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Na ovitku: Mešana kultura keratinocitov in fibroblastov pod fluorescentnim mikroskopom (glej str. 90)
On Cover: *Mixed culture of keratinocytes and fibroblasts after immunostaining under fluorescent microscope (see p. 90)*

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MICROBIAL BIOSENSORS FOR ENVIRONMENTAL MONITORING

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Microbial biosensors for environmental monitoring

Microbial biosensors are analytical devices capable of sensing substances in the environment due to the specific biological reaction of the microorganism or its parts. Construction of a microbial biosensor requires knowledge of microbial response to the specific analyte. Linking this response with the quantitative data, using a transducer, is the crucial step in the construction of a biosensor. Regarding the transducer type, biosensors are divided into electrochemical, optical biosensors and microbial fuel cells. The use of the proper configuration depends on the selection of the biosensing element. With the use of transgenic *E. coli* strains, bioluminescence or fluorescence based biosensors were developed. Microbial fuel cells enable the use of the heterogeneous microbial populations, isolated from wastewater. Different microorganisms are used for different pollutants – pesticides, heavy metals, phenolic compounds, organic waste, etc. Biosensing enables measurement of their concentration and their toxic or genotoxic effects on the microbes. Increasing environmental awareness has contributed to the increase of interest for biomonitoring. Although technologies, such as bioinformatics and genetic engineering, allow us to design complex and efficient microbial biosensors for environmental pollutants, the transfer of the laboratory work to the field still remains a problem to solve.

Key words: microbiology / environmental protection / microbial biosensors / environmental pollutants / microbial fuel cells / bioluminescence / genetics / bioinformatics / genetic engineering

Mikrobni biosenzorji za monitoring okolja

Mikrobni biosenzorji so analitske naprave, ki nam omogočajo zaznavanje snovi v okolju zaradi specifične biološke reakcije, ki poteka v mikroorganizmu ali njegovem delu. Če želimo takšno napravo uporabiti za monitoring onesnažil v okolju, moramo dobro poznati odziv mikroorganizma na specifičen analit in ga preko pretvornika signala spremeniti v kvantitativno obliko. Poznamo različne konfiguracije mikrobnih biosenzorjev glede na pretvornik signala – elektrokemijske in optične biosenzorje ter mikrobne gorivne celice. Vsaka ima svoje prednosti in slabosti, katero uporabimo, je v veliki meri odvisno od izbora biosenzorskega organizma. Transgene celice *E. coli* nam omogočajo bioluminescenčno ali fluorescenčno zaznavo, v mikrobne gorivne celice pa lahko vključimo mešane mikrobne združbe. Z izborom organizma se prilagajamo tudi onesnažilu. Med najpogostejšimi onesnažili so pesticidi, težke kovine, fenoli, organski odpadki. Z biosenzorji ne spremljamo le njihovih koncentracij v okolju, pač pa beležimo tudi toksične in genotoksične vplive analitov na mikroorganizme. S povečevanjem skrbi za okolje narašča tudi pomen mikrobnih biosenzorjev. Razvoj tehnologij, kot sta bioinformatika in genetski inženiring, nam omogoča temeljitejše in uspešnejše načrtovanje uporabe mikrobnih biosenzorjev v okoljskih aplikacijah. Izziv za prihodnje pa ostaja prenos mikrobne biosenzorske tehnologije na teren.

Ključne besede: mikrobiologija / varstvo okolja / mikrobni biosenzorji / okoljska onesnažila / mikrobne gorivne celice / bioluminescenca / genetika / bioinformatika / genetski inženiring

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1 INTRODUCTION

A biosensor is a self-contained integrated device, capable of providing specific quantitative or semi-quantitative analytical information using a biosensing element connected with a transducer (IUPAC 1996, Thevenot *et al.*, 2001). Biosensor construction, a three-step process, involves combining two elements with different characteristics. First, a biological sensing element is chosen, then a transducer is selected, and finally the biological component (detection element) is fixed to the transducer (Xu and Ying, 2011). Enzymes, antibodies, cell receptors, microorganisms, animal and plant cells or tissue cultures can be used as biorecognition components of a biosensor. Microorganisms have a huge potential for detection of a wide spectrum of chemical substances and their mixtures, they are adjustable to different reaction conditions and compared to enzymes or antibodies do not require expensive preparation processes (Shin 2010, Xu and Ying, 2011). They can be genetically modified, too. This characteristic enables the use of microbial biosensors in the fields of environmental monitoring, food safety and medicine.

2 CHOICE AND APPLICATION OF A PROPER ORGANISM

Choice of a proper microorganism for the detection of pollutants and their effects in the environment and its incorporation with the competent transducer is a key step in the development of an environmental biosensor. Bacteria and yeast are the most commonly used (Xu and Ying, 2011). The chosen microorganism must be robust and capable of specific pollutant detection in small concentrations, to ensure price efficient detection. Recently whole-cell biosensors (Chan *et al.*, 2013; Niazi *et al.*, 2008; Mulchandani and Rajesh, 2011; Anu Prathap *et al.*, 2012) and microbial fuel cells (Di Lorenzo *et al.*, 2014; Shen *et al.*, 2013; Liu *et al.*, 2014; Ayyaru and Dharmaligman, 2013) draw special attention on the field of environmental monitoring. Genetic engineering became important, too. We can manipulate organisms to improve mechanisms of analyte detection or express them in new organisms (Mulhandani and Rajesh, 2011). DNA segments coding for detection mechanisms can be transferred into model organisms with optimized growing conditions, such as *Escherichia coli* and *Saccharomyces cerevisiae*. The organism and the detection configuration should be combined properly to achieve the best possible detection of the signal.

3 BIOSENSOR CONFIGURATION

There are three main types of microbial biosensors classified, based on different signal transducers: electrochemical, optical and microbial fuel cells (Xu and Ying, 2011).

Electrochemical transducers use the change of the electric current, potential and conductivity, caused by microbial-analyte contact. They can be further divided into amperometric, potentiometric and conductometric biosensors. Amperometric microbial biosensors operate at a fixed potential with respect to a reference electrode, and then the corresponding current is obtained due to the oxidation or reduction of electroactive species at the surface of the electrode (Xu and Ying, 2011). This configuration has been described by Yong *et al.* (2011), Anu Prathap *et al.* (2012) and Wang *et al.* (2013); on the other hand, the potentiometric transducer was constructed by Mulchandani and Rajesh (2011). Transducers of this kind use ion-selective electrodes to transmit the biological signal into an electric signal. They are less sensitive, produce higher relative error and a worse linear relationship between the exporting signal and the concentration of the detected analyte (Xu and Ying, 2011). As it is obvious from their name, conductometric biosensors measure changes in conductivity of the media, caused by the target analyte. Although the conductance measurements are extremely sensitive, the detection of solution conductance is considered to be nonspecific (Xu and Ying, 2011).

Optical biosensors can be defined as sensor devices that make use of optical principles, such as bioluminescence, fluorescence and colorimetry for transduction of a biochemical interaction into a suitable output signal (Xu and Ying, 2011). The use of genetic engineering enables an expression of fluorescence and bioluminescence in the target organism. Scientists report of luciferase (Niazi *et al.*, 2008; Shin, 2010; Chan *et al.*, 2013) and green fluorescent protein (GFP) applications (Wei *et al.*, 2013; Kim *et al.*, 2015). Microbial fuel cells (MFC) are bioelectrochemical devices that produce electrical energy through the action of specific microbes (known as anodophiles), capable of transferring the electrons generated from the oxidation of organic compounds (the fuel) to an anode electrode (Di Lorenzo *et al.*, 2014). In a typical two-chamber MFC (Fig. 1), the electrons are absorbed by the anode and are transported to the cathode through an external circuit. After crossing a proton exchange membrane, the protons enter the cathodic chamber where they combine with oxygen to form water (Du *et al.*, 2007). Electric current, produced by fuel oxidation, can serve as a transducer of a microbial response to the analyte. Due to their simple design and low cost, single-chamber MFC, where the cathode is exposed to air, are extensively used in envi-

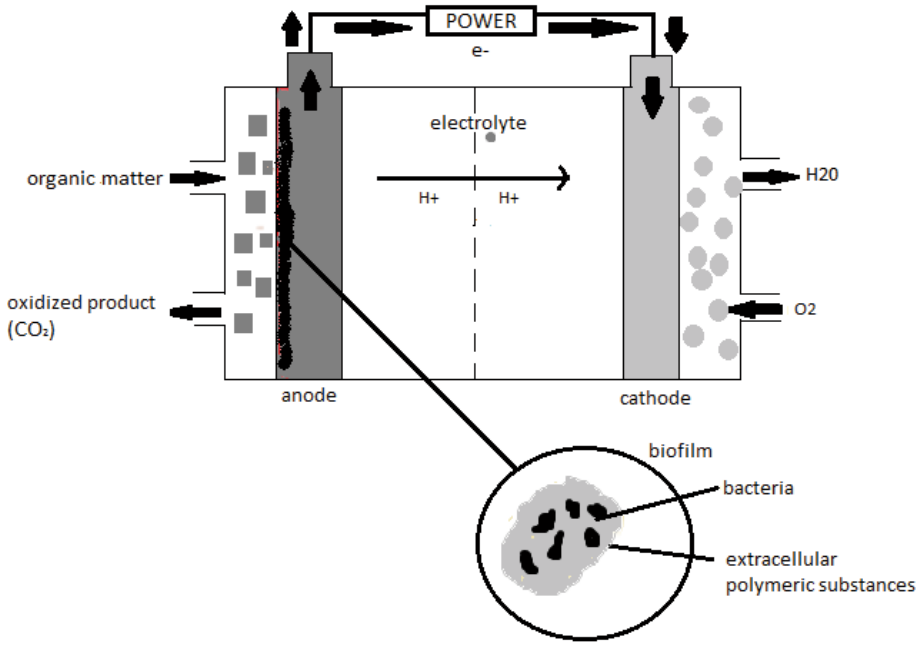

Figure 1: *A scheme of a two-chamber microbial fuel cell*
Slika 1: *Shema dvoprekatne mikrobne gorivne celice*

ronmental applications (Du *et al.*, 2007). They are useful for the detection of biochemical oxygen demand (BOD) (Liu *et al.*, 2013, Ayyaru and Dharmaligman, 2014), heavy metals and their toxicity (Shen *et al.*, 2013; Liu *et al.*, 2014; Di Lorenzo *et al.*, 2014). MFC enable the use of heterogeneous microbial populations, isolated from wastewater plants and other working MFC. This characteristic makes them very successful for the development of sensitive, specific and cost efficient biosensors.

4 ENVIRONMENTAL APPLICATIONS

Organisations, like WHO and FAO, have realised the negative effect of pollutants on human health (Bereza-Malcolm *et al.*, 2015). Together with their concern grows the need for monitoring of dangerous substances in the environment. Pollutant residues can eventually accumulate in our food and drinking water. Food quality control systems are established to prevent that kind of cases. A biosensor, used instead of conventional chemical monitoring methods, must be easy to use, cost-efficient, stable when stored and capable of the detection of small amounts of the analyte. It must have a low detection limit and a short response time (Kumar *et al.*, 2006). Transferring a working microbial biosensor under controlled laboratory conditions onto the field requires a lot of work and optimization. What follows is an overview of current trends in the field of environmental microbial monitoring.

4.1 PESTICIDES

Pesticides are chemical or biological substances meant for pests control. Considering the target organism, we can divide them into insecticides, herbicides, fungicides, bacteriocides, nematocides and others. The use of the first three listed above represent 95 % of world consumption (Aktar *et al.*, 2009). Insecticides are the most acute toxic group of pesticides. Their extensive use has a major environmental impact, resulting in water and ground accumulation. The negative effect of the pesticides rose awareness decades ago and led to the development of detection methods like gas and liquid chromatography for the monitoring of organophosphate pesticides. These methods have brought high selectivity and sensitivity, but are inappropriate for field detection, require expensive equipment and a skilled technician (Mulchandani and Rajesh, 2011). Furthermore, chemical analytical methods only provide the information of pesticide identity and quantity, but no information about their toxicity. Some biosensors are capable to detect pesticide toxicity and therefore they are suitable for their detection. Biosensors represent their alternative.

Kumar *et al.* (2006) report of an optical biosensor with bacteria *Flavobacterium* cells, adsorbed on the glass fiber. The cells express organophosphate hydrolase on their surface, an enzyme, capable of the hydrolysis of the organophosphate pesticides to the optically measurable colour products. Synthetic samples of the methyl

parathion have been tested with the biosensor and a gas chromatography analysis. Biosensoric analysis was based on the ratio between the hydrolysed methyl parathion and the amount of the colourful product – p-nitrophenol. The methods have comparable results, but the biosensoric analysis is more cost-efficient. The developed biosensor showed a lower detection limit compared to other similar devices. The same detection principle of the p-nitrophenol as a hydrolysing product of the methyl parathion, was applied by Kumar and D'Souza (2010). They immobilized bacteria *Sphingomonas* JK1 on the bottom side of the microplate and linked them with an optical plate reader, to form an optical biosensor. This system enables multiple sample detection on one plate. Biosensor can detect 4–80 μM concentrations of methyl parathion and can be reused up to 75 times. Kumar and D'Souza (2011) also report of a recombinant *E. coli*, periplasmically expressing enzymes for methyl parathion hydrolysis, as a biorecognition element. Microbial cells were immobilized on a screen printed electrode, using glutaraldehyde. The researchers observed the changes in the electric current, caused by different concentrations of the methyl parathion. Biosensor showed good selectivity – it did not react to the addition of glucose, sucrose and endosulfan; the response to the phenol and p-aminophenyl sulfate was insignificant and had good stability – it preserved 80 % of the enzymatic activity after being used in 32 reactions. Expression of the organophosphate hydrolase on the surface of the cells is efficient, its sensitivity can be improved by the application of the genetic engineering methods.

Recombinant biofilm forming bacteria *Moraxella*, containing the ICN protein from the *Pseudosomonas syringae* INA5, are capable of the detecting 1 μM methyl parathion and 0.2 μM paraoxon (Mulchandani and Rajesh, 2011). Overexpression of the linA2 gene, encoding the γ-hexachlorocyclohexane dehydrochlorinase (LinA2) in *E. coli* BL21, has been used by Anu Prathap *et al.* (2012) for the development of a sensitive, selective and fast electrochemical biosensor. LinA2 protein catalyses the dehydrochlorination of lindane into trichlorobenzene, forming HCl as a by-product and causing an increase in the conductivity of the cell microenvironment that can be detected with the pulse amperometry. Authors report about the detection limit of 2 ppt for lindane.

E. coli is among the most frequently used organisms in the field of microbial biosensors. A bioluminescent strain PGRFM, including luxCDABE operon and promotor region of the pgi gene, important for the metabolic answer to the oxidative stress, was applied by Niazi *et al.* (2008) for the construction of an optical biosensor. The sensor showed excellent response to the methyl viologen, pesticide that causes the induction of reactive oxygen species, with the detection limit of 0.6 ppm, when exposed to starving conditions. Another detection principle for the toxicity of ametryn, fenamiphos and endosulfan was reported by Yong *et al.* (2011). Amperometrically working ferricyanid was used as a redox probe to measure the overall toxicity of the chemicals on the *E. coli* respiration. Endosulfan was the most toxic, with the IC_{50} = 5.7 mg/L.

