within the framework of a program for control of BVDV infection in Denmark encompassed 16,113 dairy herds, and presence of PI animals was established in 39% of herds. In addition to identification and elimination of viraemic PI animals, the program included the following prophylactic measures: 1) only tested and non-PI confirmed animals could be introduced into the herd; 2) any seropositive pregnant heifer that was

to be introduced into the herd was quarantined until calving and could not enter the herd before the calf was tested PI negative; 3) to prevent occurrence of acute infection in the herd all newly purchase animals were isolated during three weeks; 4) animals were not grazed in areas close to herds suspected to contain PI cattle; 5) common pastures had to be free from PI animals; 6) all animals participating on exhibitions or auctions had to be tested and certified as BVDV-free and non-PI; 7) on the infected farms, the testing encompass also small ruminants; 8) field veterinarians strictly followed relevant biosecurity measures to prevent potential transmission between the herds. The herds were considered free from infection in case of absence of virus specific antibodies in blood serum of three calves older than eight months tested at least 12 months after removal of all PI animals from the herd (Bitsch and Ronsholt, 1995).

In the framework of an experimental program of BVDV infection control in Italy (the Province of Rome), which was based on identification and culling of PI animals blood sera from all animals older than one year from 174 mainly dairy herds were examined (Ferrari et al., 1999). According to the results of serological testing the herds were categorized as negative if none of the animals was tested seropositive. The herd was claimed positive if one or more animals were seropositive. Negative herds underwent repeated testing after 30 days. If the second examination revealed negative result, the herd was declared free from BVDV. To the purpose of maintaining the free status, the tests were repeated every six months including examination of bulk milk sample from the milk tanks from dairy farms or examination of blood samples on beef farms. In seropositive herds, all animals aged 6-12 months were tested for the presence of specific antibodies in serum samples. According to the results, the herds could be categorized as "with or without" recent BVDV infection. In herds without recent infection, seronegative status of 6-12 months old animals was checked at 6-month intervals. If the tested animals (6-12 months of age) have revealed permanent negative results throughout 18 months (three consequent testing at 6-month intervals), the herd could be certified as BVDV-free. In herds characterized by recent infection (one or more seropositive calves aged 6-12 months) all animals were tested for the presence of virus specific antibodies. All seronegative animals were tested for the presence of BVDV virus. The animals were categorized as PI if the virus was detected during two subsequent tests at 30-day interval. Seronegative animals were tested every six months to exclude or confirm new cases of infection. The herd was claimed BVDV-free if all 6-12 months old animals born 12 months after elimination of the last PI cattle were seronegative as well as if there were no seropositive findings in the group of seronegative animals (Ferrari et al., 1999).

More extensive program for the control and eradication of BVDV in Italy considered the potential application of programmed vaccination. In the herds with high seroprevalence, vaccination of seronegative animals before conception was practiced (Cancelloti and Carlotto, 1985). Adequate information about the status of BVDV infection in the herd was the prerequisite for implementing an adequate vaccination program. Serological and virological examination was performed with an aim of confirming and excluding of PI animals. At the same time, relevant prophylactic measures were applied, including "all in - all out" approach, frequent and thorough disinfection, quarantine and isolation as well as rigorous hygienic measures at calving. The control of BVDV infection in Italy involved attenuated vaccine, which was considered safe and effective prophylactic tool, especially in calves. The heifers on dairy farms were vaccinated at age of 12-14 months. During the period of program implementation, it was established that proper determination/identification of herd status before vaccination was of crucial importance and that vaccination cannot be considered a long-term option. Administration of attenuated and/or killed vaccine for an indefinite period and without precisely knowing of infectious status of the herd is highly hazardous and economically unacceptable.

During the past few years, the strategies for systematic eradication of BVDV infection were launched in many EU countries. The strategies were initiated either by the governments and relevant authorities or by farmer associations under government's support. The programs are similar to the aforementioned ones, with some minor modifications.

In the Republic of Serbia, the situation is vastly unfavourable – the problem and the control of BVD infection in cattle herds are absolutely neglected. Cattle owners and producers are generally left to their own devices to cope with this problem. During the past several years, there have been some attempts to establish infection control at herd level; however, such isolated attempts received any support from neither governmental authorities nor farm owners, which is partly due to frequent changes of the ownership of the farms during transition period. There were some attempts of applying the Scandinavian system relying on elimination of PI animals and improvement of biosecurity measures on the farm, as well as diverse vaccine preparations. However, neither of efforts proved successful. In that respect, this economical problem that substantially affects the competitiveness of Serbian cattle production was recognized by professionals in veterinary medicine and the management of the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection. They created the initiative for establishing an initially voluntary program for the control of BVD infection in the territory of the Republic of Serbia.

Researchers from the Scientific Veterinary Institute „Novi Sad“ (NIV-NS) have been permanently participating in the programs for monitoring of infectious animal diseases and zoonoses in Serbia. Thus, Serbian Government placed confidence on the scientific and professional competences of the researchers from NIV-NS and colleagues participating in research project No TR31084 as well as professionals from the sector of animal health protection, and initiated creation of a draft of the Program for the control, suppression and eradication of BVDV infection in Republic of Serbia. The program would be initially implemented on voluntary basis at individual farms and cattle herds, depending on the interest of the owners (Petrović et al., 2015). Considering that the Program for the control of BVDV infection combines successful methodologies developed and applied in several countries (Scandinavian, German, Italian and Slovenian control program), and to the introduced "up to date" diagnostic methods into the laboratories that will be included in animals testing (Petrović et al., 2010), it is expected to be successful in cattle herds that will voluntarily conduct the control of BVDV infection. The Program would be subsequently implemented at national level as a governmental program for the control of BVDV infection in the Republic of Serbia.

METHODOLOGIES PROPOSED FOR CONTROL, SUPPRESSION AND ERADICATION OF BVDV INFECTION

Primary goal in the control of BVDV is the prevention of prenatal infection. The procedure involves identification and elimination of persistently infected (PI) cattle from the herd (Roeder and Harkness, 1986; Baker, 1987). After elimination of PI animals, particular attention should be paid on introduction of new animals into the herd, avoiding contact infections and preventing the occurrence of transplancental infection (Baker, 1987). The central aspect of this volontary Program for control, suppression and eradication of BVDV infection relies on the establishment of herd free from BVDV infection. Certification of the herds would be coordinated and validated by the Veterinary Directorate that would also be responsible for providing an online database of certified herds with all relevant data pertaining to the herd and certification validity date.

The Program design encompasses several steps, i.e. stages of disease control, depending on the actual status of BVDV infection in a herd.

1. Stage 1: Affirming status „free from BVDV infection“;
2. Stage 2: Obtaining status „free from BVDV infection;
3. Stage 3: Maintenance of BVDV-free status of the herd and

4. Stage 4: Loss of the status „free from BVDV infection" and procedure for restoring and reclaiming the status of BVDV-free herd

The goals of this Program Proposal are:
- Reduction of economic losses in cattle herds;
- Obtaining of certified breeding material to facilitate and promote its access to international market;
- Better market price of such breeding material

Stage 1: Affirming the status „free from BVDV infection"

To affirm the BVDV-free status, the herd needs to fulfil the following conditions:

1. If infection in the herd was not detected throughout past 12 months;
2. If none of the animals manifest clinical signs that would suggest suspect BVDV infection;
3. If the holding (farm, holding yard, pasture, etc.) where the cattle herd is located is protected by either natural or artificial barriers to prevent any undesirable contact between in-farm cattle with animals, people, equipment, tools or vehicles from other farms of poor or unknown/uncontrolled status with respect to BVDV infection (prevent direct and indirect contacts between animals of different health status);
4. If newly purchased animals were introduced into the herd, they should originate from certified BVDV-free herds, or have been isolated quarantined for 30 days and proved negative after relevant testing for the presence of BVD virus and BVDV-specific antibodies (examination performed minimum 15 days after putting into quarantine)
5. If none of the animals from the herd was in contact with cattle originating from herds of unknown BVDV status or herds of poorer BVDV status;
6. If artificial insemination is performed using only semen from the Centres for artificial insemination certified as BVDV-free or from bulls confirmed as free from BVDV infection or if natural breeding is practiced, only the bulls originating from BVDV-free herds or proved BVDV-free bulls are used;
7. If two consequent serological testings for the presence of BVDV-specific antibodies performed at 6-month interval in all animals aged 7-13 months were resulted with negative finding (if there are no animals of such age, the test includes all for six months older animals - animals aged 14-20 months, etc). The period after last previous test must not exceed 6 months.

Request for certification is submitted to the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection by the owner – farmer. The Request should be previously approved / verified by relevant veterinary inspector according to health status examination (declaration of the relevant veterinary service responsible for herd status monitoring in line with aforementioned terms 1-6) and laboratory analysis pursuant to aforementioned term 7. According to the submitted request and relevant accompanying documentation, Veterinary Directorate of the Ministry of Agriculture and Environmental Protection awards the Certificate on BVDV-free status to the herd with a validity period of 12 months and enters the herd into the database (Register) of herds free from BVDV infection, which would be publicly available from the web-page of the Veterinary Directorate and is automatically updated.

Stage 2: Obtaining of status „free from BVDV infection"

If for any reason the herd cannot be declared as BVDV-free according to rules described for the Stage 1 of BVDV control or clinical manifestations or laboratory examination suggest the presence of BVDV infection in the herd, the following steps should be taken towards obtaining of BVD-free status:

1. Examine all animals in the herd for the presence of BVD virus and promptly eliminate (slaughter) all persistently infected animals (PI) from the herd;
2. During one-year period after elimination of all PI animals from the herd, examine every single newborn calf for the presence of BVDV during first week of life and immediately eliminate all virus-positive animals;
3. One year after elimination of the last PI cattle, i.e., BVD-positive animal from the herd, examine all animals aged 7-13 months for the presence of BVDV-specific antibodies and :
    a. If testing results are negative, repeat testing after 6 months including all animals aged 7-13 months. If the results of repeated testing for the presence of BVDV-specific antibodies are negative and conditions of the Stage 1 of BVDV control / terms 1-6 are fulfilled, the herd is declared as free from BVDV infection;
    b. In case of positive result in repeated testing, the procedure of suppression and eradication of BVDV infection should started from the beginning, that is, from Term 1 of this stage of BVDV control (Stage 2);
3. If there are no animals of such age in the herd at the moment of testing, the test includes all animals from the upper 6-month age category, that is, cattle aged 14-20 months

The status of a BVDV-free herd is declared if all aforementioned measures and procedures are successfully implemented, resulting in negative finding of two consequent serological testings for the presence of BVDV-specific antibodies performed at 6-month interval in all animals aged 7-13 months (if there are no animals of such age, the test includes all animals from the upper 6-month age category). Request for certification is submitted to the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection by the owner – farmer in line with conditions stipulated under the Stage 1 of BVDV control.

Stage 3: Maintenance of status „free from BVDV infection"

Maintenance of the BVDV-free herd status relies on implementation of rigorous biosecurity measures and monitoring of herd's health status by relevant veterinary service. The status is verified at 12-month intervals by serological examination of all animals aged 7-13 months for the presence of BVDV-specific antibodies. Based on the obtained data and negative serological finding the herd certificate is extended for the period of next 12 months. Besides the abovementioned, the herd must comply with all terms stipulated in the Stage 1 of BVDV control, terms 1-6.

If laboratory examination aimed at obtaining disease-free status of uncertified herd or in a certified herd reveals positive serological finding in animals aged 7-13 months, the following steps should be taken:

1. Test all animals in the herd for the presence of BVD virus and promptly eliminate (slaughter) all persistently infected animals (PI) from the herd;
2. During subsequent one-year period, examine every single newborn calf for the presence of BVDV during first week of life (immediately eliminate virus-positive animals);
3. One year after elimination of the last PI cattle, i.e., BVD-positive animal from the herd, examine all animals aged 7-13 months for the presence of BVDV-specific antibodies according to the procedure described under the Stage 1 of BVDV control and if:
    a. test results are negative, repeat testing for the presence of BVDV-specific antibodies after 6 months including all animals aged 7-13 months. If the results of repeated testing are still negative and conditions from the Stage 1 of BVDV control / terms 1-6 are fulfilled, the herd is declared free from BVDV infection and the certificate on BVDV-free status is issued;

b. the result in repeated testing is positive, the procedure of suppression and eradication of BVDV infection is started from the beginning, that is, from Term 1 of this Stage of BVDV control.

3. If there are no animals of such age in the herd at the moment of testing, the test includes all animals from the upper 6-month age category, that is, cattle aged 14-20 months, etc.

The monitoring of herd's health status is performed by relevant veterinary service or institution, which should conduct a detailed and comprehensive inspection at least twice yearly at minimum 4-month intervals. The inspection must include clinical examination of the cattle as well as control of the compliance of farm practices with the terms 1-5 stipulated in the Stage 1 of BVDV control. After the inspection, the relevant institution or veterinary service completes the appropriate Protocol on herd status, that is, whether the conditions for declaring the herd as free from BVDV infection are fulfilled or not. The Protocol is made in three copies submitted to 1) farm owner, 2) veterinary inspector and 3) responsible field veterinarian.