4.2 HEAVY METALS

Heavy metals are extensively used in several industry branches such as mining, metallurgical, electronics, electroplating and metalfinishing (Wang *et al.*, 2013). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Järup, 2003). Standard detection techniques – spectrometry, ionic chromatography, potentiometric electrodes – are expensive, sometimes time consuming and require high skilled technicians. Development of simple methods, suitable for field application, is the priority in the field of heavy metal analysis. Biosensor detection is among them.

Yüce *et al.* (2010) reported on the inclusion of the cyanobacterium *Phormidium* as the biosensing element of an amperometrical biosensor. Heat treated dead cyanobacterial biomass was mixed with carbon dust and added to a steal rod to form an electrode, capable of the detection of Pb(II) in water solution. The Ag/AgCl reference electrode and platinum wire as counter electrode were also the part of the apparatus that measures the changes in the electric field, induced by heavy metal water solution. Results showed good stability and repeatability, a hypothetical limit of detection was set for 5 X 10^{-8} M.

Microbial fuel cells (MFC) became important as well. The presence of a pollutant in wastewater can inhibit the metabolic activity of the electrochemically active bacteria, leading to the reduced electron transfer and weak current production. Single-chamber air–cathode MFC, enriched with real domestic wastewater have been applied by Shen *et al.* (2013) for the detection of Cu (II). They were interested in the response of a biofilm, formed by microorganisms in wastewater, at different flow rates. Higher feed rate causes higher shear rate in the surrounding of the MFC, leading to the overproduction of the extracellular polymeric substances and reduced biosensor sensitivity.

The effect of metals on microbes can be also measured with an oxidative stress biosensor. Ooi *et al.* (2015) constructed a biosensor, using *E. coli* DH5α™ transformed with pRSET-roGFP2 plasmid that enables fluorescent de-

tection of arsenic induced oxidative stress. The biosensor is fast, efficient and enables detection down to 0.2 µg/l of arsenic. The same microorganism was used by Arias-Barierro *et al.* (2010) for the detection of Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+} and arsenite. The described biosensor is even more sensitive and enables the detection down to $1*10^{-7}$ mg/l arsenite, 0.001 ppm copper and zinc ions, 0.01 ppm cadmium ion and 5 ppm lead ions.

A continuous flow of the analyte to the biosensor is the most recent improvement in the field of biomonitoring. Kim *et al.* (2015) incorporated *E. coli* DH5 in a microfluidic device, capable of feeding nutrients and various concentrations of heavy metals ions under continuous-feed mode, for the detection of Pb^{2+} and Cd^{2+}. The detection mechanism is based on the negative control of the GFP reporter gene, mediated by CadC-type transcriptional repressors, which bind to Pb^{2+} or Cd^{2+} divalent ions and derepress the GFP reporter promoters. They observed 3–4 fold increase in the sensitivity of the biosensor and good specificity dynamics to detect Pb^{2+} in Cd^{2+}, comparing to conventional batch-type detection modes.

An alternative approach in the construction of a biosensor for the detection of heavy metal pollution on the field enables synthetic biology. The environmental pressure – oscillation in the temperature, pH, access to the nutrients and toxicants in the environment affect a diverse set of regulatory elements, controlling the downstream signal cascade (Bereza-Malcolm *et al.*, 2015). Microbial biosensor can be constructed de novo, using regulatory elements for the production of new genetic circuits. The authors estimate that this biosensor application can solve the problem of weak specificity and the toxic nature of heavy metals to the microbial chassis in real world applications.

4.3 TOXICITY AND GENOTOXICITY

The overall effect of the pollutants on the environment cannot be determined without an estimation of their toxicity. For the measurements of toxicity of water and ground samples, we use commercially developed tests – Mictorox® and ToxAlert® with *Vibrio fischeri*, Cellsense® with *Escherichia coli* (Rodriguez-Mozaz *et al.*, 2004). They use fluorescent and amperometric detection. These systems no longer fulfil the need for monitoring of the toxicants in the environment, so the development of new methods is of great interest.

The secondary plant metabolites can show antimicrobial activity. Chan *et al.* (2013) developed two biosensors for the evaluation of aldehyde and phenolic terpenes and isothiocyanate on the microbes. The first biosensor combined the characteristics of commercial biosensors – they used *E. coli* HB101 with the luxCDABE gene from *V. fischeri*, the other biosensor consisted of *Acinetobacter baylyi* ADP1_recA_lux, transformed with the luxCDABE gene from *Photorhabdus luminescens*. These transgenic bacteria produce light in the presence of toxicants, damaging the DNA; the intensity of the light is directly correlated with the recA expression level. RecA is an essential DNA repair gene. Isothiocyanate and cinnamaldehyde are the most toxic substances for *E. coli* – they mechanically damage plasmalemma, weaken the cell metabolism and the production of the energy, but they do not activate recA *A. baylyi* – it is less plausible that they damage the microbial genome.

Many studies examine the toxicity of heavy metals. An amperometrical microbial biosensor ToxTell applies different microbial species as a biosensing element, giving the optimal results of the toxicity of the real samples (Wang *et al.*, 2013). The test organisms, *Psychrobacter* bacteria, isolated from the wastewater plant were immobilized on a polycarbonated screen printed electrode membrane. They investigated the toxicity of Cu^{2+}, Cd^{2+}, Zn^{2+}, Cr^{6+}, Hg^{2+} and Pb^{2+} to determine the EC_{50} value. The highest EC_{50} value was observed for Pb – 110 mg/l, the lowest for Hg – 0.8 mg/l. The toxicity of the metals increases with the decrease of particle size, as shown by Ivask *et al.* (2014). They investigated the toxicity of the silver nanoparticles, according to their size, to bacteria *E. coli* and *Pseudomonas fluorescens*, yeast *S. cerevisiae* and microalgae *Pseudokirchneriella subcapitata*. The latter showed the highest sensitivity. Liu *et al.* (2014) reported on the use of MFC as a real-time wastewater toxic shock biosensor. They monitored the response of the microbes, isolated from a wastewater plant, to the shocks of Cr^{6+}, Fe^{3+}, NO_3^- and sodium acetate. The growth of a biofilm on the anodic electrode was observed after five days. The biofilm enables the support and protection for electrogenic bacteria and improves the biosensor specificity – it can differentiate the chromium, iron, nitrate and sodium acetate shock. Single-chamber air–cathode MFC was used by Di Lorenzo *et al.* (2014) for the detection of cadmium. At optimal pH and temperature, the addition of cadmium in feeding water caused immediate change in the outgoing current. The biosensor enabled the detection of cadmium in the range of 1 to 50 µg/l.

In an extensive study of the application of yeast in a hypersensitive biosensor, capable of automatic detection of a broad spectrum of genotoxic pollutants, Wei *et al.* (2013) used transformed and mutated *S. cerevisiae* BY4741 cells to establish their response on genotoxic chemicals (methyl sulfonyl methane (MMS), 4-nitroquinoline-oxide (4-NQO), phleomycin, hydrogen peroxide, tert butyl hydroperoxide methil viologen, chlorambucil

Table 1: *Overview of the biosensors, according to the target analyte, used microorganism, transducer type and detection specificity*
Preglednica 1: *Pregled biosenzorjev po tarčnih analitih, uporabljenem mikroorganizmu, vrsti prevodnika in specifičnosti detekcije*

Analyte	Microorganism	Transducer type	Detection limit (LOD), EC_{50} or IC_{50}	Reference
Methyl parathion	*Flavobacterium*	Optical	LOD = 0.3 µM	Kumar *et al.* (2006)
Methyl parathion	*Sphingomonas* JK1	Optical	Detection range: 4–80 µM	Kumar and D'Souza (2010)
Methil parathion	Recombinant *E. coli*	Electrochemical	LOD = 0.5 µM	Kumar and D'Souza (2011)
Methyl parathion, paraoxon	Recombinant *Moraxella*	Electrochemical	Methil parathion: LOD = 1 µM, paraoxon: LOD = 0.2 µM	Mulchandani and Rajesh (2011)
Lindane	Recombinant *E. coli* BL21	Electrochemical	LOD = 2 ppt	Anu Prathap *et al.* (2012)
Methyl viologen	Recombinant *E. coli*	Optical	LOD = 0.6 ppm	Niazi *et al.* (2008)
DCP, ametryn, endosulfan, fenamiphos	*E. coli*	Electrochemical	IC_{50} = 5.7–22 mg/L	Yong *et al.* (2011)
Lead	*Phormidium*	Electrochemical	LOD = $2.5*10^{-8}$ M	Yüce *et al.* (2010)
Copper	Heterogeneous microbial populations	MFC	LOD = 5 ppm	Shen *et al.* (2013)
Arsenite, selenite	Recombinant *E. coli* DH5α™	Optical	Arsenite: LOD = 0.2 µg/l, selenite: LOD = 5.8 ng/l	Ooi *et al.* (2015)
Cadmium, copper, lead, zinc, arsenite	Recombinant *E. coli* DH5α™	Optical	Pb: LOD = 5 ppm, Cd: LOD = 0.01 ppm, Cu, Zn: LOD = 0.001 ppm, arsenite: LOD = $1*10^{-7}$ mg/l	Arias-Barierro *et al.* (2010)
Zinc, cadmium	Recombinant *E. coli* DH5	Optical	/	Kim *et al.* (2015)
Isothiocyanate, cinnamaldehyde	Recombinant *E. coli* HB101	Optical	/	Chan *et al.* (2013)
Isothiocyanate, cinnamaldehyde	Recombinant *Acinetobacter baylyi* ADP1	Optical	/	Chan *et al.* (2013)
Copper, cadmium, zinc, chromium, mercury, lead	*Psychrobacter*	Electrochemical	EC_{50}: 0.8–110.1 mg/l	Wang *et al.* (2013)
Silver nanoparticles	*E. coli*, *P. fluorescens*, *S. cerevisiae*, *P. subcapitata*	Optical	EC_{50}: 0.01–8.17 mg/l	Ivask *et al.* (2014)
Chromium, iron, nitrate, sodium acetate	Heterogeneous microbial populations	MFC	/	Liu *et al.* (2014)
Cadmium	Electroactive mixed bacteria	MFC	Detection range: 1–50 µg/l	Di Lorenzo *et al.* (2014)
MMS, 4-NQO, phleomycin, hydrogen peroxide, tert butyl hydroperoxide methyl viologen, chlorambucil and cisplatin	*S. cerevisiae* BY4741	Optical	4-NQO: LOD = 0.12 ng/ml MMS: LOD = 0.36 µg/ml	Wei *et al.* (2013
Zinc, copper, 3,5-DCP, benzene, toluene, bronopol	Recombinant *E. coli* HB101	Optical	$EC_{50;}$ 0.09–21.0 mg/l	Horsburgh *et al.* (2002)
Catechol	*Lactobacillus*	Electrochemical	Detection range: 0.5–5.0 mM	Sagiroglu *et al.* (2011)

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Salicylate	Recombinant *E. coli DH5α*	Optical	LOD = 0.1 μM	Shin (2010)
BOD	*P. putida* SG10	Electrochemical	Detection range: 0.5–10 mg/l	Chee (2013)
BOD	Electroactive mixed bacteria	MFC	Detection range: 100–750 ppm	Ayyaru and Dharmaligman (2014)
BOD	Heterogeneous microbial populations	MFC	Detection range: 3–164 ppm	Di Lorenzo *et al.* (2014)
BOD	Heterogeneous microbial populations	Electrochemical	Detection range: 20–450 mg/l	Vaiopoulou *et al.* (2005)

and cisplatin). Transcripts for RNR3 and HUG1 genes that work as a sensor due to their overexpression induced with DNA damage were linked with the yEGFP reporter gene, enabling the fluorescent detection. They report of improved detection at mutants with five or seven genes deleted. The highest sensitivity was observed with quintuple and septuple mutants. The septuple mutant of the HUG1 sensor gene showed the greatest sensitivity (relative sensitivity: 0.12 ng/ml for 4-NQO and 0.36 μg/ml for MMS). Real-time monitoring is a key in the cases of large spills of toxicants to ensure an immediate response and reduce the negative effects on the environment (Di Lorenzo *et al.*, 2014). On-line microbial biosensor can be used for automatized detection of toxicity. The *E. coli* HB101 cell suspension transformed with the pUCD607 plasmid with a lux CDABE insert was applied by Horsburgh *et al.* (2002) for the detection of the toxicity of environmental samples from a metal plating plant, a paper mill and a distillery. They constructed a pump system, enabling continuous flow of the cells, mixed with the samples to a light detection unit. This biosensor is sensitive on a broad spectrum of chemicals (zinc, copper, 3,5-DCP, benzene, toluene, bronopol), the EC_{50} values measured with a biosensor for zinc and bronopol were significantly more reliable than EC_{50} measured by batch mode in a cuvette. The biosensor of this kind enables quick and cheap making of environmental samples fingerprint, without the use of chemicals.

4.4 PHENOLIC COMPOUNDS

Phenolic compounds that appear in the environment originate from the paper and pulp industry and from the production of drugs, dyes, and antioxidants (Rodriguez-Mozaz *et al.*, 2004). Lyophilised cells of the bacteria *Lactobacillus*, that were immobilised on a teflon-membrane oxygen electrode, work as a practical biosensor, suitable for the detection of catechol in wastewater and dairy products (Sagiroglu *et al.*, 2011). The sensor measures the difference in the concentration of dissolved oxygen depending on the concentration of catechol and shows good sensitivity, substrate specificity, repeatability and cost-efficiency. Aromatic compounds raise special awareness due to their toxicity and environmental resistance. The microbial activation mechanism, triggering the NahR regulatory protein synthesis in the presence of salicylate, was used by Shin (2010) for the construction of a biosensor. The *E. coli* DH5α was transformed with a pNRSAL plasmid containing the nahR gene and luciferase reporter gene, for the bioluminescent detection of salicylate. The response of the mutants, introduced by side directed mutagenesis at the residues 169 and 248 of the nahR gene was compared to the response of the wild type organism. The substitution of the amino acids leads into drastic changes in the microbial response to salicylate, including the 50-fold increase of sensitivity.

4.5 BIOCHEMICAL OXYGEN DEMAND

Biochemical oxygen demand (BOD or BOD_5) can be measured by a dedicated BOD test that applies aerobic microorganisms that consume the organic compounds in water systems for biochemical decomposition (Chee, 2013). BOD represents the oxygen used for neutralisation of organic compounds in 5 days, at 20 °C. Its conventional determination is time consuming and requests an expert to achieve repeatable results (Ayyaru and Dharmaligman, 2013). The use of biosensor enables us to avoid long-lasting incubation. They are mainly appropriate for the detection of BOD in samples with high concentration of easy-degradable organic compounds. Chee (2013) used five microorganisms (*P. putida* SG10, *P. fluorescens* IAM12022, *P. putida* IAM1236, *B. subtilis* IAM12118, *T.cutaneum* IFO10466) that were immobilized on a porous cellulose-nitrate membrane of an oxygen electrode for the detection of BOD in river samples. All of the organisms were exposed to artificial wastewater and standard solutions of glucose and glutaminic acid. The most sensitive one (*P. putida* SG10 with the detection limit of 0.5 mg/l) was applied for the characterization of

river samples. This biosensoric method is comparable with the determination of BOD_5 by the standard method.

MFC are also suitable for the detection of BOD. Ayyaru and Dharmaligman (2014) report of a single chamber MFC, enriched with electrochemically active bacteria, isolated from the University of Anna water treatment plant as a suitable biosensor for the characterization of the unstable BOD. They monitored the electric current, produced by MFC at continuous feeding of the properly diluted samples of artificial wastewater. The anodic electrode senses the BOD as a current, produced by electrogenic bacteria, when in contact with organic compounds. A similar principle was used by Di Lorenzo *et al.* (2014) for the evaluation of a single-chamber air–cathode MFC with multilayer 3D printing. MFC was enriched with heterogeneous microbial populations from another working MFC. They monitored the amperometrical response of the sensor on increasing concentration of acetate in water. The biosensor enabled a fast linear detection 3–164 ppm of chemical oxygen demand (due to the acetate, used in the study, it is similar to BOD_5)

The CO2 concentration in gas phase, a by-product of microbial respiration activity during the catalysis of organic compounds, can be measured for the determination of current BOD values in wastewater samples. This principle was used by Vaiopoulou *et al.* (2005) for the development of a microbial biosensor, consisting of a conical fluidized bed reactor and cylindrical oxygen saturation chamber. The cell biomass from the activated sludge was used as an immobilized biosensing component. The biosensor was firstly calibrated in a laboratory with artificial wastewater with the addition of glucose and acetic acid and later used for the detection of BOD in a wastewater treatment plant Xianthi. The biosensor is adjustable for a broad range of wastewater.It enables the use of microbial populations from existing wastewater treatment plant and shows high activity of the immobilized cells, due to the continuous oxygen feeding.

5 SUMMARY

Microorganisms are appropriate biosensing elements for the construction of environmental pollutants biosensors. They are used for the detection of heavy metals, pesticides, phenolic compounds, BOD and toxicity or genotoxicity. MFC and whole cell biosensors are the most frequently used biosensor types. The development of genetic engineering enables organism manipulation and improved action of the sensory system. The majority of biosensors stated above, show excellent performance in laboratory conditions, but are not yet all optimized for field applications.

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EKONOMSKA ANALIZA ORGANIZIRANOSTI JAHALNEGA CENTRA; PRIMER UPORABE LINEARNEGA PROGRAMIRANJA

Jaka ŽGAJNAR [1]

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Ekonomska analiza organiziranosti jahalnega centra; primer uporabe linearnega programiranja

V prispevku analiziramo ekonomiko konjeniškega centra, ki poleg storitev šole jahanja vključuje tudi rejo in oskrbo konj. Temeljimo na predpostavkah hipotetičnega kmetijskega gospodarstva, ki deluje v osrednji Sloveniji. Priprave optimalnega načrta gospodarjenja se v prispevku lotevamo s pomočjo metod matematičnega programiranja. Z njihovo uporabo lahko v danih razmerah z različnih zornih kotov ovrednotimo razvojno perspektivnost in pokažemo na možnosti povečanja dodane vrednosti. Na podlagi maksimiranja pokritja poizkušamo odgovarjati na različna vprašanja in izzive, ki se pojavijo pri vodenju in načrtovanju takšnega konjeniškega centra. Dobljeni rezultati kažejo, da so dana cenovno-stroškovna razmerja neugodna za lastno vzrejo konj, in sicer tako za namen obnove lastne črede šolskih konj kot tudi za prodajo. Slednje odraža trenutno neugodne razmere na področju konjereje. Na takšnih gospodarstvih je oskrba konj pomembna aktivnost in preko oportunitetnega vidika določa optimalni nabor dejavnosti. Tako z vidika diverzifikacije dohodka kot dodatnega vira prihodka so zanimive tudi aktivnosti male šole jahanja za otroke. Šola jahanja, ki ima zaposlenega enega inštruktorja jahanja, lahko ob predpostavki pokritega jahališča stroške polne zaposlitve pokrije s 4,6 šolskih konjev.

Ključne besede: konji / konjeništvo / jahalni centri / ekonomska analiza / linearno programiranje

Economic analysis of the organisation of a riding centre; application of the linear programming approach

In this contribution, the economics of an equestrian centre, which in addition to a variety of riding school activities also includes breeding and livery, are analysed. We consider the conditions for a hypothetical holding operating in central Slovenia. Methods of mathematical programming are applied in order to attempt to optimise the holding's activities. Their use may in the given situation facilitate the evaluation of development prospects from different perspectives and indicate the opportunities of increasing value-added. On the basis of maximizing the gross margin, we try to address the various questions and challenges that arise in managing and planning for such an equestrian centre. The obtained results indicate that breeding is unfavourable in the given price-cost ratio, both for the renewal of the working horses herd and for sale. This reflects the current adverse situation in the field of horse breeding. Livery is an important activity on such holdings and through opportunity perspective provides an optimal set of activities. Activities of the riding school for children are interesting in terms of income diversification, as well as an additional source of revenue. A riding school with one riding instructor and an indoor arena needs 4.6 horses in order to cover the costs of full-time employment.

Key words: horses / horseback riding / economic analysis / riding centers / linear programming

1 UVOD

V zadnjih desetletjih se je uporaba konj drastično spremenila. Konj je izgubil svoj prvotni pomen kot delovna in transportna žival (Vejnovič, 2008). V današnjem času se konje uporablja predvsem kot rekreacijske živali za jahanje v prostem času, za uporabo v zdravstvene in terapevtske namene ter v vrhunskem športu (Hess in sod., 2014). Avtorji tudi izpostavljajo, da je v zadnjem obdobju ključen dejavnik povečanja staleža konj predvsem povečano povpraševanje po storitvah konjeništva za zabavo in prosti čas. Ob tem Zasada (2013) ugotavlja, da je vse večja težnja urbanega prebivalstva po preživljanju prostega časa v naravi pripeljala do razvoja kmetijskih

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gospodarstev, ki v obliki jahalnih centrov ponujajo različne konjeniške storitve. Ljudje iz mest zaradi pomanjkanja ustrezne infrastrukture, časa ter znanja o skrbi za živali, konje prepustijo v oskrbo kmetijskim gospodarstvom, ki izpolnjujejo te kriterije in s tem zagotavljajo ustrezne življenjske pogoje za živali (Birke in sod., 2010). Ta dejavnost tako na mnogih gospodarstvih že predstavlja osrednjo oziroma dopolnilno dejavnost. Ob tem Estwood in sod. (2008) navajajo različne oblike oskrbe konj, ki jih lahko srečamo v praksi. Dodaten vir prihodkov pa na takšnih gospodarstvih pogosto predstavlja ponudba jahalnih tečajev, treniranja konj in podobnih aktivnosti, vezanih na konjeniško dejavnost. Slednje je nedvomno tudi posledica dejstva, da se bistveno več ljudi vključuje v ta šport, kot pa jih dejansko ima lastnega konja (Hess in sod., 2014). Takšna kmetijska gospodarstva s šolo jahanja tako pokrijejo razliko v povpraševanju in omogočajo ježo in aktivnosti s konji tudi tistim, ki nimajo lastnega konja.

Konjeništvo v Evropi postaja pomembna gospodarska panoga. Nix (2015) ugotavlja, da gre v primeru Anglije celo za drugo najpomembnejšo zaposlitveno dejavnost na podeželju. Poleg najrazličnejših storitev jahanja in vožnje vpreg, ta vključuje tudi druge aktivnosti in sicer od izdelave in trženja opreme za jahače, voznike in konje, veterinarstva, kovaštva pa vse do pridelovanja krme oziroma nakupa krme (Estwood in sod., 2008). Elgaker in sod. (2012) ocenjujejo, da se tovrstni prihodki iz konjeništva na Švedskem uvrščajo na peto mesto med najpomembnejšimi prihodki iz kmetijstva in po njihovi oceni znašajo 5 milijard €. Podobno vrednost dosegajo tudi v Nemčiji, kjer naj bi s tremi do štirimi konji ustvarili polno delovno mesto. Nadalje pa Liljenstolpe (2009) ugotavlja, da je v Veliki Britaniji za polno delovno mesto potrebnih pet do sedem konjev.

V praksi srečamo različne oblike gospodarstev, ki se tako ali drugače ukvarjajo s konjeništvom in konjerejo. Ta gospodarstva se po strukturi in velikosti pomembno razlikujejo (Elgaker in sod., 2012). Veliko je klasičnih oziroma ljubiteljskih kmetij, ki konje uporabljajo za delo oziroma prosti čas, manj pa je kmetijskih gospodarstev, specializiranih v panogi reje konj. Slednje se pojavljajo v obliki kobilarn ali jahalnih centrov, na katerih je prisotno večje število konj (Žgajnar in Zupan, 2014). Sama struktura kmetijskega gospodarstva, gledano z vidika aktivnosti, pa navadno definira tudi usmeritev le-te bodisi v oskrbo konj, rekreacijsko jahanje, šole jahanja oziroma profesionalno treniranje konjeniških disciplin.

Jahalni centri storitve jahanja tržijo pod skupnim imenom »šola jahanja«, ki vključujejo različne programe, od individualnih ur jahanja, jahanja v skupini, terenskega jahanja, jahanja ponijev za najmlajše ipd., ki so namenjeni različnim starostnim skupinam z različnim predznanjem. V zadnjih letih vse več jahalnih centrov ponuja tudi jahalne tabore in tako imenovane konjičkove delavnice, preko katerih otroci poleg jahanja spoznavajo tudi vsakdanje delo in skrb za konje (Žagar Hribar in Žgajnar, 2015). Nabor aktivnosti kot tudi storitev je tako zelo pester. Od šole jahanja, oskrbe konj do reje in vzreje konj. Tako kot pri ostalih gospodarskih panogah pa je za uspešno in učinkovito upravljanje potrebno natančno poznavanje posamezne aktivnosti tudi s stroškovno-prihodkovnega vidika. Slednje je ključno pri izbiri kombinacij aktivnosti, ki bodo ob danih pogojih omogočila dosego najvišje ravni dohodka. Za izpolnjevanje tega cilja je poleg potrebnega znanja s področja tehnologije, reje konj, konjeništva in upravljanja takšnega kmetijskega gospodarstva, potrebno tudi natančno poznavanje in obvladovanje stroškov. Iz tega naslova se bomo v danem prispevku osredotočili na podrobno analizo priprave načrta gospodarjenja ter izbiro ustreznega nabora storitvenih aktivnosti.

Pri uspešnosti delovanja takšnega gospodarstva nedvomno pomembno vlogo igra povpraševanje po njihovih storitvah. Ob tem pa velja izpostaviti, da za trg s konjeniškimi dejavnostmi veljajo določene posebnosti. Tako Hess in sod. (2014) poudarjajo, da gre pri tovrstnih aktivnostih, vezanih na konjeništvo, v večini primerov za lokalni trg in ne za nacionalni trg, kar načeloma velja za kmetijske trge. Tako je po ekonomski teoriji trg z jahalnimi storitvami pomembno odvisen od cen posameznih storitev. Te se razlikujejo med državami kot tudi med posameznimi regijami znotraj držav. Cene so odvisne od več dejavnikov, med katerimi poleg ponudbe in povpraševanja na nekem območju, ki ga določata povprečen prihodek gospodinjstev in gostota prebivalstva, pomembno vlogo igrajo zlasti kvalitativni dejavniki, kot so izkušenost in ugled učitelja jahanja, razpoložljiva infrastruktura, ne nazadnje pa tudi lokacija centra (Hess in sod., 2014). Pomembno vlogo pri definiranju cen storitev igra tudi tradicija določenega jahalnega centra (Hess in sod. 2014). Glede na njihove analize jahalnih šol na Švedskem so ugotovili, da višje cene dosegajo centri z daljšo tradicijo. Tako so cene storitev pri novoustanovljenih oziroma mlajših centrih, ki so svoje storitve pred kratkim ponudile na trg, značilno nižje.

Povprečne cene mesečne oskrbe konj se v EU gibljejo od 150 € do 750 €, cena povprečne ure jahanja pa med 10 € in 45 €. Slovenija po opravljeni mednarodni analizi s svojimi cenami zavzema srednje vrednosti med evropskimi državami (Liljenstolpe, 2009). Ob tem pa Hess in sod. (2014) ugotavljajo, da je v bližini urbanih naselij povprečni dohodek gospodinjstva višji in posledično imajo tudi šole jahanja nekoliko višje cene svojih storitev.

K zagotavljanju čim boljšega ekonomskega položaja in blaginje kmetijskih gospodarstev oziroma jahalnih centrov so poleg znanj tehnologije, reje konj in ekono-

mike potrebne tudi dodatne informacije o dostopnosti razpoložljivih virov in stroškovno-cenovnih razmerij. Slednje lahko pridobimo tudi s pomočjo podrobnih analiz storitveno-proizvodnega načrta. Ugotavljamo, da je v Sloveniji pomanjkanje tovrstnih analiz, neupoštevanje ekonomskih načel pri analizi poslovanja na področju konjeništva pa še otežuje optimalno načrtovanje aktivnosti, s katerimi bi najbolje izkoristili dane možnosti posameznega jahalnega centra oziroma pokazali na razvojne perspektivnosti.

Eden izmed pristopov reševanja in analiziranja tovrstnih problemov je modeliranje z elektronskimi preglednicami. Modele, ki jih zapišemo v obliki elektronskih preglednic, namreč lahko enostavno rešujemo z metodami matematičnega programiranja (Powell in Baker, 2009). Izmed slednjih je za tovrstne probleme najpogosteje uporabljeno predvsem linearno programiranje (v nadaljevanju LP), kjer optimiranje poteka na podlagi minimiranja oziroma maksimiranja namenske funkcije (Žgajnar in sod., 2011). Iz rešitev LP dobimo informacije o optimalni razporeditvi proizvodnih virov, o ključnih omejujočih virih, proizvodnih dejavnikih v presežku in smiselnih vrednostih za pridobitev dodatnih enot omejenih virov (Boehlje in Eidman, 1984). LP omogoča enostavno analizo morebitnih sprememb vhodnih parametrov, kot so cene inputov oziroma opravljenih storitev. Enostaven je tudi izračun oportunitetnih stroškov ob različnih tehnoloških učinkovitostih ob izbiri alternativne aktivnosti (Winston, 2004). Dodatno pa z analizo občutljivost lahko pridobimo tudi informacije o stabilnosti dobljenih rezultatov (Hazell in Norton, 1986). Na področju analize konjeniške dejavnosti v Sloveniji je bil podoben pristop uporabljen pri diplomskem delu, ki ga je opravil Stariha (2015).

Namen danega prispevka je predstaviti razvito modelno orodje za analize na področju konjeniške dejavnosti in sicer zlasti z vidika organiziranja centra, ki se poleg različnih oblik oskrbe in vzreje konj ukvarja tudi s šolo jahanja. Na primeru hipotetičnega gospodarstva želimo pokazati na nekatere zakonitosti, ki se kažejo v danih ekonomskih razmerah in ki jih ob določeni predpostavkah lahko posplošimo tudi na druge podobne primere.