The owner is obligated to report any evident disturbance of health status of his animals that suggests potential BVDV infection as well as each case of abortion to the responsible field veterinarian, who has to perform sampling of materials for virological and serological examination and submit them to relevant laboratory for further testing for the presence of BVDV infection.

The status of a BVDV-free herd is declared if all above stated measures and procedures are completed, and if two consequent serological examinations for the presence of antibodies against BVDV performed at 6-month interval in all animals aged 7-13 months resulted with negative finding (if there are no animals of such age, the test includes all animals from the upper 6-month age category). Request for certification is submitted to the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection by the owner / farmer in line with conditions stipulated under the Stage 1 of BVDV control.

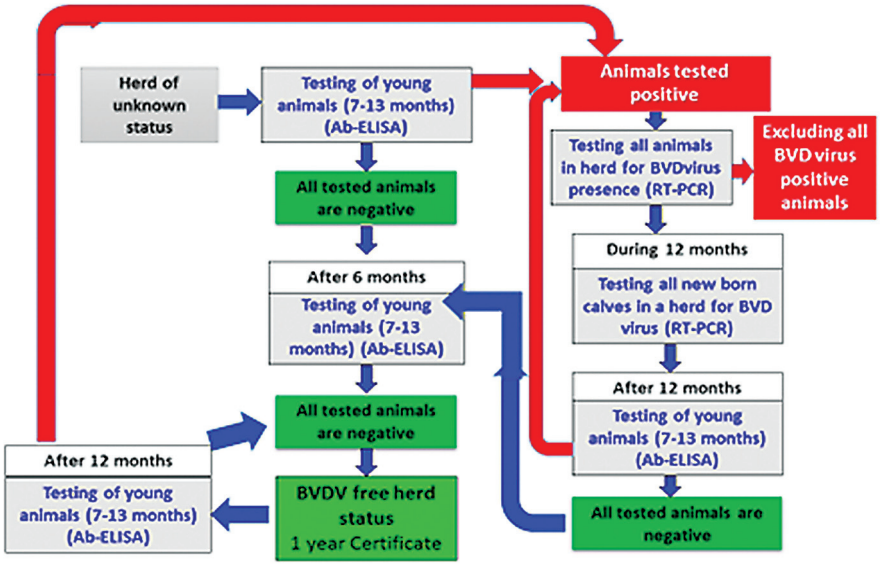
Stage 4: Loss of the status „free from BVDV infection" and procedure for restoring and reclaiming the BVDV-free herd

If it is established that the requirements for declaring the herd as free from BVDV infection are not fulfilled, the owner is issued a Decision on withdrawal of the certificate on BVDV-free status (or the validity of the certificate is not extended to the further period) by the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection.

The data on the herd that has lost its status of BVDV-free herd are deleted from the Registry of herds free from BVDV infection by the Veterinary Directorate.

To restore the lost BVDV-free status of the herd, the owner is obligated to fulfil all conditions and procedures for obtaining of the status „free from BVDV“ infection described in the Stage 2 of BVDV control.

Figure 1: Flow diagram for obtaining of BVDV-free status of the herd



Acknowledgements: This paper is a result of the research within the project TR 31084, funded by the Ministry of Education, Science and Technological Development, Republic of Serbia

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Primljeno: 25.11.2015.
Odobreno: 15.01.2016.

Original scientific paper UDK 639.2.03:639.211

ECONOMIC EVALUATION OF RAINBOW TROUT (*Oncorhynchus mykiss*) FARMING FED WITH DIFFERENT COMPOSITION FEED

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Abstract

This paper evaluates the effects of two different feeds on production results and economic performance in the rainbow trout (Oncorhynchus mykiss) farming. The first group (O-1) was fed with a standard complete pelleted feed, and the second group (O-2) was fed with combined feed consisting of 75 % pellets and 25 % of sardines. Better results were obtained with the O-2 group, while the O-1 group achieved results characteristic for local farming conditions and feed quality. Experimental group O-2 had significantly higher ($p<0,01$) growth rate when compared to group O-1. Considering current feed and final product (fish) prices in the market, better results in terms of cost-effectiveness were obtained ($E>0,00$) in O-2 group of trout fed with the combined meal.

Keywords: rainbow trout, feed, growth rate, cost-effectiveness

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UPOREDNA ANALIZA PROIZVODNIH I EKONOMSKIH POKAZATELJA U INTENZIVNOJ PROIZVODNJI KALIFORNIJSKE PASTRMKE (Oncorhynchus mykiss) HRANJENE OBROCIMA RAZLIČITOG SASTAVA

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Kratak sadržaj

U radu je ispitivan uticaj ishrane kalifornijske pastrmke (Oncorhynchus mykiss) sa dva obroka različitog sastava na proizvodno-ekonomske rezultate. Eksperiment je postavljen sa dve grupe kalifornijske pastrmke: O-1 hranjena je standardnom kompletnom peletiranom hranom i O-2 koja je hranjena kombinovanim obrokom. Bolji rezultat je ostvaren kod O-2 grupe, dok je O-1 grupa postigla rezultate karakteristične za kvalitet domaće hrane i uslove držanja u našoj zemlji. Ogledna grupa O-2 imala je signifikantno veći ($p<0,01$) ukupni prirast u odnosu na O-1. Pri važećim tržišnim cenama hraniva i konzumne ribe kao finalnog proizvoda, bolji ekonomski rezultati ($E=2,06$) postignuti su korišćenjem kombinovanog obroka kod O-2 grupe.

Ključne reči: kalifornijska pastrmka, ishrana, prirast, ekonomika.

INTRODUCTION

Fish production as well as nutrition has developed significantly in recent years (Azis at al., 2011). Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feeding represents almost half of the production costs.

Rainbow trout is one of the most important *Salmonide* species of major economic interest for commercial culturing worldwide and it is the dominant type of trout farming in the Balkans (Vranić, 2012). The type of feed and the feeding process itself are among the most important factors influencing trout growth, feed utilization and tissue composition in intensive production. *Salmonide* feed costs constitute more than 40% of the production cost (Azevedo P.A., 2004). Thus, much work has been done to create the optimal nutritional requirements, feeding process, growth and feed conversion ratios of salmonids

(Okumus at al., 2002). Much effort has been and is still put into creating feed composition best for developing balanced commercial diets that promote optimal growth and health with minimal production cost.

In order to make production cheaper in intensive farming system, it is necessary to provide cost-effective feed which can satisfy trout biological and nutritional needs. Cost-effective feed is achieved by introducing raw material substitute for more expensive feed (Weatherup R.N., 1997, Kiang, 1999, Sredanović et al., 2002). Due to the importance of increased production in salmonidae intensive farming system, in this study we evaluated the effects of substituting expensive pelleted feed with a cheaper one, and its impact on the economics of rainbow trout production.

MATERIALS AND METHODS

A 90 day feeding trial was conducted in rainbow trout pond with all necessary facilities for trout culture farming. The pond is located at an altitude over 200 m and is supplied by quality water from a river source by direct gravity. Rainbow trout (Oncorhynchus mykiss-Walbaum) with the average weight of 90 g and length of 190-210 mm was used in the experiment. The fish were divided into two experimental groups, O-1 and O-2, and placed in the separated pools, 1 320 trout in each one.

The experimental group O-1 was fed with a standard pelleted mixture, while the experimental group 0-2 was fed with mixture of 75% pellets and 25% sardines. At the beginning of the experiment the length and average weight of the fish were recorded, and at the end of experiment the weight gain, feed intake, feed conversion ratio (FCR), and the economics of production were examined.

According to the feed formulation specification and crude cost, the price of one kilogram feed for each group was calculated. Economic indicators (cost-efficiency, the cost price and the financial results) were calculated at the end of experiment over actual values and costs of production. Retail prices of certain raw materials used for the feed preparation were taken from the last quarter of year 2015, as well as the retail price of fish on the market.

The price of one kilogram of feed (pellets), for O-1 experimental group was 135,00 dinars, while the price of one kilogram of feed for O-2 experimental group was 115,00 dinars (750 gr of pellets and 250 gr of sardelas). Retail price for one kilogram of trout was 600,00 dinars.

The calculation of fish farming cost has been evaluated so that the amortization costs, farm staffing cost, indirect costs, costs of starting material and

other costs (electric energy, water aeration) were fixed for both groups, but only the cost of feed was variable.

The obtained results were analyzed using descriptive statistical values. Descriptive parameters such as arithmetic mean, standard deviation, variation interval, coefficients of variation were used. Statistically significant result between experimental groups was evaluated based on *p* value.

Statistical analysis of the results was elaborated using software GrapfPad Prism version 5.00 for Windows, GrapfPad Software, San Diego, California USA, www.graphpad.com. The results were summarized in tables and charts.

RESULTS

The trout weight gain in both groups over 90 days trial are shown in Chart 1. At the beginning of the experiment the average weight of trout in both groups were comparable, so there was no significant difference ($p>0.05$) between them. However, throughout the experiment a difference in the average trout weight occurred and by the end of the experiment the more weight was obtained in 0-2 group ($\overline{X}$= 156,78±23,31). The average weight of trout in O-1 group was ($\overline{X}$= 134,68±19,80).

The difference of the average weight of trout in the 0-2 group compared to the average weight in 0-1 group was 22,10 grams and it was statistically significant ($p<0,01$) (Table 1).

Table 1. Measure of variation of trout average weight during the experiment (gr)

	Average	**Standard deviation**	**Standard errors**	**Coefficient of variation**	**Min**	**Max**
Day 1						
O-1	88,87	8,36	0,59	9,41	75,97	102,57
O-2	88,98	8,05	0,65	10,28	75,97	102,57
Day 90						
O-1	134,68[a]	19,80	1,40	14,70	96,82	192,43
O-2	156,78[a]	23,31	1,65	14,87	102,57	213,20

the same letters a show signifficant difference (p<0,01)

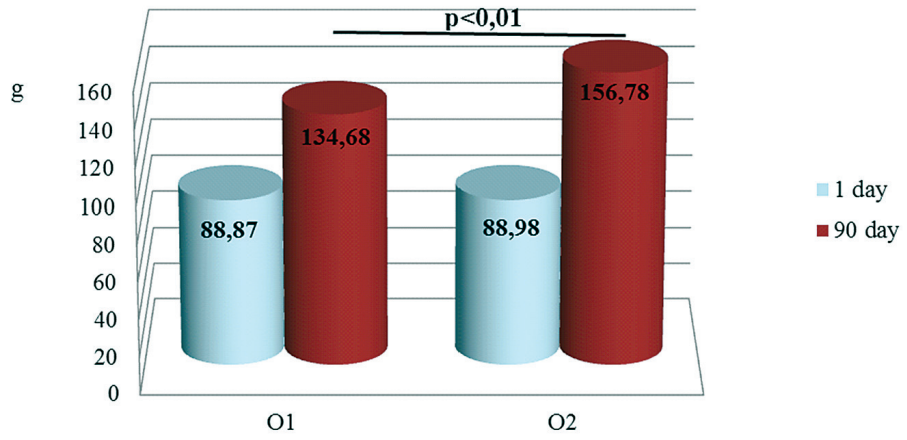

Chart 1. The average trout weight gain during the experiment

Changes in the total and daily weight gain and feed intake of trout in both groups is shown in Table 2. The better growth was achieved by trout in 0-2 group, with total weight gain of 67,80 g or 0,75 g per day, which is signifficante higher growth than the O-1 group trout ($p<0,05$).

Table 2. Total and daily change in weight gain and feed intake

	Group	
	O-1	**O-2**
Weight gain from 0 to 90 day, grams		
- Total	45,81[a]	67,80[a]
- Daily	0,51	0,75
Feed konsumption from 0 to 90 day, grams		
- Total	87,89	78,38
- Daily	0,98	0,86
FCR		
- Total	1,92	1,16

the same letters a show significant difference ($p<0,05$)
FCR-Feed conversion ratio

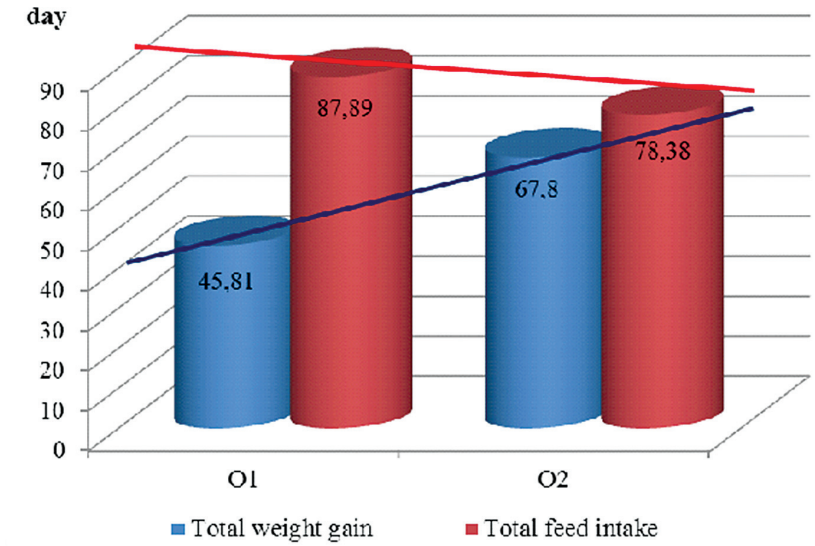

Chart 2. Total weight gain and total feed intake ratio

At the end of the experiment the total and daily feed intake of trout in 0-2 group was 78.38 and 0.86 g respectively. The less satisfying results regarding total weight gain were obtained in the O-1 group, where the total and daily feed intake was 87,89 g and 0,98 g respectively. The better feed conversion ratio was achieved in the 0-1 group (1,16).