Prispevek nadaljujemo s kratkim opisom pristopa modeliranja ter razvitega modelnega orodja za ekonomsko analizo optimalne organiziranosti aktivnosti jahalnega centra. V prvem delu na kratko opišemo strukturo modela ter ključne aktivnosti, ki lahko vstopajo v optimalno rešitev. Sledi opis ključnih skupin omejitev. Preko opisa hipotetičnega jahalnega centra podajamo osnovne predpostavke modeliranja. Tako poizkušamo na danem hipotetičnem primeru predstaviti nekatere zakonitosti, ki veljajo pri izbiri aktivnosti na takšnih gospodarstvih. Po predstavitvi rezultatov prispevek zaključujemo z diskusijo ključnih ugotovitev naše analize.

2 MATERIAL IN METODE

Problem optimalne organizacije jahalnega centra obravnavamo kot primer eno-kriterijskega problema, ki ga z vidika ekonomske teorije lahko razporedimo v skupino normativnih pristopov. Za potrebe dane analize temeljimo na pristopu omejene optimizacije in sicer deterministočnega linearnega programiranja. Z optimizacijskim potencialom LP ob danih omejitvah iščemo maksimum namenske funkcije, ki v našem primeru predstavlja najvišje pokritje (POK), ki bi ga na danem gospodarstvu ob predvidenih pogojih lahko dosegli.

Modelno orodje je razvito v Excelovem programskem okolju. To omogoča njegovo enostavno povezovanje, dopolnjevanje in prilagajanje posameznemu analiziranemu primeru. Povezave in večina potrebnih operacij je avtomatiziranih s pomočjo makrov, zapisanih v obliki kode VBA (Visual Basic for Applications) v urejevalniku za Visual Basic. Zapis osnovnih matrik za reševanje matematičnega modela kot tudi njegovo formiranje in množenje je tako sorazmerno enostavno.

Razvito modelno orodje je z vidika uporabnika odprt sistem, saj omogoča dopolnjevanje in prilagajanje posameznim analiziranim primerom. V prvi vrsti seveda s spreminjanjem proizvodnih parametrov obstoječim aktivnostim in prilagajanjem omejitev, kot tudi z dodajanjem novih aktivnosti in omejitev. Z modelnim orodjem tako lahko analiziramo različne tipe kmetijskih gospodarstev, usmerjenih v konjeništvo in konjerejo.

2.1 MATEMATIČNI ZAPIS MODELA

Namen modela je poiskati optimalen proizvodno-storitveni načrt, s katerim dosežemo najboljši finančni rezultat na ravni celotnega gospodarstva. S tem pa tudi analizirati in iskati ključne zakonitosti, ki jih je potrebno upoštevati pri načrtovanju takšnega centra. Model omogoča iskanje optimalne rešitve z maksimiranjem pokritja (*POK*). Dobljena rešitev tako odslikava odločanje gospodarja, ki je ravnodušen do tveganja in kot edino vodilo pri načrtovanju proizvodnje upošteva ekonomski vidik, seveda ob vseh danih tehnoloških zakonitostih in omejitvah razpoložljivih virov.

Matematično lahko LP zapišemo, kot je prikazano v enačbi (1):

$$\max POK = \sum_{j=1}^{n} POK_j x_j$$

tako, da je

$$\sum_{j=1}^{n} a_{ij} x_j \le b_i \qquad za\ vse\ i = 1\ do\ m$$

$$x_j \ge 0$$

pri čemer najvišje *POK* izračunamo kot vsoto pokritji posameznih storitvenih aktivnosti (POK_j), pomnoženih z obsegom j-te aktivnosti (x_j), ki vstopa v optimalni proizvodno-storitveni načrt. a_{ij} zajema tehnološko-rejske zakonitosti in pri naboru danih omejitev (b_i) omogoča iskanje maksimuma namenske funkcije (*max POK*).

2.2 OPIS AKTIVNOSTI IN NJIHOVIH ZNAČILNOSTI

Za potrebe analize smo se omejili na del najpogostejših oblik storitvenih aktivnosti na področju konjeništva in delom tudi konjereje. Model v dani verziji tako omogoča analizo zlasti rekreativnih tipov jahalnih centrov, usmerjenih v šolo jahanja, oskrbo konj ter deloma tudi lastno vzrejo konj. Ker je tokratni poudarek prispevka tudi na uporabljeni metodologiji, nabora aktivnosti nismo širili na druge tipe, ki se nanašajo predvsem na profesionalne športno-jahalne centre in specializirana gospodarstva za rejo in vzrejo konj.

Pri razvoju LP je ključno dobro poznavanje tehnoloških zakonitosti aktivnosti. Za vsako od aktivnosti, ki lahko vstopajo v optimalno rešitev, smo tako pripravili podrobno kalkulacijo v obliki ekonomsko-tehnološke karte. Iz slednje se izračunavajo potrebni tehnološki koeficienti (aij), kot tudi vzročni koeficienti (POKj) za potrebe optimizacije. Osnovne tehnološke zakonitosti, opredeljene s pomočjo tehnoloških kart, na katerih temeljijo posamezne aktivnosti, smo zasnovali po vzoru klasičnih kalkulacij pokritja. Pri analizi gospodarstev, usmerjenih v konjeništvo in konjerejo, in pri pripravi proizvodno-storitvenih načrtov je z danim modelom možno izbirati med različnimi aktivnostmi. V grobem jih lahko razdelimo na tri glavne skupine:

[1] aktivnosti oskrbe konj,
[2] aktivnosti reje in vzreje konj,
[3] aktivnosti šole jahanja (klasična šola jahanja ter aktivnosti male šole jahanja).

Prva skupina vključuje aktivnosti, ki se nanašajo na oskrbo konj. Pri tem imamo dve osnovni skupini in sicer oskrbo zasebnih konj ter oskrbo lastnih konj za potrebe šole jahanja. Aktivnosti smo opredelili do te mere, da omogočajo izbiro različnih tehnologij in sistemov reje (npr. klasični angleški boksi, prosta reja). Pri vseh rejah je predvideno, da se za nastil uporablja žagovina. Klasična oskrba konj vključuje dvakrat dnevno čiščenje boksov ter krmljenje voluminozne in močne krme. Dodatno vključuje tudi enkrat dnevno vodenje konj v izpust (215 dni v letu) ter sprehajalno napravo (200 dni v letu). Pri predvideni prosti reji predpostavljamo, da so konji uhlevljeni v posebnih skupinskih boksih z dostopom do pašnikov in so več časa zunaj. Posledično se stroški takšne oskrbe znižajo. Porabi se namreč manj nastilja, manj je dnevnega dela s čiščenjem ter v vegetacijski dobi pretežen del krmnega obroka predstavlja paša. Konj v takšni oskrbi tudi ne vodimo v sprehajalno napravo in seveda je bistveno manj premikov živali med pašniki. Tako znaša predvidena prodajna cena za klasično oskrbo 285 €, za prosto rejo 180 € oziroma za kobile z žrebetom v prosti reji 200 € mesečno.

Pri oskrbi lastnih konj za potrebe izvajanja aktivnosti šole jahanja smo dodatno vključili tudi stroške kovača, veterinarskih storitev in zavarovanja. Pri teh aktivnostih smo vključili tudi stroške dela za korekcijsko prejahovanje (skupno 30 ur letno) ustrezno usposobljene osebe, hkrati pa smo predpostavili, da se te konje vodi v sprehajalno napravo polovico manj kot zasebno uhlevljene konje.

Pri reji lastnih konj za potrebe izvajanja aktivnosti šole jahanja je ključna predpostavka, da lahko kupimo že izšolanega konja ali pa le-ta izhaja iz lastne vzreje. V obeh primerih predpostavljamo, da je konj v uporabi 10 let in sicer med petim in petnajstim letom starosti, nato ga nadomestimo z novim. Tako stroške obnove in prihodke izločene živali v prvem primeru zajamemo na ravni aktivnosti, v drugem primeru pa preko dodatnih aktivnosti reje in vzreje konj. Slednja skupina vključuje tako rejo plemenske kobile oziroma žrebice za razplod, kot tudi rejo letnika, dvoletnika, triletnika in štiriletnika. Dodatno pa vstopajo tudi aktivnosti prodaje doma vzrejenih živali in sicer v kateri koli fazi od žrebeta do štiriletnika.

Pri definiranju aktivnosti »vzreje lastnega delovnega konja« smo predpostavili, da z ujahovanjem pričnemo v četrtem letu in ga v enem letu tudi zaključimo. Osnovno ujahan konj tako lahko (i) vstopa v šolo jahanja ali pa (ii) ga prodamo po predvideni ceni. Poleg klasičnih šolskih konj smo med aktivnosti vključili tudi aktivnost reje ponija za otroke. Pri tem veljajo podobne zakonitosti, le da ponijev ne vzrejamo sami, pač pa jih lahko le dokupimo. V vseh primerih delovnih konj smo predpostavili, da konj letno lahko opravi 900 delovnih ur. Ob tem smo upoštevali, da lahko zaradi manjše poškodbe ali bolezni pride do izpada uporabe konja za določeno krajše obdobje.

Pomembno skupino storitvenih aktivnosti pa v našem modelu predstavljajo aktivnosti klasične šole jahanja in male šole jahanja za otroke. Pri snovanju teh aktivnosti smo izhajali iz izkušenj najpogosteje prisotnih oblik v tovrstnih šolah jahanja[1]. Načrtovanje in izvedba posameznih aktivnosti namreč definira tip jahalnega centra. Ta je lahko organiziran kot rekreativni ali kot profesionalni športni center, možne pa so seveda vse kombinacije med tema skrajnostma. V modelno orodje smo tako v prvem delu vključili: individualno uro jahanja (40 €), skupinsko uro jahanja (21 €) ter terensko jahanje v skupini (18 €/osebo). Pri zadnjih dveh aktivnostih smo predpostavili povprečno tri jahače na posamezni storitvi.

Drugi del pa zajema skupino aktivnosti v okviru male šole jahanja za otroke. Na tovrstnih centrih je namreč zanimiva tudi ponudba male šole jahanja oziroma jahanje ponijev, jahalni tabori med počitnicami, konjičkove delavnice (Žagar Hribar in Žgajnar, 2014; 2015) ter v zadnjem času tudi vse pogosteje organizacija rojstnodnevnih zabav za otroke. Dane aktivnosti smo tako vključili v nabor možnosti, ki lahko vstopajo v optimalno rešitev. Od tega z izjemo male šole jahanja na poniju, ki traja pol ure (15 €/storitev), konjičkove delavnice obsegajo dve uri (18 € na posamezno storitev, oziroma 110 € na paket 10 obiskov), praznovanje rojstnodnevne zabave tri ure (150 €) in tedenski tabor 8 ur dnevno (150 €).

V vseh primerih šola jahanja poteka z ustrezno usposobljeno osebo – inštruktorjem jahanja. Pri kalkuliranju stroškov dela smo ovrednotili strošek dela inštruktorja na bruto 8,5 € na uro. Prisotnost inštruktorja je obvezna tudi pri jahalnih aktivnostih, ki so vključene v jahalne tabore, konjičkove delavnice in rojstnodnevne zabave. Zaradi večjega števila otrok pri slednjih aktivnostih, pa pri izvajanju storitev sodeluje tudi dodatna oseba. Predviden strošek znaša 6,5 € na uro bruto.

2.3 NABOR OMEJITEV

Kmetijsko gospodarstvo, ki se ukvarja s konjeništvom, je tako kot vsaka gospodarska družba omejen sistem v smislu količine dostopnih proizvodno-storitvenih sredstev, kot so površine, delo in kapital v različnih oblikah (npr. hlevi, odprto jahališče, pokrito jahališče, sprehajalna naprava, različna druga oprema in stroji, čreda šolskih konj). Poleg danih notranjih omejitev pa se takšno gospodarstvo sooča tudi z zunanjimi omejitvami. V našem primeru so primer slednjih predvsem tržne omejitve, ki definirajo obseg povpraševanja po posameznih storitvah in aktivnostih jahalnega centra. Namen omejitev, ki jih vključujemo v tovrstne modele, je kar najbolje povzeti dano situacijo obravnavanega subjekta. V primeru našega modelnega orodja bi osnovni nabor omejitev (bi) lahko združili v naslednje skupine:

- Infrastrukturne omejitve (število boksov po kategorijah konj).
- Tržne omejitve – po posameznih kategorijah storitev in aktivnosti (šola jahanja in oskrba zasebnih konj).
- Omejitve ustrezno kvalificirane delovne sile (hlevar in inštruktor jahanja) na ravni leta in najem dodatne delovne sile.
- Bilance za zagotavljanje ustrezne delovne obremenitve šolskih konj.
- Bilanca živali po kategorijah v primeru razčlenjenih živinorejskih aktivnosti (reja in vzreja) ter minimalen stalež posameznih kategorij živali.

2.4 OPIS ANALIZIRANEGA PRIMERA

Uporabo modelnega orodja predstavljamo na hipotetičnem kmetijskem gospodarstvu. Predpostavljamo, da se le-to nahaja v osrednji Sloveniji, v bližini srednje velikega urbanega naselja. Na danem gospodarstvu imajo vso potrebo infrastrukturo za izvajanje šole jahanja kot tudi oskrbe konj. Sama infrastruktura za izvajanje aktivnosti šole jahanja pri naši analizi ni obravnavana kot omejitev. Predvideno je, da pri polno zasedenem hlevu kapacitete zadostujejo za vse potrebe šole jahanja, kot tudi za uporabo zasebno uhlevljenih konj.

Na strani oskrbe konj smo predvideli, da ima gospodarstvo možnost izpustov za vse konje in jih različno, glede na sezono, hlevar vodi v izpust. Med potrebno infrastrukturo imajo poleg izpustov in pašnikov tudi sprehajalno napravo za konje. Pri tem predpostavljamo, da imajo kapacitete boksov za 25 individualno uhlevljenih konj, od tega je pet primernih tudi za kobile z žrebeti. Ob tem potencialne stranke lahko izbirajo med različnimi tipi oskrbe, tudi prosto rejo v obsegu do 5 živali. Skupno tako lahko uhlevijo do 30 živali. Center ima na razpolago za izvajanje šole jahanja ustrezna jahališča kot tudi pokrito jahališče. Slednje omogoča, da lahko svoje aktivnosti izvajajo tudi v primeru slabega vremena, kar bi v nasprotnem primeru vnašalo precej negotovosti.

Pri analizi smo predvideli, da vso voluminozno krmo (seno, pašo, zeleno krmo) pridelajo na gospodarstvu. Ker ta krma z izjemo sena navadno ni predmet trgovanja, smo stroške njihove pridelave ovrednotili s pomočjo modelnih kalkulacijah Kmetijskega inštituta Slovenije. Vso ostalo močno krmo in mineralno vitaminske dodatke pa smo ovrednotili po tržnih cenah. Pri

1 Pri tem smo izhajali predvsem iz izkušenj na Pedagoško raziskovalnem centru za konjerejo Krumperk.

tem se seveda poraja vprašanje kako se spremeni morebitna organiziranost centra in aktivnosti pri načrtovanju gospodarjenja, če se spremenijo cenovno-stroškovna razmerja, tudi kot posledica, da več oziroma manj krme pridelajo sami oziroma dokupijo. Slednji vidik ni predmet obravnave v tem prispevku, je pa lahko izhodišče za nadaljnjo analizo.

Pri dani analizi smo predpostavili, da je število lastnih konj kot tudi njihova namembnost v modelu neznanka. Osnovna predpostavka je, da se na centru za potrebe šole jahanja, kot tudi reje in vzreje, redi lipicanske konje. Pri tem predpostavljamo, da je ustrezno izšolan konj za potrebe šole jahanja na trgu dostopen po ceni 12.000 €. Prav takšna je tudi predvidena prodajna cena lastno vzrejenega štiriletnega konja, ki opravi šolanje. Zaradi poenostavitev smo upoštevali, da je strošek plemenske kobile 10.000 €, nižji pa je strošek žrebice (6.000 €). Ob tem predpostavljamo, da lahko rejec proda žrebe po ceni 1.500 €, letnika po ceni 2.500 €, dvoletnika po ceni 3.000 €, triletnika preden začne z ujahovanjem pa po ceni 4.500 € [1].

Na danem gospodarstvu imajo stalno razpoložljivo delovno silo in sicer enega hlevarja (v obsegu 1.900 ur), ki skrbi za oskrbo konj, premike konj (izpust, sprehajalna naprava), čiščenje hlevov, po potrebi pa lahko sodeluje tudi kot pomočnik pri nekaterih aktivnostih male šole jahanja. Pri tem smo pavšalno ocenili tudi obseg dela, ki vključuje pripravo krme na lastnih površinah, razvoz gnoja ter ostalih potrebnih opravil. V obsegu 1.900 ur letno pa je na razpolago tudi inštruktorja jahanja, ki izvaja aktivnosti klasične šole jahanja in male šole jahanja ter po potrebi ujahuje in prejahuje lastne konje. V primeru dodatnih potreb po delovni sili lahko le-to gospodarstvo tudi najame. Pri tem smo predpostavili, da gre za ustrezno usposobljeno delovno silo, pri čemer dodaten strošek posamezne ure v izhodiščnem scenariju znaša 5,5 € za hlevarska opravila in 7,8 € za pomoč pri šoli jahanja.

2.5 SCENARIJSKA ANALIZA

S pomočjo razvitega modelnega orodja smo za namen danega prispevka analizirali tri izhodiščne primere. V prvem primeru (A) predpostavljamo, da imamo opravka z gospodarstvom, ki v nabor svojih aktivnosti lahko vključiti oskrbo zasebnih konj, ter rejo in vzrejo lastnih konj za prodajo. Prisoten je učitelj, ki je vešč ujahovanja konj in je tako ključen 'produkt', poleg storitev oskrbe, predvsem ujahan konj. Seveda pa lahko prodajo tudi še neujahane mlade živali, v kateri koli fazi od žrebeta do ujahanega štiriletnika. V drugem primeru (B) smo analizirali primer, ko ima kmetijsko gospodarstvo možnost dodatno izvajati tudi aktivnosti klasične šole jahanja za odrasle, bodisi na lastno vzrejenem konju ali pa na kupljenem šolskem konju. V tretjem primeru (C) pa smo nabor možnih aktivnosti razširili tudi na malo šolo jahanja za otroke.

Dodatno smo analizirali, kako se spremeni optimalni proizvodni načrt pri različnih postavkah najetega dela hlevarja in inštruktorja jahanja. Pri analizi smo predpostavili tri možnosti in sicer:

(1) kmetijsko gospodarstvo nima možnosti najema dodatne delovne sile,
(2) kmetijsko gospodarstvo lahko najame dodatno delovno silo, pri čemer strošek dodatne hlevarske ure znaša 5,5 € ter inštruktorske ure 7,8 €.
(3) V zadnjem primeru pa smo predpostavili, da se zniža zgolj strošek hlevarske ure za 0,3 €/h.

3 REZULTATI IN RAZPRAVA

V nadaljevanju prikazujemo rezultate analize hipotetičnega gospodarstva (pregl. 1). Ker pri prvem (A) in drugem (B) proizvodnem načrtu razpoložljiva delovna sila ni bila izkoriščena, posledično ni razlik med rezultati ob predpostavkah (1) in (2). Zato za ta dva primera prikazujemo zgolj rezultate ob predpostavki (2).

Kot izhaja iz preglednice 1, na višino realiziranih prihodkov pomembno vpliva nabor aktivnosti, ki vstopajo v optimalno rešitev. V prvem primeru (A2) tako gospodarstvo dosega skupne prihodke iz naslova oskrbe zasebnih konj in proste reje v višini 44.750 €. Ob danih stroških bi tako gospodarstvo doseglo POK v višini dobrih 15 tisoč €. Zanimiv je rezultat, ki kaže, da bi z možnostjo najema dodatne delovne sile (hlevarja) stalež povečali do zapolnitve razpoložljivih kapacitet (skupno 30 konj), kar bi prihodke zvišalo za dobrih 46 %, vendar zaradi hkratnega povišanja spremenljivih stroškov, bi se doseženo POK zvišalo zgolj za 1 %. Zanimiv rezultat, ki ga nakazuje prvi scenarij (A2 in A3), kjer predpostavljamo, da se na takšnem centru lahko izvaja le oskrba zasebnih konj (klasična in prosta reja) ter reja in vzreja konj, slednja ob dani stroškovno-cenovnih razmerjih ne vstopa v optimalno rešitev. Ta v takšnem primeru vključuje le aktivnosti oskrbe zasebnih konj, pri čemer je potrebno poudariti, da se v vseh primerih (tudi B in C) v rešitev vključuje zgolj klasična prosta reja, ne pa tudi prosta reja kobile z žrebetom. Za slednjo znaša zmanjšan strošek v vseh treh primerih –430 €, za kolikor bi se poslabšalo skupno doseženo pokritje, če bi aktivnost vstopala v optimalno rešitev.

1 Predpostavljene cene ne odražajo podrobne analize razmer na trgu, pač pa so določene hipotetično za potrebe dane analize.

Preglednica 1: *Optimalen proizvodni načrt organiziranosti jahalnega centra pri različnih izhodiščih in različnih stroških najete delovne sile*
Table 1: *Optimal production plans to organise riding centre with different assumptions and different costs of hired labour*

	Doseženi rezultati pri različnih izhodiščih						
	A2	A3	B2	B3	C1	C2	C3
Ekonomski kazalniki na ravni KMG (€)							
Skupni prihodki	44.750	96.300	106.652	156.987	119.518	131.679	181.436
Skupni VC	29.735	81.119	52.556	102.728	58.592	69.031	118.627
Skupni strošek krme	12.692	27.904	12.302	27.156	12.175	11.881	26.564
Strošek nastilja	3.450,7	7.972,6	3.557,3	7.972,6	3.564,7	3.607,9	7.972,6
Strošek dela hlevarja	12.350	25.747	12.350	25.431	12.350	12.350	25.281
Strošek dela inštruktorja	0	0	13.912	13.912	16.150	20.349	20.349
Pokritje	15.014	15.181	54.096	54.258	60.926	62.649	62.809
Seznam aktivnosti							
Vzreja in reja (št.)*							
Oskrba konj (št.)							
Klasična oskrba	9,9	25,0	5,7	20,4	5,9	4,8	19,3
Prosta reja – oskrba	5,0	5,0	5,0	5,0	5,0	5,0	5,0
Delavni konj (št.)							
Šolski konj (nakup)	0,0	0,0	4,6	4,6	4,0	4,6	4,6
Šolski konj (vzreja)	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Poni	0,0	0,0	0,0	0,0	0,4	1,0	1,0
Aktivnosti šole jahanja (h)							
Individualna ura jahanja			500,0	500,0	500,0	500,0	500,0
Jahanje v skupini			2.100,0	2.100,0	2.100,0	2.100,0	2.100,0
Terensko jahanje – skupina			600,0	600,0	160,4	600,0	600,0
Mala šola jahanja					0,4	300,0	300,0
KD – posamično vplačilo					150,0	150,0	150,0
KD – paketna storitev					60,0	60,0	60,0
Jahalni tabor					4,0	4,0	4,0
Rojstno dneva zabava					20,0	20,0	20,0
Delovna sila (h)							
Hlevar	1.900,0	1.900,0	1.900,0	1.900,0	1.900,0	1.900,0	1.900,0
Inštruktor	0,0	0,0	1.636,7	1.636,7	1.900,0	1.900,0	1.900,0
Najem delovne sile – hlevar	0,0	2.576,3	0,0	2.515,5	0,0	0,0	2.486,6
Najem delovne sile – inštruktor	0,0	0,0	0,0	0,0	0,0	538,3	538,3
Delo hlevarja – razčlenjeno (h)							
Čiščenje boksov	664,7	1.581,7	686,3	1.581,7	687,8	696,6	1.581,7
Nastiljanje boksov	211,6	486,7	218,1	486,7	218,5	221,1	486,7
Čiščenje hleva	151,2	334,6	155,5	334,6	155,8	157,6	334,6
Krmljenje skupaj	302,4	669,2	311,0	669,2	311,6	315,1	669,2
Premiki živali – izpust	305,4	737,5	315,6	737,5	316,3	320,4	737,5
Sprehajalna naprava	264,7	666,7	213,5	605,9	209,9	189,1	577,1

Legenda: KD – konjičkove delavnice; VC – spremenljivi stroški;* Ker nobena od aktivnosti reje in vzreje ne vstopa v rešitev, smo jih pri prikazu izpustili; A – možne samo aktivnosti reje in vzreje, B – vključuje dodatno aktivnosti šole jahanja, C – pogoji B ter možnost aktivnosti male šole jahanja; (1) ni možnosti najema dodatne delovne sile; (2) možnost najema dodatnega hlevarja (5,5 €/h) ter dodatne inštruktorske ure (7,8 €/h); (3) isti pogoji, kot v primeru 2, le da strošek dodatne ure hlevarja znaša 5,2 €/h.

Iz rezultatov (pregl. 1) zelo jasno izhaja, da v danih cenovno-stroškovnih razmerjih in predvideni tehnologiji, da so konji ves čas vzreje individualno uhlevljeni v boksih, reja ekonomsko ni upravičena. Tako v nobenem od predvidenih scenarijev reja in vzreja ne vstopata v optimalno rešitev, zato rezultatov po posameznih kategorijah posebej tudi ne prikazujemo. Senčne cene kažejo na problem visokih oportunitenih stroškov. Z dodatnimi analizami smo ugotovili, da se aktualnost sicer nekoliko izboljša, če uspemo pomembno znižati stroške reje (zlasti delo hlevarja, deloma pa tudi krmnega obroka). Denimo v primeru predpostavk C, bi to pomenilo skoraj 50 % znižanje stroškov štiriletne vzreje. Oziroma bi v takšnem primeru moral vzrejeni konj dosegati sorazmerno visoko prodajno ceno, da bi se gospodarstvu splačalo vzrejati živali bodisi za prodajo oziroma lastne šolske konje. Modelni rezultati kažejo, da v kolikor bi bil v danih razmerah ustrezno izšolan šolski konj dosegljiv po ceni 12.000 €, se lastna vzreja in ujahovanje ne splačata (pregl. 1). Ta vstopi v rešitev šele, če se predvidena nabavna cena šolskega konja zviša. Zanimivo je, da je zmanjšan strošek, izračunan pri postoptimalni analizi, bistveno višji pri uporabi takšnega konja za šolo jahanja (7.761 €), kot pa če bi ujahanega prodali (465 €). Seveda bi bil rezultat drugačen, v kolikor bi predpostavili, da je strošek dela inštruktorja stalni strošek. Vendar, ker naš model temelji na konceptu POK, takšna analiza ni možna. Slednje lepo odslikava trenutne razmere na področju konjereje, ki jih je gospodarska kriza nedvomno še poslabšala.

V kolikor predpostavimo, da ima gospodarstvo tudi ustrezno povpraševanje po storitvah šole jahanja za odrasle (scenarij B), se doseženo POK na gospodarstvu izboljša za dobrih 72 %, prihodki pa v obeh primerih (2 in 3) presežejo 100 tisoč €. Tudi v tem primeru se pokaže ekonomsko upravičena potreba po dodatnem delu le v primeru nižjega stroška dela (5,2 €/h), se pa v obeh primerih B2 in B3 stalež privatno uhlevljenih konj zniža na račun lastno uhlevljenih šolskih konj (4,6 konjev). Število slednjih determinira predvideno povpraševanje po storitvah šole jahanja. To je v našem primeru zunanja (eksogena) spremenljivka in jo preprosto določimo. Ker je povpraševanje nižje od razpoložljivih letno ocenjenih ur inštruktorja jahanja, je pričakovano, da omejitve niso omejujoče in lahko opravimo vse storitve. S pomočjo postoptimalne analize smo tako ugotovili, da znaša senčna cena vsake dodatne enote povpraševanja po storitvah ob pogojih B (isto za 2 in 3) za individualno uro jahanja 25,87 €, 10,66 € za skupinsko uro jahanja ter 6,25 € za posamezno uro skupinske ježe. Kar logično nakazuje tudi na to, da bi se s povečanim povpraševanjem dosežen rezultat izboljšal, oziroma koliko lahko odštejemo za dodatno enoto povpraševanja po danih storitvah. Iz tega vidika je zanimivo, da se pri pogojih (C) te vrednosti znižajo, kar je seveda posledica oportunitenega vidika aktivnosti male šole jahanja. Nadalje je zanimivo, da se tudi v primeru pogojev (B) stalež zasebno uhlevljenih konj poveča, če je dodatno delo moč dobiti po ceni, manjši ali enaki 5,2 €/h (B2). Vendar z dodatnim izračunom lahko ugotovimo, da se doseženo POK na vloženo povprečno uro zniža z dobrih 15 € na 8,96 €.

Vse bolj pogosto pa na kmetijskih gospodarstvih, usmerjenih v konjeništvo, lahko srečamo poleg že analiziranih aktivnosti tudi najrazličnejše aktivnosti male šole jahanja (C), kar lahko pomembno izboljša dosežen rezultat. Kot izhaja iz preglednice 1, v vseh treh primerih predvideni prihodki presežejo 100 tisoč €. Za ta primer prikazujemo tudi scenarij, kjer predpostavljamo, da ni možno najeti dodatne delovne sile. Če primerjamo C1 in B2, lahko vidimo, da je skupni stalež (nekoliko poenostavljeno) enak, se pa na račun manjšega števila šolskih konj vključi tudi poni. Slednje lepo kaže na pomen oportunitetnega vidika. Tako se pomembno zniža obseg izvajanja terenske ježe na zgolj 26,7 %, vendar se do zapolnitve kapacitet inštruktor jahanja vključi v storitve male šole jahanja. In sicer tiste, katerih cenovno-stroškovno razmerje je ugodnejše. Torej skoraj nič male šole jahanja (30 min ježe na poniju), v celoti pa ostale predvidene (skupinske) storitve. Problem, ki se sicer pojavi v praksi, je predvsem ta, da je pri skupinskih aktivnostih večji riziko prepozne odpovedi oziroma stranka ne pride, kar pa pomembno poslabša (predpostavljeno) ekonomiko. Seveda pa bi bil v takšnem primeru pričakovano ta trend obraten ali vsaj manj izrazit. Nadalje je razvidno, da v kolikor imamo možnost najema dodatne delovne sile, se v optimalni načrt vključijo vse aktivnosti in v primeru nižjega stroška hlevarja tudi v tem primeru pride do popolne zapolnitve razpoložljivih kapacitet za oskrbo konj. Senčna cena je tudi v tem primeru enaka kot v primeru B.