Feed costs were calculated as the result of price and quantity of feed used (O-1 group 13.349,00 dinars.; O-2 group 10.394,00 dinars). Production value was calculated as the result of the gained fish weight (O-1 group 106.667,00 dinars, O-2 group 124.170,00 dinars) and retail fish price per kilogram (600,00 din/kg). The financial result is the difference between production value and production costs, and cost-effectiveness is a ratio between the value and cost.

Table 3. Financial data generated by groups

Results	O-1	O-2
Total cost	63.349	60.394
Production value	106.667	124.170
Financial result	+43.318	+63.776
Cost price kg	356,33	291,83
Cost-effectiveness ratio	1,68	2,06

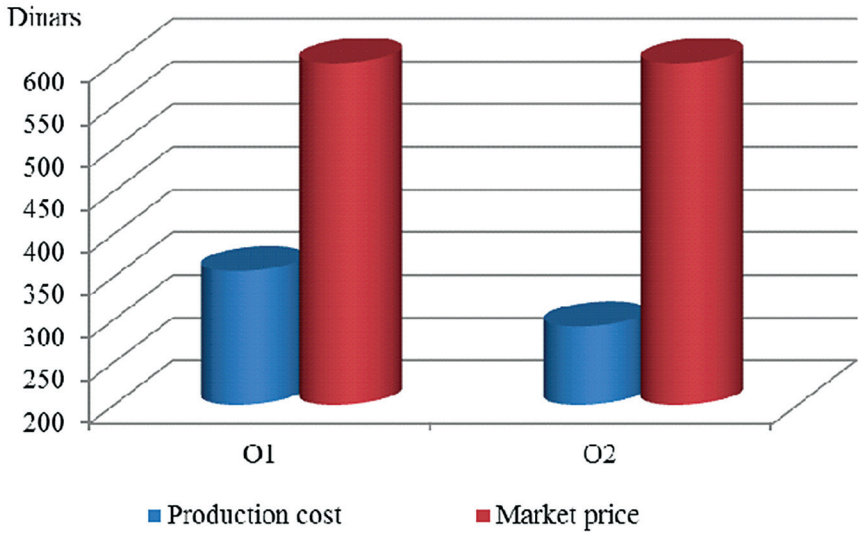

Chart 3 Production cost and market price ratio per kg

Production value in O-2 group is 16,41% higher from the production value in O-1 group. Financial result in both group was positive. The kilogram of fresh trout in O-2 group had lower cost price when compared to O-1 (291,83,00 dinars). Cost-effectiveness ratio was better in O-2 group (2,06), while in O-1 group was 1,68.

DISCUSSION

Optimal feeding is considered as one of the most important factors in intensive farming systems, whereby fish growth is entirely dependent upon the external provision of a nutritionally complete high quality diet (Tacon and al., 1997).

Common problem facing fish farm operation is the need to obtain a balance between rapid fish growth and optimal use of the supplied feed (Gokcek et al., 2008). Since the feed cost accounts approximately for 40-60% of the operating costs in intensive farming systems (Agung, 2004), the economic viability of the farming operation depends on the feed composition, its nutritional value as well as price of raw materials used in the feed preparation. Although biological needs of fish in terms of protein, energy, vitamin and mineral composition of meals are established, researchers are changing the composition of meals, depending on the types of nutrients available, their costs, production

process, and the physical characteristics of the pellets used in order to get the best and economical final production results (Alanara, 1992; Tacon, 1993; De Silva et al. 1995).

The goal of this study was to determine the effects obtained by using two different type of feed in rainbow trout (Oncorhynchus mykiss) farming. The production cost were analyzed, and the results of experimental groups have been compared. Food for the experimental group O-1 consisted of trout pellet mixture with standard raw material composition that provided all the essential nutrients as recommended for this trout category (NRC, 1993). Feed for the 0-2 group had a wider ratio of proteins both of animal and plant origin and a lower amount of energy. Based on the trout weight at the beginning and at the end of the experiment, the total and daily weight gain in both groups were calculated.

The achieved results showed that there were significant differences between the 0-2 group and the O-1 group ($p < 0.05$). Results of average daily weight gain of trout in other studies ranged from 0,7 to 1, 2 g (Kulišić and al., 1987; Mijailović et al., 1990; Apostolski et al., 1983; Dalbelo, 1986). However, daily weight gain in our experiment with combined meal was 0,75 g, and according to Kulišić et al. (1986) daily weight gain was 0,66 g, while Mijailović et al. (1990) examining the impact of the combined meal (pellet +/- fish guts) on the performance achieved higher daily weight gain (2,14 g) compared to our results.

Feed conversion ratio is one of the best indicators of production effectiveness as well as the feed quality influence on the achieved weight gain. Feed conversion ratio in O-1 group was 1,92 and in O-2 was 1,16. According to the results of Kulišić et al. (1986) who examined the effect of combined meal (pellet + worms), the feeding coefficient of 1,31 was achieved, which is slightly lower than the result obtained in our experiment where meal based on pellets + sardines (1,16) was used. Steffens (1994) research showed that poultry by-product feed is suitable as a partial or complete replacement, but that complete substitution of feed by poultry by-product and feather meal resulted in reduced weight gain and feed utilization. Fish fed diets with 27% combined poultry by-product and feather meal without amino acid supplementation had feed conversion ratio of 1,15.

Considering obtained results it can be concluded that trout feeding with combined meal (pellet + sardines) has nutritional and biological justification. The feeding coefficient in the O-1 group fed with a standard pelleted food was similar to the results obtained by Apostolski et al. (1983) of 1,88 to 2,03, while Mijailovic et al. (1990) reported a conversion coefficient of 2,21, and Dalbelo (1986) of 2,10 to 2,24.

Obtained results point that the use of fresh animal feed (O-2) compared to the palette feed (O-1) lowers the cost of feeding. The obtained financial indicators showed that feeding mechanism of group O-2 gives better results in terms of trout growth. This fact points that usage of fresh animal feeding, especially in combination with dry industrial pallets has biological, nutritional and economical justification.

CONCLUSION

Based on the obtained results the following was concluded:

1. At the and of the experiment the average trout weight in group O-2 (156,78± 23,31) was significantlly higher ($p<0,01$) then the average trout weight in group O-1(134,68±19,80).
2. The average weight gain of traout in group O-2 was statistically significant ($p<0,05$) when compared to the average weight gain in group O-1.
3. The cost-efficiency of production of trout in group O-2 was 2,06 while the ratio in group O-1 was 1,68.

AKNOWLEDGMETS

This work is part of research work under the project No. TR 31 011 funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Primljeno: 28.11.2015.
Odobreno: 15.01.2016.

Professional work UDK 639.1:598.617(497.113)"2014/2015"

RESULTS OF HUNTING THE RINGED PHEASANTS IN HUNTING GROUNDS OF AP VOJVODINA (SERBIA) IN 2014 and 2015

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Abstract

The paper presents the results of two-year harvest of ringed pheasants in hunting grounds of Autonomous Province of Vojvodina. The results obtained in the first year of research indicate that 19.67% (236) from total number of ringed and previously released pheasants (1200) were caught. The results for the entire observed period revealed that 1,499 pheasants were bagged, 399 of which were ringed. These results show that the share of ringed pheasants in the total number of harvested pheasants was 26.62%, while the share of harvested ringed pheasants of the total number of entered ringed pheasant was 15.72%, meaning that one of six released pheasants were bagged. Data related to pheasant chicks that were released in the hunting ground immediately before each hunt showed that the percentage of harvested ringed pheasants during the hunting season was 76.44%, which indicates high percentage of harvesting ringed pheasant (observed for two-year period).

Keywords: pheasant, artificial breeding, catch percent

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REZULTATI ODSTRELA PRSTENOVANIH FAZANA U LOVIŠTIMA AP VOJVODINE (SRBIJA) 2014 i 2015. GODINE

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Kratak sadržaj

U radu su prikazani rezultati dvogodišnjeg istraživanja odstrela prstenovanih fazana u lovištima AP Vojvodine. Rezultati u prvoj godini ukazuju da je izlovljeno 19,66% (236) u odnosu na ukupan broj prstenovanih i prethodno puštenih fazana (1.200). Rezultati za celokupan posmatrani period pokazuju da je ukupno odstreljeno 1.499 fazana od kojih je prstenovano bilo 399, što znači da je udeo prstenovanih u ukupnom broju izlovljenih fazana iznosio 26,62%, a da je udeo izlovljenih prstenovanih fazana od ukupnog broja unetih prstenovanih fazana u lovišta iznosio 15,72%. U ovakvom načinu pristupa tehnologije puštanja fazana u lovišta odstreljeno svako šesto fazansko pile koje je ispušteno. Podaci koji se odnose na fazančiće koji su puštani u lovište neposredno pre svakog lova pokazali su da je procenat izlova prstenovanih fazana u toku lovne sezone je iznosio je za dve posmatrane lovne godine (2014. i 2015) u iznosu od 76,44%, što ukazuje na visok procenat izlova prstenovanih fazana.

Ključne reči: fazan, veštačka proizvodnja, procenat odstrela

INTRODUCTION

Pheasant is one of the main game species in most of Europe and that has led to the need for artificial breeding of this game. During the 1960's, the number of partridges in Central, Eastern and South Europe began to decrease, which led to mass production of pheasants and to its organized release into hunting grounds. This practice has continued until present days in most of European countries, although in Serbia the number of released pheasant has decreased in the last 15 years.

There are relatively small number of studies addressing weather conditions or causes of losses of released pheasants from artificial production or losses due to intensive agricultural production using modern equipment and pesticides (Burger 1966, Hessler et al., 1970). However, the pheasant is an important factor in maintaining diversity in agricultural systems and one of few game species which protection may be of financial importance.

The aim of this research is to establish the percentage of usage of seasonal pheasant populations (the populations where a significant number of pheasant chicks was released) and to establish influence of seasonal factors and ways of hunting on sustainability of this game species.

MATERIAL AND METHOD

This research was conducted during 2013/14 and 2014/15 in several hunting grounds in Vojvodina (2013/14 - Kovačica, Ada, Maradik, Rusko Selo, Sonta and 2014/15 - Vrsac, Sid, Zitiste, Vrsac, Čerević and Sremska Mitrovica). Hunting grounds were characterized by different habitat conditions and pheasant chicks were of different age. At the age of 7-8 weeks, pheasants were ringed using plastic rings in different colors (depending on a hunters association) and transferred into shelters in hunting grounds. At the age of 12-13 weeks, they were released into an open part of the hunting ground. First hunt was two months later (mid-October) and it was followed with 9-12 hunting days in each hunting ground. After every hunt, all pheasant bags were counted, males and females separately, as well as the number of ringed pheasant. The obtained data were processed and presented in tables.

RESULTS AND DISCUSSION

During the hunting season, all ringed pheasants were recorded and results of the research (percentage of bagged pheasants that were released into hunting grounds two months before the start of the hunting season) are shown in Tables 1,2,3,4 and 5.

Ring color was characteristic for every hunting ground in order to avoid errors in the calculation of harvest from individual hunting grounds. The number of hunting days per hunting ground ranged from 9-12, while number of hunters and hunting method differed significantly between hunting grounds. Total number of hunting days during the 2013/14 season was 54, while total number of hunting participants was 724. Total number of hunting days during the 2014/15 season was 90 with the total number of hunters being 800.