Tudi pri scenariju (C) bi gospodarstvo kljub sicer precej različnemu obsegu dela doseglo zelo podobno višino skupnega POK. Posledično je pričakovano, da se doseženo POK na vloženo uro poslabša. Ob pogojih C1 bi znašalo 16,03 € na vloženo uro, pri možnosti dodatnega najema delovne sile (C2) pa 14,44 €. V kolikor bi strošek dela znašal manj ali enako 5,2 € na dodatno uro hlevarja, se stalež živali poveča do maksimuma, kar pa zniža doseženo pokritje na opravljeno povprečno uro na 9,2 €.

4 ZAKLJUČKI

V prispevku smo predstavili razvito modelno orodje za analizo optimalne organiziranosti jahalnega centra na hipotetičnem primeru. Uporabljen pristop matematičnega programiranja, ki temelji na LP, se je izkazal kot učinkovit, saj omogoča študiranje in analiziranje zakoni-

tosti na kmetijskih gospodarstvih, umerjenih v konjerejo in konjeništvo. Poslovanje hipotetičnega konjeniškega centra smo ovrednotili v različnih okoliščinah s pomočjo treh osnovnih scenarijev (A, B in C). Za vsak scenarij smo izdelali osnovno analizo poslovanja, iz katere izhaja, katere aktivnosti lahko vstopajo v optimalno rešitev in kakšen je njihov predviden obseg. Proizvodni načrt posameznega scenarija smo nato nadgradili s postoptimalno analizo, iz katere dobimo informacije o občutljivosti dobljene rešitve na morebitne spremembe, hkrati pa tudi, koliko lahko odštejemo za dodatno enoto posameznega omejenega resursa oziroma za koliko bi se morala spremeniti višina stroškov ali prodajne cene, da bi določena aktivnost vstopala v rešitev.

Rezultati kažejo na nekatere zakonitosti, ki jih lahko opazimo tudi v praksi. Tako želimo izpostaviti predvsem trenutno izrazito neugodne pogoje za rejo in vzrejo konj na takšnih centrih. Pri tem je potrebno iskati možnosti čim cenejše tehnologije, kar uhlevljanje v boksu zagotovo ni. Z izrazitim znižanjem stroškov vzreje in ob ustrezno visokih prodajnih cenah je to lahko tudi ekonomsko upravičeno. Izkaže se, da imajo stroški dela lahko pomemben vpliv na obseg optimalne oskrbe konj. Seveda se obseg dela med gospodarstvi lahko precej razlikuje, ključno pa je predvsem, kako dolge poti mora hlevar pri posameznem opravilu opraviti. Z aktivnostmi šole jahanja lahko značilno izboljšamo ekonomiko na konkretnem gospodarstvu. POK se lahko izboljša tudi za 72 %. Pri tem pa je zelo pomembno, da stalež šolskih konj prilagodimo kapacitetam inštruktorja jahanja. Izkaže se, da so za polno zaposlitev dovolj 4,6 konj.

Da bo z modelom možno analizirati tudi ostala gospodarstva, usmerjena v konjeništvo, bo v nadaljnji fazi razvoja modelnega orodja potrebno razširiti nabor aktivnosti. Pri nadaljnjem razvoju modelnega orodja bi bilo zanimivo vključiti tudi vpliv sezone in razčleniti povpraševanje kot tudi razpoložljive kapacitete preko tedna in vikenda. Namreč med temi obdobji prihaja do pomembnih razlik v obsegu povpraševanja na eni strani, po drugi strani pa tudi razpoložljivi kapaciteti infrastrukture. Slednje je po naših pričakovanjih izraziteje na tovrstnih centrih, kot smo ga obravnavali v naši analizi, v primerjavi s profesionalnimi športnimi centri. Poleg predstavljenega pa dodaten izziv predstavlja tudi vzreja konj ter iskanje dovoljene višine stroškov. Po eni strani je potrebno najti ekonomsko vzdržno rešitev, po drugi strani pa gre za izrazit problem vezanega kapitala, katerega bi lahko obravnavali tudi z dinamičnimi metodami vrednotenja naložb, kar vzreja in ujahovanje konj zagotovo je.

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ESTABLISHMENT OF PRIMARY KERATINOCYTE CULTURE FROM HORSE TISSUE BIOPSATES

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Establishment of primary keratinocyte culture from horse tissue biopsates

Primary cell lines established from skin tissue can be used in immunological, proteomic and genomic studies as *in vitro* skin models. The goal of our study was to establish a primary keratinocyte cell culture from tissue biopsates of two horses. The primary keratinocyte cell culture was obtained by mechanical and enzymatic dissociation and with explant culture method. The result was a heterogeneous primary culture comprised of keratinocytes and fibroblasts. To distinguish epithelial and mesenchymal cells immunofluorescent characterisation was performed, using antibodies against cytokeratin 14 and vimentin. We successfully at attained a primary cell line of keratinocytes, which could potentially be used to study equine skin diseases, as an animal model for human diseases, and for cosmetic and therapeutic product testing.

Key words: molecular biology / primary cell culture / skin / horses / keratinocytes

Vzpostavitev primarne celične kulture konjskih keratinocitov iz biopsij

Primarna celična linija iz kožnega tkiva lahko služi za imunološke, proteomske in genomske raziskave kot *in vitro* model kože. Cilj naše študije je bila vzpostavitev primarne celične linije keratinocitov iz biopsije kožnega tkiva dveh konjev. Primarno celično kulturo smo pridobili z mehansko in encimsko razgradnjo tkiva in z metodo izraščanja celic. Rezultat je bila heterogena kultura keratinocitov in fibroblastov. Za imunološko karakterizacijo smo uporabili protitelesa proti citokeratinu 14 in vimentinu ter poskusili obogatiti delež keratinocitov v kulturi. Uspešno smo pridobili primarno celično linijo keratinocitov, ki je potencialno uporabna za preučevanje kožnih bolezni konjev, kot živalski model za preučevanje človeških bolezni in za testiranje kozmetičnih izdelkov ter zdravil.

Ključne besede: molekularna biologija / primarna celična kultura / koža / konji / keratinocite

1 INTRODUCTION

Because of the economic value and the number of animals (in 2013 there were almost 60 million registered horses) (Faostat, 2015), horses (*Equus caballus*) represent a group of economically important animals. A significant share of veterinary research has focused on researching and treating diseases in horses. In some cases, a horse also became an animal model for human diseases, for example in melanoma studies (Seltenhammer *et al.*, 2004) and muscle, joints, tendons, ligaments, and bone injuries (Nagy *et al.*, 2014). In research that is focusing on analysing various diseases, their mechanisms of action, and subsequent physiological changes and potential treatments, the use of *in vitro* models is crucial. It enables the application of immunological, genomic and proteomic studies on a tissue culture of an organ, excluding the systemic and environmental effects and providing more comparable results as in *in vivo* studies. Additionally, *in vitro* experiments are more reasonably priced and less ethically controversial.

The skin represents the largest organ in mam-

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malian body that is divided in three major layers: dermis, epidermis and subcutis. The major cell type in the outer layer of skin are keratinocytes, which represent a protective layer. The most prevalent cell type in dermis are fibroblasts, which produce different growth factors, needed for proliferation of keratinocytes. The types of cells found in subcutis are macrophages, fibroblasts and adipose cells (Orazizadeh et. al., 2015). Research on horse skin cell lines resulted in a range of applications, including establishment of primary keratinocyte cultures for researching extracellular matrix proteins, associated with skin disseases (Visser and Pollit, 2010) and development of an equine skin-equivalent model that could be used in regenerative medicine, pathogenesis research, and biological and pharmacological studies (Cerrato *et al.*, 2014). However, very few protocols for preparation and sustainment of horse primary cell lines have been developed (*e.g.* Wunn *et al.*, 1999; Aasen and Bellmonte, 2010).

The purpose of this study was to establish a primary horse skin epithelial cell culture and to characterise cell types present in the derived culture. The study is interesting for researchers studying degenerative skin and hair diseases in horses and possibly in other species. For example, in human medicine optimisation of protocols for sustaining keratinocyte cell lines became important as a part of production process of skin substitute that is used for treatment after burns and other larger tissue damages. Various diseases in horse, such as hereditary equine regional dermal asthenia (HERDA), alopecia areata (AA) and incontinentia pigmenti (IP), known in different breeds, are associated with skin lesions, hair or mane loss and other related health problems. Detecting skin and hair diseases is usually a big economic burden for breeders. At the same time, these diseases are mostly incurable, largely due to the lack of information about the illness. Skin models employing primary cells could be used for pathogenesis research and understanding of such diseases and could be useful as a starting point in drug screening/testing studies (Guo and Jahoda, 2009).

2 MATERIALS AND METHODS

2.1 ESTABLISHMENT AND PROPAGATION OF THE CELL CULTURE

Skin biopsates containing epidermis and dermis were aseptically removed from neck area below the mane of two Appaloosa breed horses (*Equus caballus*). From each horse two samples of approximately 2 cm^2 were taken and transferred into phosphate buffered saline (PBS) with added antibiotics (100 mg/mL streptomycin and 100 mg/mL penicillin). Tissue was stored at 4 °C during transport and processed immediately. Enzymatic and tissue explant methods were used for establishment of primary cell culture.

For enzymatic digestion, the tissue biopsates were incubated in dispase II (2.4 U/mL) at 4 °C for 18 hours. After incubation of tissue epidermis was separated from dermis. The epidermis was cut into small pieces and incubated in 0.25 % trypsin with 0.05 % EDTA for 30 minutes at 37 °C (Vissar and Pollitt, 2010). During the incubation, the samples were shook every several minutes. Trypsin was inactivated with addition of medium containing serum. The liquid phase was separated from the remaining tissue pieces and centrifuged at 1000 rpm for 10 minutes to collect the dissociated cells in suspension for seeding. Fresh trypsin can be added to the processed tissue and the process repeated for several times, until the tissue is fully dissociated. The collected cells were seeded in untreated plastic culture flasks and Geltrex (Gibco)-covered culture flasks. Thin-layer Geltrex method (described in the manufacturer's protocol) was used to cover plastic surface with a mixture of DMEM and Geltrex in 1:100 ratio. DMEM/F12 (D6421, Sigma) supplemented with 10 % FBS, 10 ng/mL EGF, 5 µg/mL insulin, 0.4 µg/mL hydrocortisone, 0.1 µg/10 mL cholera toxin, 2 mmol/L glutamine, and 5 µg transferrin was used to grow the cells (growth medium). The cells were grown at 37 °C and 5 % CO_2.

Differential trypsinisation was used to obtain enriched keratinocyte culture. The primary culture, containing keratinocytes and fibroblasts, was incubated in a solution of 0.125 % trypsin-EDTA for several minutes, with continuous microscopic observation. When most of the fibroblasts detached the reaction was stopped. The solution containing fibroblasts was removed, while keratinocytes remain attached. Because of the difference in the adhesion potential of the two predominant cell types, we were able to obtain two separate, almost pure keratinocyte and fibroblast cell lines.

Solution of 0.25 % trypsin-EDTA was used to detach the cells for subculturing. The cells were split in a 1:5 ratio and cultivated further in the growth medium. A mixture of basic medium, 20 % FBS and 10 % DMSO was used for freezing the cells. The cells were frozen in a freezing container that provides a cooling rate of 1 °C per minute, kept at −80 °C overnight, and transferred in liquid nitrogen for long term storage.

Alternatively, for explant culture method tissue was mechanically minced and small parts of the tissue seeded directly in culture dishes, containing the growth medium. All other procedures were the same as described previously.

2.2 IMMUNOFLUORESCENT STAINING

The cells, grown for seven days, were washed in PBS and fixed in 4 % formalin for 5 minutes. Cells were permeabilised with 1 % Triton-X in PBS and incubated for 20 minutes. The fixed cells were washed with PBS and blocked with 10 % goat serum for 30 minutes at room temperature. Monoclonal mouse (Santa Cruz Biotech sc-53253) and polyclonal rabbit (Covance PRB-155P) antibodies against cytokeratin 14 and monoclonal mouse antibody against vimentin (Santa Cruz Biotech sc-73262) were used to distinguish between keratinocytes and mesenchymal (fibroblast) cells. The cells were incubated overnight at 4 °C in a solution of primary antibodies (1:100). After three 5-minute washes in PBS, they were incubated for 60 minutes in a dilution (1:200) of labelled secondary goat-anti-rabbit antibody (Invitrogen, Alexafluor 488, A11008) to visualize attachment of polyclonal cytokeratin 14 and in goat-anti-mouse (Alexafluor 594, A11037) antibody to visualize attachment of primary mouse monoclonal antibodies (cytokeratin 14 and vimentin). After incubation in secondary antibodies, the cells were washed again for several times in PBS and counterstained with DAPI (1:1000) for 3 minutes to visualize nuclear DNA. After washing in PBS, the cells were observed under fluorescent microscope (Nikon Eclipse TE, 2000).

3 RESULTS AND DISCUSSION

Tissue explant and enzymatic method were used for establishment of the primary keratinocyte cell culture. The enzymatic dissociation of the tissue resulted in a heterogeneous mixture of cells, predominantly consisting of keratinocytes and fibroblasts. Adherent cells that explanted from attached pieces of the tissue consisted mainly of keratinocytes, however the yield of cells was much lower, compared to enzymatic method. In tissue explant method, cell outgrowths growing from mechanically processed tissue were observed after several days; *e.g.* five days as showed in Figure 1. In enzymatic method, the dissociated cells attached, but the proliferation rate was low in the first few days. After several days in culture the cells started to proliferate and formed islands of densely packed keratinocytes and more sparsely growing fibroblasts (Fig. 2a). The cells attached to both – Geltrex-matrix and plastic surface, however, proliferation was faster on Geltrex. Witte and Kao (2005) showed that extracellular matrix is extremely important in cell adhesion and proliferation. It can also affect the development of epithelial cells, their morphogenetic activity and even survival. Evidence suggests that the absence of appropriate matrix might be the reason for progressive necrosis of epithelial cells on plastic surface (Freeman *et al*., 1978).

After 7 days of cultivation, keratinocytes formed large clusters (Fig. 2b). Orazizadeh *et al*., 2015 compared enzymatic and explant methods for human foreskin derived keratinocytes in the absence of feeder layer. They noticed that after enzymatic treatment no attached cells were found after 7 to 10 days. With explant method they found separated keratinocytes after 24 hours that latter formed large clusters of cells. Our findings differ from that, namely, the yield of cells was higher when enzymatic method was used. After 10 days in culture we observed large colonies of keratinocytes, surrounded by fibroblasts. Witte and Kao (2005) reported that growth and adhesion of keratinocytes can be increased in the presence of mesenchymal cells (fibroblasts). Interleukin 1 beta (IL-1β) is released by keratinocytes and stimulates production of diverse fibroblast growth factors (FGF) in mesenchymal cells, including keratinocyte growth factor (KGF) that enhances migration and proliferation of keratinocytes (Witte and Kao, 2005).