Table 1. Results of hunting of pheasants that were released into hunting grounds two months before the hunting season 2013/14

Hunting grounds	Hunting days	Hunters	Released ringed pheasants	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed released
Kovačica	12	63	200	28	19	67.86	9.50
Ada	9	80	200	77	37	48.05	18.50
Maradik	12	180	200	119	54	45.38	27.00
Rusko Selo	11	401	600	209	126	60.29	21.00
Total	54	724	1200	433	236	54.50	19.67

Table 2. Results of hunting pheasants that were released into hunting grounds two months before the hunting season 2013/14, according to sex

Hunting grounds	Bagged, total			Bagged, ringed		
	Total	Male	Female	Total	Male	Female
Kovačica	28	19	9	19	14	5
Ada	77	54	23	37	28	9
Maradik	119	89	30	54	41	13
Rusko Selo	209	169	40	126	74	52
Total:	433	331	102	236	157	79

Table 3. Results of hunting ringed pheasants released into hunting grounds in Vojvodina in 2015

Hunting grounds	Hunting days	Hunters	Released ringed pheasants	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed released
Bačka Topola	7	66	200	96	33	34.38	16.50
Morović (Šid)	7	24	112	22	10	45.45	8.93
Višnjićevo (Šid)	9	50	110	49	22	44.90	20.00
Jamena (Šid)	10	65	120	34	17	50.00	14.17
Šid	26	139	342	105	49	46.67	14.33
Čestereg	9	102	100	42	11	26.19	11.00
Kupinovo	6	79	196	38	17	44.74	8.67
Vršac	5	42	100	38	4	10.53	4.00
Sr. Mitrovica	37	372	400	750	49	6.53	12.25
Total A	90	800	1,338	1,069	163	15.25	12.18
ADULTS							
Čerević	11	204	100	177	77	43.50	77.00
Čestereg	10	108	149	99	99	100	66.44
Petrovaradin	11	250	543	423	423	100	77.90
Total B	32	562	792	699	599	85.69	75.63
Total (A+B)	122	1,362	2,130	1,768	762	43.10	35.77

Table 4. Results of hunting ringed pheasants realesed into hunting grounds in Vojvodina in 2015

Hunting grounds	Hunting days	Hunters	Released ringed pheasants	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed realesed
Bačka Topola	7	66	200	96	33	34.38	16.50
Morović (Šid)	7	24	112	22	10	45.45	8.93
Višnjićevo (Šid)	9	50	110	49	22	44.90	20.00
Jamena (Šid)	10	65	120	34	17	50.00	14.17
Šid	26	139	342	105	49	46.67	14.33
Čestereg	9	102	100	42	11	26.19	11.00
Kupinovo	6	79	196	38	17	44.74	8.67
Vršac	5	42	100	38	4	10.53	4.00
Sremska Mitrovica	37	372	400	750	49	6.53	12.25
Total	90	800	1,338	1,069	163	15.25	12.18

Table 5. Results of hunting ringed pheasants released into hunting grounds in Vojvodina in 2014 and 2015

Hunting grounds	Hunting days	Hunters	Released ringed pheasants	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed released
2014	54	724	1,200	433	236	54.50	19.67
2015	90	800	1,338	1,069	163	15.25	12.18
Total	144	1,524	2,538	1,499	399	26.62	15.72

The total bag for 2013/14 and 2014/15 was 1,499 pheasants. From that number, 399 pheasants were ringed, which means that the share of ringed pheasants in total number of bagged pheasants was 26.62%. The share of bagged ringed pheasants in the total number of released ringed pheasant was 15.72%. The real indicator of catch is 15.72% pointing to conclusion that in such approach and technology for releasing pheasants into hunting grounds, one of six released chicks was bagged.

The research of Pekić (Pekić, 1960) pointed on high percentage of pheasant losses in terms of the natural environment - 72% (during the hunting season 9% was bagged and for whole year 28% survived). Hanuš (Hanuš, 1985) also preformed a research on percentage of released pheasants' bag. These results showed that percentage of released pheasant bag ranged from 22.2% (minimum) to 55.6% (maximum). Catch of two-years-old pheasants was only 2.7% and the percentage of the catch of three-years-old pheasants was completely negligible.

According to the research performed in Ireland (Robertson, 1986; 1988) in controlled conditions with released 5-weeks-old pheasants, it was found that 69% of these pheasant died or disappeared until age of 12 weeks (the causes included: 45% foxes, 13% pesticide, 12% traffic and 4% other causes, while for 26% of losses the cause was unknown). The current research in hunting grounds in Vojvodina showed that the percentage of pheasant bag compared to released pheasants was very variable. Main reasons were diverse conditions on hunting site and mistakes in sheltering pheasants. Some research (Ristic et al., 1995; Zeremski et al., 1999) showed that the percentage of released pheasants' bag ranged from 7. 40% to 39.30%.

In this research, very small percentage of caught pheasants (9.50%) was recorded in the hunting grounds of „Hunting Club Kovačica". Main reason for this was the fact that hunting activities were organized near the settlement. Better percentage of caught pheasants (60.29%) was observed in hunting ground of „Hunting Club Rusko Selo", but the total number of hunters during the hunting season was far larger than in other clubs (401).

The research in the hunting ground Sonta, Čerević and Čestereg, where pheasants were released immediately before hunt, showed significantly higher percentage of ringed-pheasants bag (Table 6, 7 and 8).

Table 6. Results of hunting ringed pheasants released immediately before hunt (Sonta) in 2014

	Released pheasants	Bagged	% of bagged
Non ringed	185	125	67.57
Ringed	170	131	77.06
Total	355	256	72.11

Table 7. Results of hunting ringed pheasants released into hunting grounds in Vojvodina in 2015

Hunting grounds	Hunting days	Hunters	Released ringed pheasants	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed released
Čerević	11	204	100	177	77	43.50	77.00
Čestereg	10	108	149	99	99	100	66.44
Petrova-radin	11	250	543	423	423	100	77.90
Total	32	562	792	699	599	85.69	75.63

Table 8. Results of hunting ringed pheasants released into hunting grounds in Vojvodina in 2014 and 2015

Hunting grounds	Hunting days	Hunters	Released ringed pheasants	Bagged, total	Bagged, ringed	% of ringed from total catch	% of ringed bagged, from total ringed released
2014	26	185	180	256	131	51.17	72.78
2015	32	562	792	699	599	85.69	75.63
Total	58	747	972	955	730	76.44	75.10

Data from Tables 6, 7 and 8 are related to pheasants that were released in the hunting ground immediately before each hunt during the pheasant hunting season. There were 58 organized hunts and 792 ringed-pheasants were released. During the hunting season 2014 and 2015, the percentage of ringed-pheasants bag was 76.44%, which indicates high percentage of harvesting ringed-pheasants.

Hanuš and Fischer (1983) pointed out the importance of respecting all principles in parenting techniques and releasing, since with the optimal environmental conditions and proper care of pheasants, the catch of 50% of released males can be achieved. This is not the maximum score because there are hunting areas where the catch exceeds 60%, but on average it is around 35% compared to the total number of released pheasant chickens.

According to the research of Ristic (2005), around 5.4 million pheasants were released in the hunting grounds in Vojvodina, mostly aged 5-6 and 7-8 weeks. For the period from 1973 to 2000, the percentage of catch ranged from the minimum recorded in 2000 (10.54%) to a maximum of 28.77% in 1973.

CONCLUSION

Analyzed results indicate following conclusions:

- When it comes to the survival of artificially produced, released and harvested pheasants: they should be kept in shelters, which are made according to prescribed standards and provide good conditions for pheasant, since the average percentage of harvest in relation to the released pheasants two months before the hunt was 19.67% in 2014 and 12.18% in 2015. Average percentage for both years was only 15.72%;
- Pheasant hunt should be carried out from the beginning of the hunting season and hunting ground should rest for at least seven days after each hunt.
- For proper sheltering, hunting grounds with stable shelters are more appropriate: the results are better with pheasant chicks between 5 and 6 weeks of age, while in hunting grounds with temporary shelters it is better to use 7-8 weeks old chicks.
- In holdings where habitats lack perennial plants and the crops are harvested quickly, it is recommended to build enclosures for keeping pheasants until the hunting season and to release birds into hunting grounds immediately before hunt. In such way, the losses are minimized and catch percentage is higher - in our research it is 76.44%, which is considered excellent result.

Acknowledgment

This research was supported and funded by Ministry of Education, Science and Technological Development of Republic of Serbia, project No. TR -31084.

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Primljeno: 25.12.2015.
Odobreno: 15.01.2016.

Original scientific paper UDK 619:616-089.5636.8

MONITORING OF POSTOPERATIVE COURSE AFTER STERILIZATION IN CATS OF DIFFERENT REPRODUCTIVE STATUS AFTER THE USE OF XYLASINE/KETAMINE ANESTHESIA

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Abstract

Surgical sterilization (ovariohysterectomy) is one of the most common surgical procedures in small veterinary practice because it represents the safest way to control the population of cats. The aim of this study is to compare postoperative recovery in three groups of cats (estrous, anestrous and gravid cats) after performed ovariohysterectomy using combination of xylasine/ketamine anesthesia and, on the basis of obtained data, determine whether there are some significant differences in postoperative recovery in these three groups of cats. This study included 45 cats that were divided into three groups. First group consisted of 15 cats in estrous, second group consisted of 15 cats in anestrous and the third group consisted of 15 gravid cats. Ovariohysterectomy was performed in private veterinary clinic. Anesthetic protocol was the same for all cats, that is, combination of xylasine and ketamine at appropriate doses according to body weight. Parameters that were followed through postoperative course were appetite, body temperature, appearance of the wound and activity. Data was collected on the first and third day after surgery. Analysis of the collected data shows relatively uniform results among groups of cats considering all followed parameters. Results in this study did not show any significant differences in response to xylasine/ketamine anesthesia among cats of different reproductive status through monitoring of postoperative course.

Key words: anesthesia, cats, postoperative course, reproductive status

MONITORING POSTOPERATIVNOG TOKA NAKON STERILIZACIJE MAČAKA RAZLIČITOG REPRODUKTIVNOG STATUSA PRIMENOM KSILAZIN/KETAMIN ANESTEZIJE

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Kratak sadržaj

Hirurška sterilizacija (ovariohisterektomija) je jedna od najčešće izvođenih hirurških procedura u maloj praksi zato što predstavlja najsigurniji način kontrole populacije mačaka. Cilj ovog rada bio je da uporedi tok postoperativnog oporavka kod tri grupe mačaka (estrične, anestrične i gravidne mačke) nakon izvršene sterilizacije primenom kombinacije ksilazin/ketamin anestezije i da se na osnovu prikupljenih podataka utvrdi postoje li značajne razlike u postoperativnom oporavku kod ove tri grupe mačaka. Ovo istraživanje obuhvatilo je 45 mačaka podeljenih u tri grupe. Prva grupa obuhvatila je 15 mačaka u estrusu, druga grupa 15 mačaka u anestrusu i treća grupa 15 gravidnih mačaka. Sterilizacija je izvršena u privatnoj veterinarskoj ambulanti. Anestetički protokol je bio isti za sve mačke, kombinacija ksilazina i ketamina u odgovarajućim dozama spram telesne mase životinje. Parametri praćeni tokom postoperativnog toka bili su apetit, telesna temperatura, izgled rane i aktivnost. Podaci su prikupljani prvog i trećeg dana postoperativno. Analiza prikupljenih podataka pokazuje relativno ujednačene rezultate između tri grupe mačaka posmatrano za sve praćene parametre. Tokom monitoringa postoperativnog toka rezultati u ovom radu nisu pokazali značajne razlike u odgovoru na primenu ksilazin/ketamin anestezije među mečkama različitog reproduktivnog statusa.

Ključne reči: anestezija, mačke, postoperativni tok, reproduktivni status

INTRODUCTION

Surgical sterilization (ovariohysterectomy) is one of the most common surgical procedures in small veterinary practice because it represents the safest way to control the population of cats *(Stančić I. at al, 2015)*. The importance of cat population control is shown in a study (*Animal Shelter Reporting Study*)

that reported that some 4 to 9.5 million cats are euthanized annually in USA (*Kirpensteijn J., 2008*). A TNR (trap-neuter-return) program for stray animal population control has been first developed in Europe during 1970s, and later in the USA. Through this program, stray animals are humanly trapped, sterilized and medically treated and then returned to the locations where they were found. Because of the lack of information about these animals and inability to catch them again, many cats are sterilized during lactation, estrous and pregnancy. Besides the cat population control, other benefits of sterilization include prevention of diseases and tumors of reproductive organs.

Surgical complication rates associated with ovariohysterectomy in healthy dogs and cats have been reported to range from 6.2% to 20.6%, depending on surgeon experience. The majority of complications is mild and generally consists of incisional inflammation or gastrointestinal tract upset. Incision complications are more common in larger animals and animals with longer surgery and anesthesia times (*De Tora M., McCarthy J., 2011*). Problems associated with incisional healing are some of the most frequently reported complications following OVH surgery. It is interesting to note that the incidence of incisional swelling, wound infection, and abdominal dehiscence has not decreased over the past 40 years (*Adin C., 2011*). Wide range of complications is due to different definition of complications in different studies. Most frequent complications are mild and require only care of the owner and no veterinary intervention. The incidence of complications is lower in younger animals, where one study identified fewer complications in animals in puberty, and other 2 years of age as limit for handling sterilization without complications (*Flaggella and Aronsohn, 1994; Pollari et al., 1996; Howe L.M., 1997; Romagnoli S., 2008*). In a study, which included 98 puppies and 98 kittens brought to sterilization, complications due to anesthesia were 0% in both groups (*Flaggella and Aronsohn, 1993, 1994*).

In surgical practice, it is sometimes impossible to predict the duration of surgery. A number of complications that can occur during surgery can prolong duration of the procedure for even up to several hours. It is not uncommon that due to disorder of homeostatic mechanisms, recovery from anesthesia can take many hours (*Spasojević Kosić Lj., Trailović D., 2011*). Anesthesia of feral cats presents unique challenges. Feral cats are usually of unknown age, history, body weight, and health status. Depending on the situation, they may be malnourished, ill, or heavily parasitized. The wild temperament of feral cats prevents examination prior to chemical immobilization and increases the stress associated with trapping and transportation. The ideal anesthetic agent for feral cats would be delivered in a small volume, induce rapid immobili-

zation, provide a surgical plane of anesthesia, have a predictable and sufficient duration of effect, have a rapid recovery, provide adequate postoperative analgesia, and have a wide margin of safety. Xylazine is a mixed α1- and α2-adrenergic receptor agonist. Xylazine contributes short duration analgesia, sedation, and muscle relaxation (*Cistola A.M. et al., 2004*). The analgesic effects of xylazine are not dose dependent, lasting only 20 minutes even when xylazine is administered in high doses. The effects of xylazine can be reversed with a α2-adrenergic receptor antagonist such as yohimbine. Because of the short analgesic effects of xylazine, yohimbine reverses the residual sedative effects produced by xylazine (*Williams L.S. et al., 2002*). Ketamine hydrochloride is classified as a short-acting dissociative anesthetic that is used for chemical restraint, anesthesia induction, and surgical anesthesia in cats (*Saywer et al. 1993*). It is a rapid-acting general anesthetic that has significant analgesic activity and lacks cardiopulmonary depressant effects (*Plumb, 2005*). In the past, ketamine has been recommended for most surgical procedures in cats, including abdominal surgery (*Evans et al., 1972*). Lack of complete muscular relaxation makes ketamine unsuitable as a sole anesthetic agent (*Bill, 2006*). Recommended doses vary depending on desired depth of anesthesia, route of administration, and the use of other anesthetics concomitantly. In cats, ketamine can be given in doses ranging from 2-33 mg/kg, although doses of 50 mg/kg have been used without fatalities (*Arnbjerg, 1979; Wright, 1982*). Duration of anesthesia is approximately 30 to 45 minutes (*Lumb and Jones, 1973*). Ketamine offers many advantages. The route of administration is versatile as it can be administered subcutaneously, intravenously, intramuscularly, orally, and rectally (*Wright, 1982; Hanna et al., 1988; Wetzel and Ramsay, 1998*).

Considering the facts that many cats are sterilized during estrous or pregnancy, either because of TNR program or the lack of knowledge of their owners, we created this study to monitor postoperative course in cats of different reproductive status during several days after sterilization. The aim of this study is to compare postoperative recovery in three groups of cats (estrous, anestrous and gravid cats) after preformed ovariohysterectomy using combination of xylasine/ketamine anesthesia and on the basis of obtained data determine whether there are some significant differences in postoperative recovery in these three groups of cats.

MATERIAL AND METHODS

This study included 45 cats that were divided into three groups according to the appearance of their uterus in situ during surgery. First group consisted

of 15 cats in estrous, second group consisted of 15 cats in anestrous and the third group consisted of 15 gravid cats. Ovariohysterectomy was performed in private veterinary clinic. Preoperative procedure included clinical examination and withholding of food and water. The technique of sterilization used in these cats was through ventral midline with removal of the uterus and ovaries. Anesthetic protocol included premedication with xylasine (*Xylased*®) at a dose of 2mg/kg i/m., and 15 minutes later intramuscular application of ketamine (*Ketamidor*®) at a dose of 10 mg/kg. Parameters that were followed through postoperative course were appetite, body temperature, appearance of the wound and activity. Abdominal wall was closed in two layers, peritoneum and muscles with simple continuous pattern and the skin with simple interrupted pattern with use of absorbable suture (*Vicryl 3-0*). On the first and third day after surgery, all cats were brought to clinic for postoperative therapies – amoxicillin (*Clamoxyl LA*®) at a dose of 15 mg/kg.

RESULTS AND DISCUSSION

Analysis of the data collected on first and second postoperative day is shown in percentages. On the first day post surgery, 33.33% of gravid, 33.33% of estrous and 40% of anestrous cats did not consume any amount of food. On the third day post surgery, the results revealed that all anestrous cats had appetite while 6.67% of gravid and 6.67% of estrous cats had no appetite at all (Fig 1).

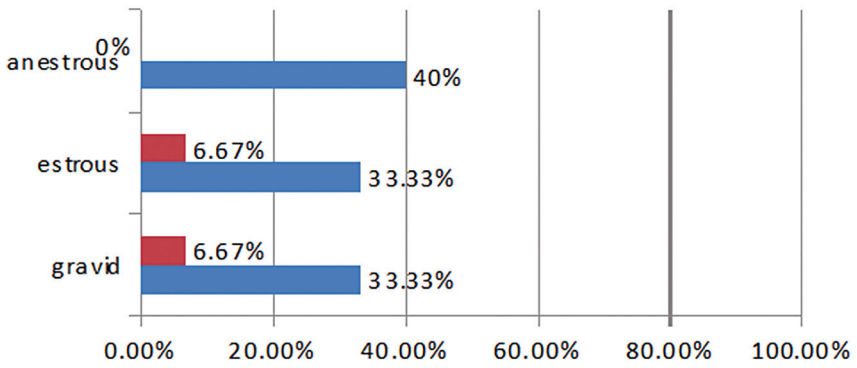

Fig 1. Lack of appetite

13.33% of gravid, 66.67% of estrous and 26.67% of anestrous cats had fever (body temperature higher than 39.2 °C) on the fist day post surgery. Situation on the third day was more uniform as it can be seen in Fig 2, where 46.67% (7) of gravid, 40% of estrous and 33.33% of anestrous cats had fever.

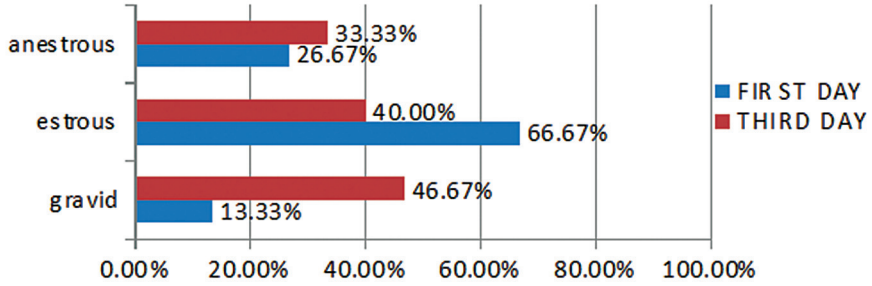

Fig 2. Body temperature above 39,2 °C

On the first day postoperatively, the highest activity rate was observed among anestrous cats, where 80% of cats were active. 66.67% of estrous cats showed activity and only 53.33% of gravid cats showed activity on the first day. All estrous cats were active on the third day, while 93.33% of gravid and anestrous cats showed activity on that day (Fig 3).

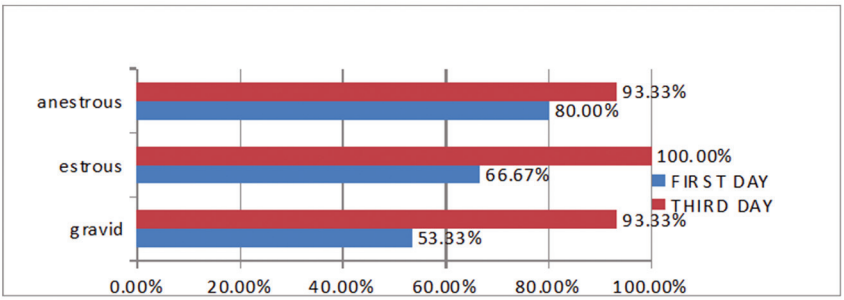

Fig 3. Activity

Monitoring of wound healing showed following results. On the first day postoperatively, 93.33% of all cats presented with proper wound and only

6.67% of cats had dry wound with mild swelling at the incision site. On the third day postoperatively, proper and dry swollen wounds were observed in 44.45% and 42.22% of cats, respectively, whereas 13.33% of cats manifested wound that was inflamed, swollen and with presence of discharge. Comparative analysis among three groups of cats revealed mostly uniform results, where on the first postoperative day 86.67% of gravid, 100% of estrous and 93.33% of anestrous cats had proper wounds. On the third day postoperatively, proper wounds were seen in 46.67% of gravid, 46.67% of estrous and 40% of anestrous cats (Fig 4).

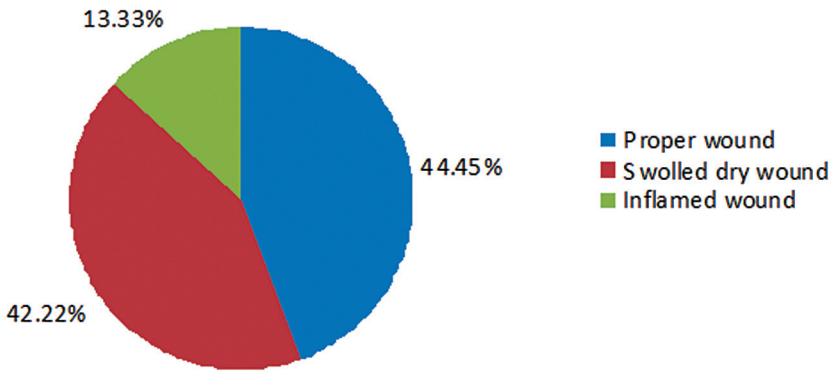

Fig 4. Appearance of the wound on third day postoperatively

Perioperative mortality is extremely low in feral cats anesthetized with TKX–tiletamine/xylasine/ ketamine combination (0.3%). In a study that included 4427 sterilized cats, total mortality rate of 0.35% was found, and the mortality rate suspected to be attributable solely to anesthesia was 0.23% (*Williams et al., 2002*). In our study, none of the cats died due to complications of any kind after surgery. It can be assumed that female cats, undergoing a more invasive surgery than males, do not receive adequate duration of postoperative analgesia from a single dose of TKX (*Cistola et al., 2004*). This can be the reason why cats showed increased attention to surgical wound as the days went by. Complications of surgical incision site are most frequent complications after sterilization and can range from 1% to 4%. Other complications can include dehiscence, wound abscesses, anorexia, lethargy and slow recovery (*Pollari, Bonnet, 1996*). Complications as anorexia, lethargy and inflammation of incision site are all seen in our study. Distribution of these complications among three groups of cats of different reproductive status is very uniform

and shows no significant differences among groups. According to experience of veterinary surgeon, cats and dogs that are pregnant or in heat can be safely sterilized despite those conditions (*Appel 2001, Looney et al., 2008*). Results obtained in our study did not show any differences in response to xylasine/ketamine anesthesia among cats of different reproductive status.

CONCLUSION

On the basis of the obtained results, we can get following conclusions:

1. Monitoring of postoperative course in cats of different reproductive status revealed no significant differences in response to xylasine/ketamine anesthesia among these three group of cats
2. Postoperative complications after performed sterilization are more dependent on postoperative care and handling than on reproductive status of the cats

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Primljeno: 25.12.2015.
Odobreno: 15.01.2016.

Professional work UDK 619:636.5

HEAT STRESS IN POULTRY INDUSTRY

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Abstract

The results of our 15-year long research on the effects of global warming in our region that relies on the database obtained from the Republic Hydrometeorological Service of the Republic of Serbia strongly suggested changes in the pattern of disease events associated with inevitable occurrence of heat stress in poultry regardless of the species and category. Having in mind the accelerating global warming and geographic position of Serbia the occurrence of frequent cyclical and intense meteorological extremes is expected (*Kapetanov et al., 2011; Kapetanov et al., 2013; Kapetanov et al., 2013*). In our country and surrounding regions characterized by continental climate, the risk from the heat stress in poultry production is limited to summer months. Still, summer period has been getting longer and is associated with increasing trend of tropical days and annual trend of global warming spreading of 0.2° geographic latitude. Heat stress of poultry is one of the most challenging problems of poultry production causing substantial damages and affecting all parameters of production performance and is often associated with sudden and massive deaths. Having in mind the aforementioned data, the objective of our research was monitoring of changes in mortality structure associated with temperature oscillations during production process with an aim of emphasizing some major sources, consequences and preventive measures related to heat stress.

Key words: heat stress, poultry, prevention

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TOPLOTNI STRES U ŽIVINARSKOJ PROIZVODNjI

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Kratak sadržaj

Tokom naših petnaestogodišnjih istraživanja, koja se odnose na uticaj globalnog otopljavanja u okruženju, pri čemu su korišćeni podaci Republičkog Hidrometeorološkog Zavoda Srbije, došli smo do rezultata koji ukazuju na izmenjenu strukturu pojavljivanja bolesti uz neizostavno prisustvo toplotnog stresa u živinarstvu, bez obzira na vrstu i kategoriju. Imajući u vidu nastupajuće globalno otopljavanje, koje uzima maha, i geografski položaj Srbije zasigurno treba očekivati češća ciklična, pa čak i intezivnija pojavljivanja meteoroloških ekstrema (*Kapetanov i sar., 2011; Kapetanov i sar., 2013; Kapetanov i sar., 2013*)**.** U našoj zemlji, kao i svim zemljama okruženja koje imaju kontinentalnu klimu, mogućnost toplotnog stresa u živinarskoj proizvodnji postoji samo u jednom delu godine - leti. Međutim, letnji period kao godišnje doba traje duže sa povećanjem broja tropskih dana i sa godišnjim trendom širenja globalnog otopljvanja za $0{,}2^0$ gegrafske širine. Toplotni stres živine je jedan od problema koji u živinarstvu prouzrokuje velike štete, narušavajući sve proizvodne perfomanse i koga prate nagla i masovna uginuća. S obzirom da nam je letnja sezona prošla, podsećanje na osnovne uzroke, posledice i načine sprečavanja toplotnog stresa i izvučena iskustva mogla bi biti od velike koristi.