Fibroblasts are normally unwanted in primary cell cultures. Fibroblast contamination in the culture was decreased with mechanical removal of dermis in the skin biopsate (using scalpel) and with incubation in dispase II that enabled physical separation of epidermal and dermal tissue. For additional separation of keratinocytes from fibroblasts the differential trypsinisation was used. In case of tissue explant method, we obtained a relatively pure keratinocyte culture. Guo and Jahonda (2009) reported that fibroblasts migrate out of adult skin explant later than keratinocytes. With well-timed removal of tissue pieces from the culture, we were able to avoid fibroblast contamination.

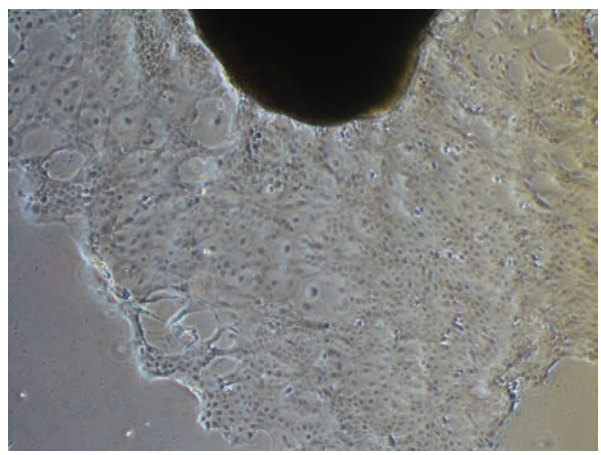

Figure 1: *Explant culture of keratinocytes with visible piece of the attached skin tissue (magnification x 40)*
Slika 1: *Kultura keratinocitov, pridobljena z metodo izraščanja celic, z vidnim delom kožnega tkiva (40 x povečava)*

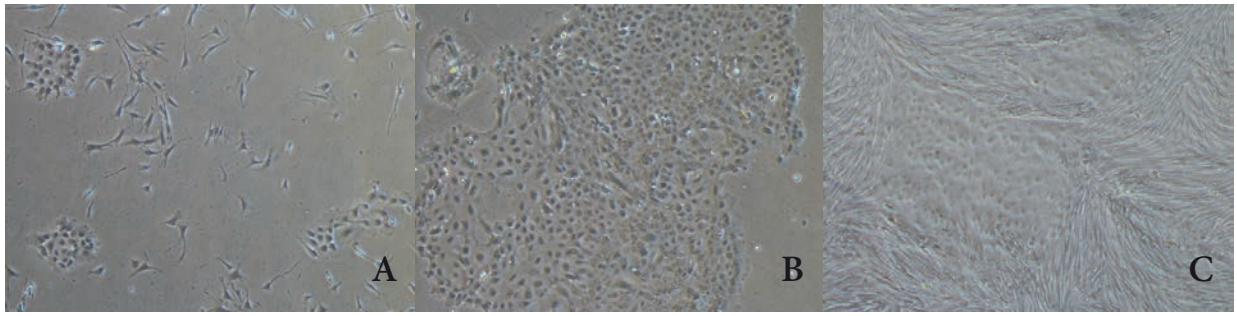

Figure 2: *Keratinocytes and fibroblasts in the culture established with enzymatic method during day 2 (A, magnification x 40), day 7 (B, magnification x 40), and day 10 (C, magnification x 40)*
Slika 2: *Kultura keratinocitov in fibroblastov, pridobljena z encimsko metodo drugi dan (A, 40 x povečava), 7 dan (B, 40 x povečava) in 10 dan (40 x povečava)*

Cells were characterised based on morphological characteristics and by immunofluorescent staining. Antibodies against cytokeratin 14, distinct for keratinocytes, and antibody against vimentin, distinct for fibroblasts, were used for basic characterisation. Cytokeratins are cytoskeletal proteins distinctive for epithelial cells. Cytokeratin 14 forms hemidesmosomes together with cytokeratin 5, the structures on the inner basal surface of keratinocytes that attach cells to the extracellular matrix, and consequently maintain integrity of the basal layer (Nobusawa et. al., 2014). Positive staining against vimentin is often used for identification of fibroblasts. It is a type III cytoskeletal intermediate filament (IF) that is expressed in mesenchymal cells. Furthermore, vimentin is used in identifying cells that undergo an epithelial-to-mesenchymal transition (EMT). During EMT epithelial cells drastically change their shape and motile behaviour on the point of differentiating into mesenchymal cells (Mendez *et al*., 2010).

Immunofluorescent co-staining showed presence of both markers in the culture – epithelial (keratinocytes) and mesenchymal (fibroblast) cells (Fig. 3). Epithelial cell morphology and expression of cytokeratin 14 proves that cells in the primary culture belong to keratinocyte lineages. Vimentin was expressed only in fibroblasts, which can also be recognised by their elongated spindle shaped morphology (Vissar and Pollit, 2010).

We found that keratinocytes from equine skin were able to proliferate in the primary culture for at least four passages, without any visible change in the morphology and proliferation rate, estimated by the time needed to overgrow the growth surface between the passages. Cells adhere well to Geltrex, which we used instead of collagen type I (Vissar and Pollit, 2010), but can attach and proliferate also on plastic surface. The established cell culture can be frozen and recovers with approximately 80 % viability rate. The cells are available for further research, for example, as a research model of horse skin diseases, product testing and toxicity screens in pharmaceutical and cosmetic industry, or as an animal model for studying certain human skin diseases.

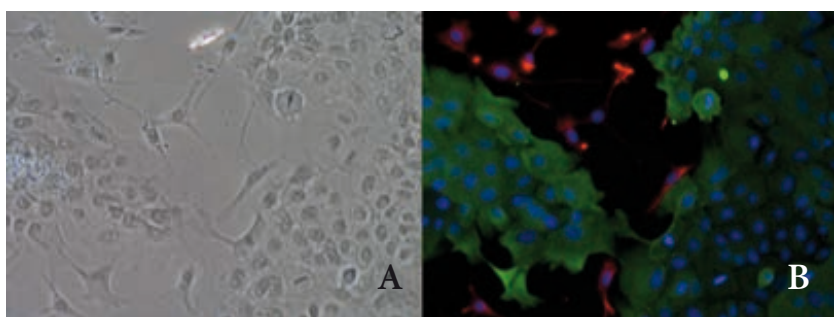

Figure 2: *Mixed culture of keratinocytes and fibroblasts after immunostaining under bright field (A) and fluorescent (B) illumination (magnification x 200). Keratinocytes stained with antibodies against cytokeratin 14 (green) and fibroblasts stained with antibodies against vimentin (red). Nuclei were counterstained with DAPI (4', 6-diamidine-2'-phenylindole dihydrochloride).*
Slika 2: *Mešana kultura keratinocitov in fibroblastov pod svetlobnim (A) in fluorescentnim mikroskopom (B) (magnification x 200). Keratinociti so obarvani s protitelesom proti citokeratinu 14 (zelena), fibroblasti pa s protitelesom proti vimentinu (rdeča). Jedra so obarvana z DAPI (4', 6-diamidin-2'fenilindol dihidroklorid).*

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ANALYSIS OF BACKFAT THICKNESS IN ON-FARM TESTED GILTS IN SLOVENIA USING REACTION NORMS

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Analysis of backfat thickness in on-farm tested gilts in Slovenia using reaction norms

Animals have the ability to respond to differences in environment, which can be called phenotypic plasticity or environment sensitivity. Robust animal, that show little variability across environments, are less sensitive. Therefore, the aim of this study was to investigate genotype by environment interaction for backfat thickness using reaction norm. Random regression model have become common for analyzing data on individuals over time. Reaction norms were predicted for 239 sires. Results show how sires differ across environments for backfat thickness.

Key words: pigs / gilts / genetics / environment / maternal genotype / backfat thickness / reaction norms / Slovenia

Reakcijske norme za debelino hrbtne slanine pri testiranih mladicah v Sloveniji

Živali imajo možnost, da se na razlike v okolju odzivajo različno. To imenujemo fenotipska plastičnost ali okoljska občutljivost. Robustne živali, ki kažejo majhno variabilnost med okolji, so manj občutljive. Namen prispevka je z reakcijskimi normami preučiti interakcijo med genotipom in okoljem za debelino hrbtne slanine. Za analizo podatkov smo uporabili model z naključno regresijo, reakcijske norme smo napovedali 239 očetom. Rezultati kažejo, kako se preučevana lastnost za posameznega očeta skozi okolja spreminja.

Ključne besede: prašiči / mladice / genetika / okolje / maternalni genotip / debelina hrbtne slanine / reakcijske norme / Slovenija

1 INTRODUCTION

The ability of living organisms to respond to changes in their environments is called phenotypic plasticity or environmental sensitivity (de Jong and Bijma, 2002). Genotypes with highly variable production across different environments are characterized as 'plastic'. However, genotypes with little variability across environments are called as 'robust'. Population under high selection pressure become more sensitive and as such, robustness has rapidly become a term with high interest in animal production (Knap, 2005; ten Napel *et al.*, 2006). Robustness is not a trait which is easily measured. Thus, there are many definitions for it. Among others, it is defined as the ability of even production potential through a wide range of environments. Robust animas should be less sensitive to stress and are expected to recover more quickly than less robust animals. This indicates that robust animals function well under a wide range of environments.

Phenotypic plasticity is related to genotype by environment interaction. In pig breeding, genotype by environment interaction could reduce genetic improvement if breeding values for any trait used as breeding goal are predicted on records obtained in specific test environments. However, productive animals are raised in different environments. A key problem is to decide under which conditions animals should be tested and how genotype by environment interaction can be included in selection procedure (de Jong and Bijma, 2002). The evaluation of genotypes in only one environment cannot be used to predict the performance of pigs reared in different environments whenever genotype by environment

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interaction exists. Genetic evaluation requires a possibility of sire ranking in each environment.

Kolmodin *et al.* (2002) applied random regression models as reaction norm models to express performance of a genotype as a function of the environment. If different genotypes have different reaction norms, then there is genotype by environment interaction (de Jong and Bijma, 2002). However, these models require definition of environment constraints on a continuous scale. Random regression models have become common for the analysis of longitudinal data or repeated records on individuals over time (Schaeffer, 2004). Some studies on genotype by environment interaction using reaction norm models have been reported for litter size in pigs (Knap and Su, 2008), for growth traits in pigs (Hermesch *et al.*, 2008; Li and Hermesch, 2012), and for milk production in dairy cattle (Kolmodin *et al.*, 2002; Shariati *et al.*, 2007, Logar at al., 2007).

The objective of this study was to apply reaction norm model to evaluate genotype by environment interaction for backfat thickness in gilts in Slovenia.

2 MATERIAL AND METHODS

Individual records and pedigree information were collected for 19053 on-farm tested animals. Data comprising Slovenian Landrace – line 11, Slovenian Large White 22 and Hybrids 22x11 (21) and 11x22 (12) gilts were routinely recorded from 2000 to 2013. Animals were raised on 46 family farms under production conditions. Records were included if body weight was at least 80 kg, and age at the end of the test was at most 300 days. The environmental variable was defined as herd-year average of daily gain. After editing, 18805 records were obtained on gilts weighing on average 108.8 kg at the end of test (Table 1). The average backfat thickness was 10.5 mm (±2.2). The observations were spread over 1864 herd-year seasons. The pedigree file contained animals with records and up to five generations of ancestors. There were 239 different sires included. The variable use to characterize the environment of animal was defined as deviation of herd-year daily gain on test and backfat thickness.

Table 1: *Descriptive statistics for body weight, daily gain, and backfat thickness (N = 18805)*
Preglednica 1: *Opisna statistika za telesno maso, dnevni prirast in debelino hrbtne slanine (N = 18805)*

Variable	Mean	SD	Min.	Max.
Body weight (kg)	108.8	13.8	80	200
Daily gain (g/day)	554	46	375	708
Backfat (mm)	10.5	2.2	4	24

Linear regression was applied to describe random sire effect over environments. The following mixed model was used:

$$y_{ijklmno} = \mu + G_i + S_j + R_k + b(x_{ijklmn} - \bar{x}) + h_k + s_{0im} + s_{1im} + w_{ijklmn} + l_{ijklmn} + e_{ijklmno} \quad (1)$$

where $y_{ijklmno}$ is a trait, μ is overall mean for trait. The fixed part of model included genotype G_i (i = 1, 2, 3, 4), season S_j (j = 1, 2, … 160) and herd R_k (k = 1, 2, … 46). Animal weight at the end of test (x_{ijklmn}) was described by linear regression with b as linear regression coefficient. Random part of model included common herd-year environment (h_{kl}), common litter environment (l_{ijklmn}), intercept (level) of reaction norm for sire (s_{0im}), random regression coefficient (slope) for sire (s_{1im}) with environmental variable (w_{ijklmn}) expressed as deviation of herd-year average of daily gain. Variable ($e_{ijklmno}$) is random residual.

Sire effect $\mathbf{s}_0$ and $\mathbf{s}_1$ were assumed to be normally distributed with mean zero and covariance structure:

$$\text{var}\begin{bmatrix}\mathbf{s}_0\\ \mathbf{s}_1\end{bmatrix} = \begin{bmatrix}\sigma^2_{s0} & \sigma_{s01}\\ \sigma_{s01} & \sigma^2_{s1}\end{bmatrix} \otimes \mathbf{A} = \mathbf{S}_0 \otimes \mathbf{A} = \frac{1}{4}\begin{bmatrix}\sigma^2_{\alpha 0} & \sigma_{\alpha 01}\\ \sigma_{\alpha 01} & \sigma^2_{\alpha 1}\end{bmatrix} \otimes \mathbf{A} \quad (2)$$

where $\mathbf{s}_0$ and $\mathbf{s}_1$ are vectors of unknown parameters for intercept and slope in random regression for sire effect. Genetic variance was partitioned into three components: variance for level ($\sigma^2_{\alpha 0}$), for slope ($\sigma^2_{\alpha 1}$), and covariance between the two ($\sigma_{\alpha 01}$). Matrix **A** is the additive relationship matrix for sire and the matrix $\mathbf{S}_0$ is equal to one quarter of the genetic variance matrix for level and slope.

The environmental variable herd-year averages were grouped into six classes allowing for heterogeneous residual variances across environments. The observations were assigned to classes by their environment value. The lowest values formed first class and the highest values formed the sixth class. The classes had equal range of environmental variables. The residuals were assumed to be independently distributed with mean zero and variance σ^2_{te} within environmental class ***t***.

In the reaction norm models, breeding values are described by linear regression with level and slope as parameters. Predicted breeding values within environment w_t could be expressed as predicted offspring performance $POP_{im/wt}$; Kolmodin *et al.*, 2002) calculated as:

$$POP_{im/wt} = s_{0im} + s_{1im} w_t \quad (3)$$

SAS software (SAS Inst. Inc., 2008) was used for data editing and finalization of the results. Dispersion parameters were estimated using residual maximum likelihood methodology as applied in VCE-5 (Groeneveld *et al.*, 2010).