Ključne reči: :

INTRODUCTION

Main heat sources in housing facility include external temperature of the air released into the room, emission from heated roofs and walls and heat released by the birds themselves. External air temperature is quite beyond our influence; however, certain measures for cooling walls and roofs should be taken. Still, the heat released by the birds themselves represents the most dominant heat source in the facility. Complete poultry feed mixtures are energy-rich – they are often compared with a chocolate-glazed cake. The energy in complete feed formulations should provide basic physiological function of the body (breathing, blood circulation, maintenance of body temperature, immunity, etc.), that is, both maintenance and productive functions. Poultry is con-

sidered highly effective species as only 25% of dietary energy is necessary for satisfying their maintenance demands. However, 75% of energy is an excess, which is released by birds to maintain normal body temperature.

When the temperature in poultry house exceeds 27^0 C, the birds begin to feel uncomfortable and start panting. Ambient temperature in poultry house above 27^0 C makes birds feel uncomfortable and they start panting, whereas temperatures above 30^0 C make proper heat release highly difficult. It is particularly problematic when high temperatures are associated with high air humidity.

Hence, heat stress of poultry is one of the most challenging problems of poultry production causing substantial damages and affecting all parameters of production performance and is often associated with sudden and massive deaths. Having in mind the aforementioned data, the objective of our research was monitoring of changes in mortality structure associated with temperature oscillations during production process with an aim of emphasizing some major sources, consequences and preventive measures related to heat stress

MATERIAL AND METHODS

Throughout the period of several years, the presence of diverse bacterial and viral diseases as well as production characteristics was monitored in the territory of South Bačka and Srem Districts. The monitoring encompassed the mortality structure and production performance of poultry of light and heavy genotype including different categories and age of birds in the industrial production system.

The baseline for presenting the achieved production performance was the Table dating from 1995, which depicts the structure of economic losses associated with particular diseases in broiler production. Another table depicts the mortality structure in broiler chickens at present time characterized by evident climatic changes due to global warming. The tables used for comparing mortality structure and economic losses pertain to summer production lots.

Production features were assessed using following parameters: feed conversion, mortality, final weight and length of fattening period. The presence of infectious diseases was confirmed using laboratory assays as well as bacterial, serological and virological examination. Technopathies observed during the production process along with laboratory examinations were of importance for determining mortality structure. All aforementioned parameters were monitored throughout the cited time period, whereas hydrometeorological conditions, i.e., temperature oscillations, were variables influencing the poultry production process.

RESULTS AND DISCUSSION

During the research period, presence of diverse viral, bacterial and parasitic diseases as well as diseases of other etiologies were monitored, including Pseudo-Poultry Plague, Gamboro Disease, Salmonellosis, E.coli, Coccidiosis, Uricosis, technological fallout and diseases of unknown etiology (Table 1).

Table 1 Structure of economic losses in a summer production lot according to the disease

1995

Disease type	Disease (%)	Economic losses (%)
Salmonellosis	1.19	1.19
Uricosis	2.23	2.23
Omphalitis	2.96	2.96
Technological fallout	11.5	11.5
Coccidiosis	25.00	25.00
Mycotoxicosis	-	-
Coli infection	32.94	32.94
Apoplexia	8.74	8.74
Gamboro disease	-	-
Other	15.39	15.39
TOTAL	100	100

Table 2 displays the data on monitoring production features and mortality structure during the research period. Analysis of mortality structure indicated the presence of some diseases that had not been previously recorded, such as Mycotoxicosis, Aspergillosis, Pox, Histomoniasis and heat shock and their participation in economic losses is presented (Table 2).

Table 2. *Structure of economic losses in a summer production lot according to the disease* in 2015

Disease type	Disease (%)	Economic losses (%)
Salmonellosis	1.10	1.10
Uricosis	1.29	2.29
Technological fallout	10.0	10.00
Coli infection	30.90	30.90
Apoplexia	10.78	10.78
Gamboro disease	-	-
Mycotoxicosis	25.25	25.25
Aspergillosis	2.96	2.96
Pox	1.30	1.3
Histomoniasis	1.25	1.25
Heat shock	15.39	15.39
TOTAL	100	100

As obvious in presented tables, some of the diseases were less prevalent in the period of less pronounced heat stress. Contrary to that, the period of increased temperatures is associated with higher incidence of some diseases. Also, our research revealed decreased antimicrobial susceptibility of particular bacterial species, which is likely due to increased application of antimicrobial drugs on the farms. The administration of antibiotics has commonly been unjustified as the diseases were provoked by heat stress (*Stojanov et al., 2008*). This problem is closely related to the implementation of appropriate prophylactic measures, since climatic changes induced variations in the presence and geographical distribution of particular disease-vectors that were formerly unknown.

Understanding of the physiology of poultry and their potential defense mechanisms is the prerequisite for adequate prevention of heat stress.

POULTRY DEFENSE MECHANISM AGAINST HEAT STRESS

1. **Emission** – certain portion of heat is released through emission (radiation) by releasing the temperature into the environment via the skin. To increase temperature release by emission poultry often *lift* their *wings* away from their bodies to expose the skin areas that have least feathers. Such behavior can be effective only provided that external temperature is lower than body temperature. If the temperature difference is small, i.e., the air overheated, the effects of cooling by radiation is negligible. Airflow can be very helpful, i.e. "moving air" surrounding the poultry actually "removes" the warmest layer immediately around the body and enables further release of the heat. As this most effective way of releasing heat to maintain the body temperature is directly linked with appropriate ventilation, it should be taken into consideration when planning the ventilation capacity of the housing facility. Heat release by radiation is not possible when poultry is overcrowded or the space is too small, which prevents the airflow around the body. These issues should be considered when planning the population density and the rate of meat production per m^2!
2. **Evaporation (panting)** – Regulation of heat-balance by evaporation starts after other way of cooling fails. The bird starts to pant slowly and then increases the intensity and speed of panting. In this manner, the birds can release over 60% of moist air from the body via the lungs and thus decrease the body temperature (Fig. 1)

Figure 1. High temperature of 34^0 C measured in housing premise

Highly saturated air (high relative humidity in the premises) reduces the effectiveness of panting and evaporative heat loss. Panting requires energy use, thus, even if the heat stress has been reduced production results will be jeopardized in this period (*Levent and Porter, 2005; Simmons et al., 1996*).

3. **Conduction (heat transfer)** – some part of the heat can be transmitted through skin contact with cooler surfaces. The birds lean against cold wall or watering system or (if kept in cages) against cage walls. Dry and loose litter enables the poultry to reach the cold floor beneath and lay down on it. Wet litter only contributes to additional heating! Anyway, the heat release by conduction is relatively weak unless floor cooling systems are available.
4. **Reducing feed intake** - Under conditions of high ambient temperature the poultry reduce the feed intake to decrease heat stress, which negatively affects the production performance.
5. **Increase water consumption** –In conditions of increased environmental temperature water intake significantly increases as a consequence of great water loss through panting. Moreover, bird's metabolism undergoes changes by increasing kidney activity to survive with heat stress – water uptake and output is increased.

In addition to understanding of poultry physiology and their defense mechanisms against increased temperature, implementation of appropriate measures for alleviating heat stress in poultry houses are required.

MEASURES FOR ALLEVIATING HEAT STRESS

1. **Density** is considered one of the crucial factors. The overcrowding in poultry production facilities is harmful in many ways – huge number of birds releases more heat, great density of poultry does not allow to birds to keep distance from each other thus reducing the effectiveness of heat release by radiation. If the birds are too close to each other the effects of heat release are reduced by even 40%, which is apparent in overcrowded premises. The airflow surrounding the birds, with the purpose of "taking away" excess heat around the body is obstructed in case of too dense bird population. In that respect, to obtain good production performance, i.e., final body weight of chickens, the number of birds should be carefully planned during summer months!

Ventilation is plays crucial role. To adequately handle the heat wave, the capacity of the ventilation system needs to be adjusted to the number of bird po-

pulation and the exhaust fans must be functional and properly positioned. Good ventilation system should be designed and installed as an integral part of a construction project, but it can be additionally adjusted and upgraded if necessary. The number and capacity of exhaust fans should be adjusted to the number, size and position of air intakes. The airflow rate also contributes to heat removal around the birds, which is one of the major advantages of tunnel ventilation system. Airflow is the prerequisite for **s**uccessful cooling. An adequate adjustment of airflow rate with poultry species and age is of critical importance during hot summer season (*Donald, 2000*). Optimal airflow in production facilities is and imperative when speaking of tunnel ventilation, especially to alleviate negative effects of high humidity resulting from evaporation heat release and in adult poultry, which is covered with feathers and thus more difficultly remove the air surrounding the body. Considering the characteristics of young birds such as rather low humidity, limited amount of heat they produce, lack of feathers and lesser need for space, an adequate airflow rate for young birds is somewhat lower and should provide a comfortable and pleasant environment. Too much (high speed) of airflow is n*ot comfortab*le for birds, thus, it is not recommended in normal environmental conditions. The first symptom of an inadequate fan speed and high airflow rate is dehydration in young birds.

Installation of an alarm system, which activates in case that fans stop to operate can prevent huge damage because in the period of extreme heats, one should react PROMPTLY and FASTLY! Thus, the alarm system is highly valuable along with the system that provides *automatic activation* for the *generator in case of power supply problems.*

Pad cooling systems are installed on the air inlet openings and provide cooling by air flowing into the house through the pad moisten with cold water. **Evaporative cooling pads** are currently the most effective and efficient systems for cooling poultry houses and is installed at many farms. (Figure 2)

Figure 2. Evaporative pad cooling system

The only potential problem with this system might be the humidity, which mustn't be too high. According to some calculations, with each degree of temperature lowering the humidity increases for 4.5%. In that respect, in conditions of relatively high humidity, the system is not effective as it will introduce not only cool but also humid air, thus preventing appropriate heat release by panting.

Quite often during summertime, outside temperatures are 80^0 F – 26.66^0 C, and relative humidity 80%. Then, the temperature increases (late morning, afternoon) whereas relative humidity gradually decreases. During the night, the situation is opposite. It is important to know, in which period the relative humidity increases, e.g., from 10.00 p.m. to 10.00 a.m., when running of a pad cooling system would not be beneficial because it is difficult to evaporate water into air and get much cooling when the air already is 80 % full of moisture *(Czarick and Lacy, 2000)*. In any case, the system should not operate continuously for 24 hours, except in cases of tropical temperatures throughout the day and night. Additional nighttime cooling might be necessary for older birds or birds 2 weeks before slaughter *(Dozier et al., 2005)*. In order to get efficient performance from a pad system, provide proper maintenance and operation. Allowing the pads to dry out overnight or at least once a day is highly important to prevent problems such as algae, collection of scale and dirt, which can reduce the life of the pads and water pump (Figure 3).

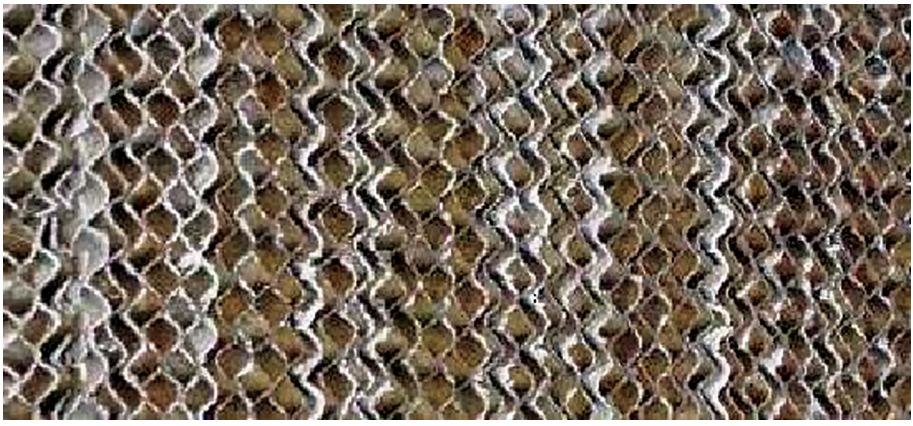

Figure 3. Scale deposition on the pads

A common question is, "When should the pads start running?" Often, farmers tend to run pads too soon (at lower temperatures) in an attempt to do what they think is best for their birds. In a poultry room with adequate

air speed (minimum of 500 m on the foot; 600–700 m per minute is better), there is little benefit to running pads before about 82–85 °F (27.77-29.44 °C) (*Dozier et al., 2005*). Running the pads likely is counterproductive in terms of ambient conditions, production performances and overall health status of the flock (*Campbell et al., 2006*). Pad cooling system is complementary with tunnel ventilation and relies on the large volume of airflow produced by tunnel fans, which improves sensible heat release from the birds (*Donald et al., 2000; Donald, 2000*). To ensure proper functioning of a pad cooling system, it is important not to install sensors near the pads (Fig. 4), as this will tend to increase the possibility of pads coming on at too high a temperature for birds in the tunnel fan end (Fig. 5).

Figure 4. Grouping of chickens near the pads toward the cool place

Figure 5. Reduced number of chickens at the end of the tunnel because of high temperature

2. **Installation of evaporation cooling systems - foggers**.

Foggers spray droplets of cold water under high pressure forming a fog above the chickens thus cooling the surrounding air. These systems are applicable only in conditions of low humidity otherwise the droplets will not transport excess heat out of the room but leave a lot of additional moisture and wet the litter and the birds. It is of great importance to adjust the foggers with ventilation system and to properly maintain the equipment. The system is quite cost effective and efficient. However, it requires professional and permanent surveillance. Otherwise, in conditions of high air humidity and poor maintenance of the system, the owners often complain that:

- Often on hot afternoons, when they decide to turn on the foggers to improve the pad cooling, after some time they pick up a number of dead birds. It is due to the fact that running foggers actually increases the humidity and cannot remove excess heat and humidity from the production room
- Increase in humidity by fogger as well as lack of adequate maintenance of the ventilator can reduce the airflow for even 20% and more. Windspeed decrease from 500 feet per minute to only 300 feet per minute prevents proper heat release causing the birds to show signs of heat stress at temperatures as low as 78 °F (25.55° C) (*Czarick and Fairchild, 2003; Berma, 2008*).
- High humidity of the air in the production facility can cause poor paw quality and generate production of ammonia from the litter
- Hard water results in scale deposits in the system and nozzle obstruction, which may cause wetting of the litter

3. **Installation of in-house recirculation fans** – air mixers. If any of aforementioned cooling systems is not feasible, increase of air velocity might be helpful. It can be accomplished by installing additional in-house fans, which are simple air-mixers that only accelerate the airflow. In this manner, the temperature of the air is not reduced but the airflow is increased, which can additionally cool the birds. Adequate (sufficient) number and proper positioning of fans inside the room is of crucial importance, as the birds will crowd around the fans with highest airflow. Any crowding of chickens could be detrimental. Migration fences can be used as a temporary solution to prevent too many birds to migrate towards one cooler until the appropriate fan regimen is accomplished.

Floor cooling systems combined with tunnel ventilation

Conduction (heat conduction) – some part of the heat can be transmitted

through skin contact with cooler surfaces. Dry and loose litter enables the poultry to reach the cold floor beneath and lay down on it. Floor cooling operates similar to floor heating, in which *cold water* flows *through* the underfloor pipe system during the summer. The combination of underfloor cooling and tunnel ventilation system is highly effective and most promising solution. Some of its basic advantages are: the system is relatively cheap, easy to maintain and energy-saving. It should be emphasized that the system does not increase the air humidity in the poultry house. As compared with the systems known so far, combined cooling systems proved highly effective in terms of improving the production performances in poultry industry, and statistically significant differences were reported (*Donald et al., 2000*).

Figure 6. Proper distribution of chickens in the poultry house

EFFECTS OF APPROPRIATE FEED AND WATER SUPPLY REGIMENT DURING THE PERIOD OF HEAT STRESS

4. **Water.** During the heat stress, sufficient amounts of fresh cold water must be available to chickens. Cool water is required to enable heat release from the body. It is also necessary to provide additional waterers as the birds will consume more water. Water tanks should be properly insulated to prevent warming. It has already been emphasized that heat stress can induce heavy panting. Increased respiration rate results in release of substantial amounts of carbon dioxide and disturbance of blood acid-base balance as a consequence of decreased bicarbonate levels.
5. **Food.** With an increase of ambient temperature, the poultry will reduce the feed intake. Digestion and metabolism are associated with energy release and heating of the body, thus, the birds naturally avoid eating throughout the day. Feed removal not only prior to the hottest part of the day but some

6-8 hour before (period required for digesting the feed) has been shown to be beneficial. In laying hens, dietary regimen should consist of 1/3 of the ration early in the morning and 2/3 late in the afternoon. Offering a night-time feeding is a good practice, as it will improve the quality of the eggshell. The composition of the feed mix should be properly adjusted and reformulated. Protein contributes more metabolic heat than fats, thus, it is necessary to formulate diets with lower protein levels and to utilize synthetic amino acids, especially methionine and lysine and increase the energy content rather through fats than carbohydrates. Hence, energy:protein ratio should be increased in favor of energy. Such formulations are readily digestible and easily metabolized with reduced heat release. Increased fat content requires supplementation of E vitamin as a stabilizer. Feeding management during extreme heat should consider highly digestible feed formulations. During heat stress pelleted feed should be preferred as it requires less energy for digestion.

Reduced feed intake and feed formulation adjustments will naturally result in changes of weight gain; however, this is negligible damage when compared to potential loss because of massive death. The same stands for the laying hens with an additional problem related to eggshell quality. The release of carbon dioxide through panting induces the increase of blood pH and consequent decrease in calcium bicarbonate levels, which is essential for eggshell formation. Disturbed mechanism of Ca transport in the blood contributes to poorer quality of eggshell rather than the level of calcium. Thus, supplementation of calcium to feed formulation will not be effective. S*upplementation* of *electrolytes* in the drinking *water* or feed is well accepted practice to compensate the loss. Some authors recommend supplementation of sodium bicarbonate, ammonium chloride and potassium chloride, and vitamins C, E and B2 are recommended, also.

CONCLUSIONS

Monitoring of the presence and occurrence of particular infectious diseases during the research period as well as monitoring of production features and mortality structure revealed distinct changes. Considering that production on farms encompassed by this research involves modern technological procedures, the observed changes, above all those pertaining to the characteristics and structure of the mortality, are likely to be attributed to the effects of heat stress. In order to obtain more accurate data that would provide more

precise conclusions further research is necessary along with the establishment of specific measurement system for assessing the effects of climatic changes on the observed parameters in poultry production.

Understanding of physiological features of poultry determining their defense mechanism against heat stress as well as the methods for alleviating impact of heat stress in poultry production will contribute to solving the problems and optimizing production features. It will also enable establishment of standards for measurement and monitoring of climatic changes and their effects on poultry production.

ACKNOWLEDGEMENTS

This investigation was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project No. TR 031071.

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Primljeno: 25.12.2015.
Odobreno: 15.01.2016.

Original scientific paper UDK 619:616.988:636.4

RESISTANCE TO FLUOROQUINOLONES IN *ESCHERICHIA COLI* FROM PIGS

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Abstract

The resistance of *Escherichia coli* (*E. coli*) to fluoroquinolones has become a serious issue on large pig farms worldwide, since these antimicrobial agents are widely used in the control of various diseases such as neonatal diarrhea, post weaning diarrhea, the edema disease as well as others. Being a frequent inhabitant of the digestive tract, *E.coli* is often exposed to antimicrobial agents, which are used to treat various infections. Uncontrolled application of fluoroquinolones has led to the emergence of resistant pathogenic strains as well as commensals. The spread of resistant strains is mostly found in animal and human food production chains, which are potentially huge threat for the general population. The resistance to fluoroquinolones may very often be combined with the resistance to other classes of antibiotics. Therefore, the use of fluoroquinolones for treating uncomplicated infections in pigs must be under strict control or completely banned. In this paper, we compared the results from available literature addressing the prevalence of antimicrobial resistance to fluoroquinolones in *E. coli* strains both worldwide and in countries from the nearby region.

Key words: Escherichia coli, resistance, fluoroquinolones, pigs

REZISTENCIJA *ESCHERICHIA COLI* NA FLUOROHINOLONE KOD SVINJA

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Kratak sadržaj

Rezistencija *Escherichia coli* prema fluorohinolonima postala je ozbiljan problem na velikim farmama svinja u svetu jer se ovi preparati koriste za suzbijanje različitih bolesti, kao što su neonatalna dijareja (neonatal diarrhea), dijareja odlučene prasadi (postweaning diarrhea), edemska bolest (edema disease) i druge. Kao redovan stanovnik digestivnog trakta *E. coli* je često izložena antimikrobnim agenasima koji se koriste u tretmanu različitih infekcija. Nekontrolisana terapija fluorohinolonima dovela je do pojave rezistencije patogenih sojeva, ali i komensala. Širenje rezistentnih sojeva najviše je zastupljeno u lancima proizvodnje hrane za ljude i životinje što predstavlja veliku opasnost po čovečanstvo. Veoma često rezistencija na fluorohinolone može biti udružena sa rezistencijom na ostale klase antibiotika. Upotreba fluorohinolona u tretmanu nekomplikovanih infekcija u svinjarstvu mora biti strogo kontrolisana, ili potpuno zabranjena. U ovom radu upoređeni su rezultati iz dostupne literature o prevalenciji antimikrobne rezistencije na fluorohinolone kod sojeva *Escherichia coli* u svetu i državama iz neposrednog okruženja.

Ključne reči: Escherichia coli, rezistencija, fluorohinoloni, svinje

INTRODUCTION

Intensive pig farming sometimes requires the use of antimicrobial agents for the therapy of various bacterial infections. If the antimicrobial treatment begins without the prior bacteriological examination of the diseased pigs, and if the use of antibiotics is excessive, it may lead to resistance in certain strains of bacteria (Henderiksen et al., 2008). The widespread use of antimicrobial agents has caused the mobilization of bacterial specific mechanisms of resistance in order to survive in the environment (Velhner et al., 2010). In addition to the pathogenic bacteria, commensal microorganisms may also become resistant to antibiotics by creating the possibility for horizontal spread of the resistance genes (Schiearch et al., 2009). Meat and meat products produced for consumption from animals infected with bacteria resistant to antibiotics pre-

sent the major threat to public health (Thorsteinsdottir et al., 2008). In addition to this, multidrug resistant microorganisms have a tendency to expand intercontinentally, which means that some of the bacteria have a unique phenotype and genotype, and may be discovered by using molecular typing methods. The detection of resistance genes and specific mutations on targeted genes could be useful for the epidemiological analysis (Velhner et al., 2010). For example, dihydrofolate reductase genes such as *dfrA1* are more frequent in *E. coli* isolates from Europe while the *dfrA17* and *dfrA12* are more prevalent in Korea and Australia (Blahna et al., 2006). The sequence type ST131 of the *E. coli* resistant to fluoroquinolones and the extended spectrum beta lactam drugs with the CTX-M15 gene is widespread around the world causing urinary tract and bloodstream infections (Petty et al., 2014).

E. coli is a Gram negative bacteria, which belongs to the *Enterobacteriaceae* family and can cause several illnesses in humans and animals. *E.coli* is normally present in the microbiota of the digestive tract in humans and animals, where it is exposed to the influence of various antimicrobial agents, which are used for either therapy or prevention. Consequently, *E. coli* has developed numerous mechanisms, thanks to which it can withstand their influence, where the most important mechanism of resistance is the one towards the fluoroquinolones, due to their intensive and uncontrollable use (Yue et al., 2008). Fluoroquinolones are antimicrobial agents used for the therapy in both humans and animals. Their mode of action is to inhibit topoisomerase enzymes. The resistance develops due to the point mutations on genes which encode the topoisomerase (Hopkins et al., 2005).

MUTATIONS ON THE TOPOISOMERASE GENES

Mutations that cause the development of resistance to fluoroquinolones are located on a protein segment called the quinolone resistance-determining region (QRDR). The *gyrA* and *gyrB* genes encode the gyrase A enzyme. The QRDR is located between amino acids Ala67-Gln106 on the *gyrA* and Asp426-Lys447 on the *gyrB* gene. Single or double mutants are often found on codons 83 and/or 87. The *parC* and *parE* genes encoding the enzyme topoisomerase IV, are the secondary target for quinolone in Gram-negative bacteria. In isolates which demonstrate increased resistance to fluoroquinolones, mutations on all four genes may be found (Hopkins et al., 2005). Resistance to fluoroquinolones can often result in a cross resistance to other antibiotics such as β-lactams, tetracyclines, chloramphenicol and others. Consequently, the health of people and animals may be seriously endangered, if the use of antimicrobial agents should be needed for treatment (Hopkins at al., 2005).

THE SITUATION WORLDWIDE

The *E coli* resistance to fluoroquinolones has become a serious issue on pig farms across the world, since this drug is used for treating several diseases such as neonatal diarrhea, post weaning diarrhea, the edema disease and others. In order to reduce the economical losses caused by these diseases, fluoroquinolones are used in veterinary medicine, which has resulted in the acquisition of the resistance of pathogenic strains of *E coli* and of commensals (Huang et al., 2014). Table 1 shows the licensed quinolones and fluoroquinolones used in livestock production in various regions in the world.

Table 1. Quinolones and fluoroquinolones most commonly used in the livestock industry in various regions around the world (adapted from Webber and Piddock 2001).

Regions	Quinolone type
Europe	Enrofloxacin Flumequine Marbofloxacin Danofloxacin
USA	Enrofloxacin
Japan	Enrofloxacin Danofloxacin Orbifloxacin Difloxacin Ofloxacin Oxolinic acid
Asia	Enrofloxacin Danofloxacin Ciprofloxacin
Latin America	Enrofloxacin Ciprofloxacin Danofloxacin Norfloxacin Flumequine
Canada	None
Australia	None

The most recent research has shown that in China, the *E coli* resistance to fluoroquinolones in farm animals, especially pigs, prevails in comparison

to other countries. High level of resistance to these microbial agents arose as a result of the application of prophylactic antimicrobial therapy during the period of seven days. Because of this established situation, Huan et al. (2014) investigated the effect of ciprofloxacin administered in feed on the dynamics of acquiring the resistance of *E coli* strains isolated from pig feces of the Landrace, Duroc and Yorkshire breed. According to these results, the minimal inhibitory concentration (MIC) for ciprofloxacin in the experimental group, after three days of application, increased from ≥0.5mg/L to ≥8mg/L, while it was stable in the control group ≥1mg/L as well as in the experimental group before the application. After the sixteenth day of application, the MIC for the experimental group was ≥128mg/L. Using the PCR method, point mutations were detected on the *gyrA* and *parC* genes.

According to the research results obtained by the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM), in *E. coli* (n=358) strains derived from pigs, the resistance to fluoroquinolones (enrofloxacin and ofloxacin) was not established. In most of the isolates (n=267), the MIC ≥0.05mg/L to enrofloxacin was noticed, whereas MIC value for ofloxacin was ≥0.1mg/L (n=230) (Kijima-Tanaka et al., 2003).

A study that examined antimicrobial resistance of zoonotic enteric bacteria *Campylobacter* spp., *E. coli* and *Enterococci* spp. isolated from pig meat and carcasses was conducted in two regions in Australia (New South Wales and South Australia). The resistance to ciprofloxacin has not been established in isolated *E. coli* strains (Hart et al., 2004).

During the year 2004, resistance to ciprofloxacin was not established in *E. coli* strains isolated from feces on 20 pig farms in Canada (in the Alberta state). In the total number of the *E. coli* isolates (n=1439), which were included in this study, the MIC to ciprofloxacin was ≥0.0015mg/L in 1426 of the isolates. The highest MIC of ≥0.5mg/L was observed in one of the examined *E. coli* isolates (Rosengren at al., 2008).

THE SITUATION IN EUROPE

The resistance to fluoroquinolones is mostly observed in multidrug resistant bacteria. Consequently, the therapy for animals and humans is compromised, so caution when using antimicrobial drugs is strongly advised. In some European countries, the use of fluoroquinolones is banned in the animal husbandry.

The legislation in Denmark from 2002 restricts the use of fluoroquinolones in animal husbandry, unless for treating infections, where pathogens are resistant to all the other antimicrobials. According to data from Hammerum

et al. (2007), the consumption of fluoroquinolones in 2001 was around 114 kg. After implementation of this law, the use of fluoroquinolones was reduced to 18 kg in 2005. With the 2005 legislation, the use of fluoroquinolones and cephalosporin's was completely prohibited in livestock production, with special emphasis on pigs, because pig production shares 82% of the total animal production in Denmark. The implementation of this law makes Denmark the only country in Europe where the use of fluoroquinolones (ciprofloxacin, enrofloxacin, difloxacin, and marbofloxacin) is banned in the livestock industry, which was reported by Hendriksen et al. (2008).

In the period 2002-2004, Hendriksen et al. (2008) conducted a study aimed at continuous monitoring of antimicrobial resistance of pathogenic bacteria and commensals, where 12 EU Member States were involved. This was the first report on bacterial resistance to antimicrobial agents in pigs, which also confirmed the resistance of *E. coli* (isolated from pig feces) towards fluoroquniolones (ciprofloxacin and enrofloxacin). While during these three years there was no evidence of *E. coli* resistance to fluoroquinolones in Denmark, Portugal had the highest average prevalence of resistance being 48.0% for the period 2002-2004. Spain is just behind Portugal with an average prevalence of resistance of 14.7%, while in other countries the prevalence of resistance was in the range from 1.3% to 6.5%. Data for the Netherlands was not available, while Lithuania, Norway and Switzerland are lacking evidence for all three years (Table 2).

Table 2: The prevalence of the resistance to ciprofloxacin and enrofloxacin for *E. coli* isolated from diseased pigs in different European countries during 2002-2004., expressed in % (adapted from Hendriksen at al., 2008).

The prevalence of resistance of *E. coli* in some European countries												
Year	B	DK	E	FIN	F	LV	NL	N	P	ES	S	CH
2002	3.3	0.0	8.0	4.0	4.7	-	-	0.0	76.0*	15.0	7.0	-
2003	1.1	0.0	2.0	1.5	6.2	22.0	-	-	38.0*	14.9	0.0	1.3
2004	2.9	0.0	2.0	0.0	5.5	-	-	0.0	30.0*	14.2	6.0	0.0
Average	2.43	0.0	4.0	1.83	5.46	22.0	-	0.0	48.0	14.7	6.5	1.3

B Belgium DK Denmark E England and Wales, FIN Finland, F-France, LV-Lithuania, NL-Netherlands, Norway N-P-Portugal, EC-Spain, Sweden S-CH-Switzerland; *enrofloxacin

According to data from the European Food Safety Authority (EFSA) from 2013, significant decrease of the prevalence of resistance to ciprofloxacin was established in *E. coli* strains isolated from pigs in Holland. While in 2009 the prevalence of the resistance was around 10%, it was not found in any of the 289 *E. coli* strains examined in 2013.

The results obtained by Guerra et al. (2003) revealed that 61% of the analyzed *E. coli* isolates (n = 42) originating from cattle, pigs and poultry in Germany demonstrated no resistance to ciprofloxacin, i.e., the MIC was in the range from ≥0.012mg/L to ≥2mg/L. In 39% of the isolates, the resistance to ciprofloxacin (MIC ≥4mg / L) and the presence of a double mutant on the *gyrA* and *parC* was determined.

The use of antibiotics in the UK on annual basis amounts to 440-480 tons. The half of this amount belongs to tetracyclines, while the other half is shared between sulfonamides, aminoglycosides, β-lactams, macrolides and fluoroquinolones. The prevalence of antimicrobial resistance is different for various domestic animals. The highest resistance is established for tetracyclines used in pig production. There is still no official data on the prevalence of resistance to ciprofloxacin for *E. coli*, isolated from pigs. However from total 2480 of the isolates 0.6% was resistant to nalidixic acid (Enne et al., 2008).

Testing of the resistance to ciprofloxacin and enrofloxacin in Iceland was a part of the research of Thorsteinsdottir et al. (2008) conducted from October 2005 to May 2007. The research objectives was to evaluate the frequency of resistance occurrence and the genetic similarity of *E. coli* strains isolated from healthy pigs and broilers, pig and broiler meat from slaughterhouses, and human isolates. The resistance prevalence is shown in table 3.

Table 3. The prevalence of ciprofloxacin and enrofloxacin resistance of *E. coli* isolated from pigs and pig meat in Iceland expressed in % (adapted from Thorsteinsdottir et al., 2008).

	The source of *E. coli*	
Antibiotic	feces n=109	meat n=50
Ciprofloxacin	14 (12.8)	4 (8.0)
Enrofloxacin	13 (11.9)	3 (6.0)

According to the data available from the literature, the resistance to fluoroquinolones of *E. coli* isolated from the feces of healthy and infected pigs in Europe is higher than in other countries worldwide.

THE SITUATION IN THE REGION

Enrofloxacin and flumequin are the most frequently used quinolones in the livestock industry in Serbia and countries in the region. There is no precise data about the prevalence of the *E. coli* resistance towards quinolones and fluoroquinolones in the surrounding region, except for Croatia and Hungary.

The research conducted by Habrun et al. (2011) in the Republic of Croatia demonstrated an increase in the prevalence of the *E. coli* resistance to fluoroquinolones. In 1990, the resistance was not established, while in 1996 it increased to 7.5%. During the years 1997 and 1998, the prevalence of enrofloxacin-resistance reached 29.7% . The testing conducted on eight pig farms (2005-2007) on 256 *E. coli* isolates isolated from organs of dead piglets with clinical signs of diarrhea revealed an increase of 39% in the resistance prevalence, also towards enrofloxacin, while 11% of the isolates showed intermediate sensitivity. The authors concluded that the increase in the prevalence of the resistance resulted from an excessive use of fluoroquinolones (Habrun et al. 2010).

According to EFSA data from 2013, in Hungary, the prevalence of resistance to ciprofloxacin of *E. coli* isolated from pigs (n = 152) was 9.2%. Hungary is the only country in our region, which uses ciprofloxacin in the livestock industry.

CONCLUSION

Fluoroquinolones are one of the most important classes of antimicrobial agents used for treatment of various infections in swine production. Their uncontrolled use in veterinary medicine may lead to the development of antimicrobial resistance of zoonotic pathogens. Meat and meat products from such animals may present a major reservoir for resistant strains and the source of infection for humans. The transmission of resistant bacterial strains through food and between humans and animals presents a major threat for public health. The use of fluoroquinolones should be limited when treating less dangerous infections or completely prohibited in the livestock industry. Despite numerous studies, the effects of different amino acid substitutions in topoisomerase genes are still not fully understood. In order to overcome the problem of resistance, all antimicrobial agents must be used with caution, especially fluoroquinolones, and the code of practice for issuing prescription for antibiotics through must be respected.

ACKNOWLEDGMENT

This work has been funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project number TR 31071.

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Primljeno: 20.11.2015.
Odobreno: 15.01.2016.

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4. **Članak u zborniku radova sa naučno-stručnog skupa:**
Valčić M., Lazić S., Rašić Z.: Mesto i uloga terenskog veterinara u epizootiološkom radu.
U: Dragiša R.Trailović, urednik, Zbornik radova, X regionalno savetovanje iz kliničke patologije i terapije životinja, 1-5. septembar, Kragujevac, Beograd: Fakultet veterinarske medicine, 2008, 75-82.

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The paper should be in Word program, Latin characters, size 12 pt, Times New Roman, double spaced. Left and right margins 20 mm, top and foot margins 30 mm, paper size A4. If special symbols are used, use font Symbol. The manuscript should be submitted on paper size A4, but also in electronic form. Pagination on the right side, starting from the title page. References and notes are cited in the text by authors' names, followed by the year of publication. If there are more than two authors, only the name of the first author is written followed by the abbreviation "i sar." (Vidić i sar., 2004).

If the paper is part of a project, name the financier and the project number at the end.

Title page

On the title page the following should be written:

- the title of the paper in capital letters, without underlining and abbreviations
- the names of the authors (first and second name, followed by a comma).

Above the second name place a number that denotes the institution where the author works:

- full name of the institutions should be given.

- at the bottom of the page write E-mail address of one author, for correspondence.

Summary

Every paper should be followed by a summary (300 words). Beside the title, name of the authors and institutions, it should contain the most important facts from the paper and three to eight key words.

Text

All the subtitles write in bold capital letters. Use short and concise sentences. Name the drugs as their International Nonproprietary Names (so called generic names). If the name of a specific drug is to be stressed, name it together with the producer (in brackets). The names of devices write as used in trade (name of the producer in brackets). When using an abbreviation for the first time, write the words that stand for. Abbreviations cannot be used in the title and summary. Text should not be longer than 8 pages. Avoid long enclosures.

Tables number with the Arabic numerals (above the table). Use Times New Roman, 12 pt, single space, without indentation. If abbreviations are used, give an explanation bellow the table.

Graphs number with the Arabic numerals (below the graph). Use Times New Roman, 12 pt, single space, without indentation. If abbreviations are used, give an explanation bellow the graph..

Scheme number with the Arabic numerals (bellow the scheme). Use Times New Roman, 10 pt, single space, without indentation. If abbreviations are used, give an explanation bellow the graph.

Photographs number with the Arabic numerals (bellow the photo). Only original photographs can be used (black and white). On the back side write ordinal number of the photo and mark the top of the photo.

Headings

Headings in the paper are**: Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusion and Literature.**

Introduction points on the most important, i.e. most recent data regarding the topic with a short presentation of the aims of this research.

Material and Methods. Here describe the conditions in the experiment, name the used methods, material and animals.

Results. The results are displayed through tables or graphs, numbered with ordinal numbers and with an explanation what the photo, table or graph shows.

Discussion. Here give analyses of the obtained results comparing to the results and opinions of other authors, pointing the importance of this research, without giving a conclusion.

Conclusion. Here the authors gives his final conclusions.

Literature. The author should list the references, preferably the most recent one. References should be numbered with Arabic numerals, one under the other, written in alphabetical order according to the surname of the first author. In general, the number of references is not limited, but it is advisable to write 15 references.

Examples of references:

1. **Articles in journals:**

Stojanović D., Maličević Ž., Ašanin R.: The use a new model for the investigation of sepsis. Acta Veterinaria, 52, 2/3, 125-131, 2002

2. **Books:**

Qinn P.: Clinical Veterinary Microbiology. London, Mosby, 1998

3. **Chapters in books:**

Vidić B., Boboš S., Lako B., Lončarević A.: Dijagnostika bruceloze. U: Aleksandar Lončarević, Bruceloza svinja, Beograd: Poljoprivredni fakultet, 2000, str.47-49

4. **Articles in proceedings:**

Valčić M., Lazić S., Rašić Z.: Mesto i uloga terenskog veterinara u epizootiološkom radu.

U: Dragiša R.Trailović, urednik, Zbornik radova, X regionalno savetovanje iz kliničke patologije i terapije životinja, 1-5. septembar, Kragujevac, Beograd: Fakultet veterinarske medicine, 2008, 75-82

Note

A paper that is not in accordance to the aforementioned instructions will not be sent for a review and will be returned to the authors for corrections.

Address of the journal

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