3 RESULTS AND DISCUSSION

Estimates of genetic (co)variances and correlations together with residual variance estimates in the reaction norm model are shown in table 2.

Estimates of residual variances decrease progressively with increasing environmental variable for backfat thickness suggesting heterogeneous residual variances. In better environments, testing conditions of gilts are more standardized and the gilts express more uniformity as in barren environments.

Estimated sire variance for slope was 23.84 (mm/(g/day))^2. Genetic correlation between level and slope was estimated to 0.14, which is an indication of potential re-ranking. As long as the genetic variance of the reaction norm slope is greater than zero and genetic correlation among environments is smaller than one, there is genotype by environment interaction (de Jong and Bijma, 2002). Genetic correlation between the reaction norm of level and slope far from 1 will cause re-ranking of animals among environments (Su *et al.*, 2006).

Reaction norm was used also to describe genetic variability of pig carcass weight as a function of heat stress of crossbreed pigs in North Carolina (Zumbach *et al.*, 2008). They estimated negative correlation between the intercept and slope. This could be due to increased sensitivity of animals to heat stress.

Sire effects for 43 sires are illustrated by linear regression on Figure 1 showing re-ranking of sires across environments. For backfat thickness, the best animals have the most negative breeding values predicted, because selection is for thinner backfat. Sires A and B are very sensitive, in other words not robust to the environmental changes. Sire A is more superior with less backfat thickness in rich environment (Fig. 1, right) and is ranked on the 27th place only in the most barren environment (Fig. 1, left), while sire B fits better to barren than rich environment. Both are very specific and not well suited for the whole specter of environmental conditions. Nevertheless, sire D performed well and has excellent as well as steady genetic merit across all environments. Sire C has a robust genotype as well. Its breeding values seems to be more or less constant over environments considered, but the level of breeding value is worse that the level for sire D. In general, re-ranking observed was greater than expected in such small population.

Re-ranking of boars in organic and conventional pig production was studied in Swedish Landrace (Wallenbeck *et al.*, 2009) for growth rate and backfat thickness. Wallenbeck *et al.* (2009) found re-ranking of boars between the two production systems. In their study, the best boar in the conventional environment (boar X) was ranked on

Table 2: *Variance components and genetic correlations between level and slope for backfat thickness*
Preglednica 2: *Komponente variance in genetska korelacija med stopnjo in naklonom za debelino hrbtne slanine*

(Co)variance component		Estimate ± SEE	Genetic correlation
Sire	level	0.30 ± 0.04	0.14 ± 0.13
	slope	23.84 ± 7.26	
Residual variances in class	1	2.14 ± 0.04	
	2	2.63 ± 0.13	
	3	1.63 ± 0.02	
	4	1.53 ± 0.02	
	5	1.37 ± 0.03	
	6	1.20 ± 0.07	

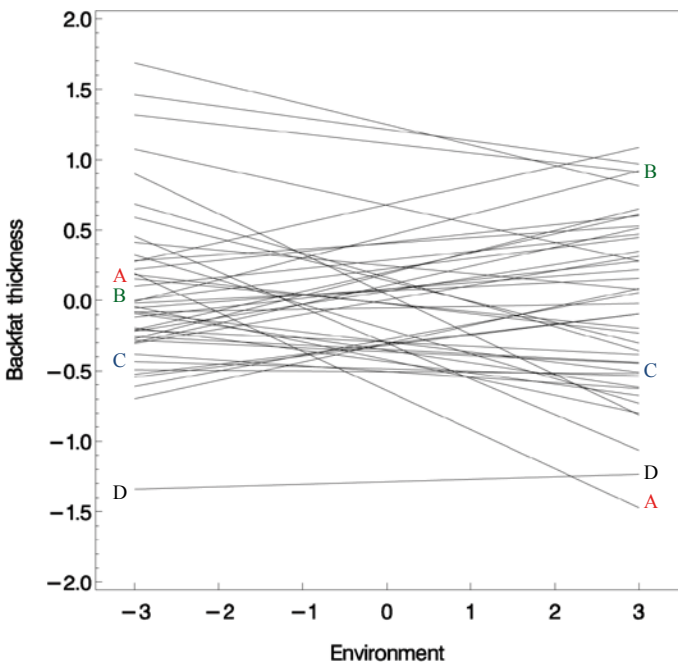

Figure 1: *Reaction norms for sample of 43 sires for backfat thickness (mm) in different herd environments (x-axe shows deviation from average environment in SD units of herd-year daily gain average)*
Slika 1: *Reakcijske norme za debelino hrbtne slanine (mm) v različnih okoljih pri 43 očetih (na x-osi je prikazan odklon od povprečnega okolja, ki je definirano kot povprečni dnevni prirast v čredi znotraj leta)*

place 11th in the organic environment. At the same time, the lowest ranking boar in the conventional environment was almost as good as boar boar X in the organic environment. But, the positive correlations mean that boars that are ranked highly on conventional breeding value are in many cases ranked highly on organic breeding value. This indicates that interactions GxE are week.

Reaction norms were used to investigate sire by environment interactions for growth rate and backfat thickness in Australian pigs (Li and Hermesch, 2012). Li and Hermesch (2012) reported, that Large White was the most sensitive breed for growth rate and backfat thickness, while Duroc was the most robust breed across their production systems.

4 CONCLUSIONS

Robustness of gilts in Slovenia was studied using reaction norm model. Analyses were performed using random regression model for backfat thickness. Breeding value as well as ranking of some sires (example sire A and B) changed over environments. There was a group of sires which breeding values and rank did not change much over environments. Overall results proved existence of genotype by environment interaction. This information is useful to setup strategic performance recording procedures for genetic improvement of productivity and robustness.

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EFFECT OF DIFFERENT LITTER MATERIALS ON FOOT PAD DERMATITIS, HOCK BURN AND FEATHER COVERAGE IN BROILER CHICKENS [1]

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Effect of different litter materials on foot pad dermatitis, hock burn and feather coverage in broiler chickens

An experiment was conducted to evaluate the feather coverage on breast and thighs in meat type chickens, reared on three different types of litter materials, including wood shavings, shredded paper and chopped wheat straw. The experiment lasted for 72 days. A total of 447 one-day-old meat type chickens were randomly allotted to the three litter materials, giving 149 birds per litter. On days 24, 47 and 72 of the experimental period, feathering on the breast and thighs was scored according to a 1–4 scale. In addition, the severity of foot pad dermatitis (FPD) and hock burn (HB) was evaluated at the same days by attributing scores from 1–3. Litter characteristics (moisture content, water absorbing capacity, bulk density, pH) were determined at the end of the experiment. Severity of FPD varied significantly ($P < 0.016$) among the litter materials with chopped straw showing the highest severity scores and wood shavings showing the lowest. Litter material had no effect ($P > 0.016$) on the severity of HB and on thigh feathering. Birds reared on chopped wheat straw presented the lowest breast feather coverage. Straw litter had the highest moisture content and pH value. In conclusion, wood shavings proved to be best litter material for meat type chickens rearing among the three tested litter materials.

Key words: poultry / broiler chickens / litter / foot pad dermatitis / hock burn / feather coverage

Vpliv različnih vrst nastila na pojav vnetja kože na blazinicah stopal, vnetja kože skočnih sklepov ter na operjenost pri pitovnih piščancih

Poskus je bil zasnovan z namenom ocenitve operjenosti področja prsi in beder pitovnih piščancev, vzrejenih na treh vrstah nastila, vključujoč lesne oblance, razrezan papir in rezano pšenično slamo. Poskus je trajal 72 dni. Štiristo sedeminštirideset en dan starih pitovnih piščancev je bilo naključno razdeljenih v 3 skupine, 149 živali na posamezno vrsto nastila. V poskusnem obdobju smo trikrat (24., 47. in 72. dan) ocenili operjenost področja prsi in beder z uporabo lestvice z ocenami od 1 do 4. Istočasno smo ocenili intenzivnost vnetja kože na blazinicah stopal in intenzivnost vnetja kože na skočnih sklepih z uporabo lestvice z ocenami od 1 do 3. Na koncu poskusa smo vzorcem nastila določili gostoto, vsebnost vode, pH vrednost ter sposobnost zadrževanja vode. Intenzivnost vnetja kože na blazinicah stopal je bila značilno različna ($p < 0,016$) med posameznimi vrstami nastila in sicer so najvišje (najslabše) ocene dosegli piščanci, uhlevljeni na rezani pšenični slami, in najnižje (najboljše) piščanci, uhlevljeni na lesnih oblancih. Vrsta nastila ni značilno vplivala ($p < 0,016$) niti na operjenost področja beder niti na pojavljanje vnetij kože na skočnih sklepih. Pri piščancih, uhlevljenih na pšenični slami, je bila opažena najslabša operjenost področja prsi. Vzorec nastila s pšenično slamo je imel najvišjo vsebnost vode ter najvišjo pH vrednost. Izmed treh proučevanih materialov so se kot najboljši material za nastiljanje v rejah pitovnih piščancev izkazali lesni oblanci.

Ključne besede: perutnina / pitovni piščanci / nastil / blazinice stopal / skočni sklepi / vnetje kože / operjenost

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1 INTRODUCTION

Foot pad dermatitis (FPD), hock burn (HB) and breast blisters (BB) are dermatological problems with similar pathologies and are collectively known as a contact dermatitis (Greene *et al.*, 1985). Contact dermatitis is an ulcerative condition of the skin affecting the plantar surface of the feet (FPD), the hock (HB) and the breast (BB) (Haslam *et al.*, 2007). It is seen in growing broiler chickens and turkeys, and in broiler parents. Some lesions are superficial, whereas others progress to deep ulcers and cause discomfort and pain (Cengiz *et al.*, 2011). Besides the negative effect on welfare, different forms of contact dermatitis affect farmer income, and in the future it is likely to have increasing importance in terms of legislation. Several major factors are associated with the occurrence of contact dermatitis including type, depth and condition of litter, stocking density, feed composition, light and climate (Meluzzi and Sirri, 2009).

Of these, litter may be the most important because meat type chickens spend most of their time on the litter and their foot pads, hock and breast are in constant contact with the material on the floor. Therefore, if the type, quantity and quality of litter material are not optimal there is a considerable risk that birds will develop contact dermatitis and breast blisters (Meluzzi and Sirri, 2009). Various types of litter materials are used in different countries. In Slovenia, wood shavings and sawdust are the most common materials used as litter in commercial broiler production. However, these preferred litter materials are becoming limited in supply and expensive. Therefore, appropriate substitutions need to be considered. Various forms of recycled paper and chopped straw have proven to be good litter materials in research and commercial situations. With those idea in view, the present study was designed to evaluate the effects of different litter substrates on feather coverage and on the severity of FPD and HB in meat type chickens.

2 MATERIAL AND METHODS

The experiment was carried out at a poultry research station (Biotechnical Faculty, University of Ljubljana, Slovenia) with the approval of the Commission for Animal Experiments of the Institution. Four hundred forty seven newly hatched parent stock chicks of Slovenian traditional meat type breed »Slovenian Late Feathering Hen« were randomly assigned, as mixed sex, to the three litter materials (wood shavings, chopped wheat straw, shredded paper), giving 149 birds per litter (pen). Each group was kept in a pen measuring 30 m^2 resulting in a flock density of 5 birds m^{-2}. Experimental pens were located side-by-side within the same environmentally controlled poultry house. Feeder and drinker spaces were identical in each pen. Day old chicks were individually identified by toe clipping. The depth of litter in all pens was approximately 8–10 cm. The brooding temperatures were kept at 32 to 33 °C from day 1 to 7; thereafter, the temperature was reduced by 3 °C/week until it reached 21 to 23 °C, and was maintained at that temperature thereafter. During the first month, all birds consumed feed for *ad libitum* intake. From the age of 30 days all birds were fed on restricted rations according to breeder recommendations. The feed was supplied in a single daily feed and was generally consumed in less than 20 minutes. The chickens were given a standard grower feed (11.9 MJ ME/kg, 20.0 % CP) containing an anticoccidial additive. Access to water was unlimited. Fresh litter materials were added to pens whenever damp litter resulting from excessive drinking was observed. The photoperiod was 23 h light (L) and 1 h dark (D) during the first week. Thereafter, light was decreased by 3 to 4 h/week to 8 L:16 D at week 8. The trial lasted 72 days. The severity (i.e., extent of lesions) of FPD and HB and the degree of feathering on the breast and thighs were determined at 24, 47 and 72 days. At 24 and 47 days approximately 50–60 birds per pen were randomly chosen, while at 72 days of age all birds were evaluated. The foot pad and hock lesions were assessed using a 3-point scoring system, in which 1 = no lesions; no or very small superficial lesions, slight discoloration on a limited area, mild thickening of the skin; 2 = mild lesion; discoloration of the foot pad, superficial lesions, dark papillae; and 3 = severe lesion; ulcers or scabs, signs of haemorrhages or swollen foot pads. The foot pad and hock scores were evaluated by using the mean of both feet. Breast and thighs feathering were scored according to the following 1–4 score scale: 1 = skin is not visible, given area completely feathered; 2 = less than 25 % of feathers missing from a given area; 3 = 25–50 % of feathers missing from a given area; and 4 = more than 50 % of feathers missing from a given area. At the end of experiment, litter samples were collected from five randomly chosen locations within each pen and thoroughly mixed to obtain material representative of the entire pen. Each litter material was analyzed for moisture content, pH value and moisture retention capacity. Litter samples were dried at 100 °C for 18 hours to determine moisture content which is expressed on a fresh matter basis. The pH of each litter type was measured after litter samples of nearly 10 g were suspended for 30 min in 100 mL of distilled water. pH was recorded until constant values were obtained. In order to determine water absorbing

Table 1: *Scores of FPD and HB severity and feathering scores on the breast and thighs of meat type chickens reared on different litter materials over 72 days*
Preglednica 1: *Ocene poškodb kože na blazinicah stopal oziroma na področju skočnih sklepov in ocene operjenosti področja prsi oziroma beder pri pitovnih piščancih, rejenih 72 dni na različnih vrstah nastila*

		FPD scores		HB scores		Feathering scores on the breast		Feathering scores on the thighs	
Experimental group	Number of chickens	Mean value	P value	Mean value	P value	Mean value	P value	Mean value	P value
Wood shavings	243	1.00[a]	0.0001	1.00[a]	1.000	1.62[a]	0.0001	1.46[a]	0.449
Shredded paper	254	1.15[b]		1.00[a]		1.81[a]		1.50[a]	
Chopped wheat straw	245	1.48[c]		1.00[a]		2.71		1.57[a]	

[a,b,c] Means followed by the same letters in the same column are not significantly different (P > 0.016)

capacity, dried samples of pure litter materials were weighed and placed in pans. Moisture holding capacity was determined by filling the pan with water and letting it stand for 90 minutes. Excess water was then drained for 3 minutes and the sample was then weighed again. The percentage of water absorbed was then calculated on dry matter basis. The results were analysed using the statistical SAS program (SAS Institute, 2008). Ordinal variables (feather scores, foot pad and hock scores) were analyzed using Shapiro-Wilk test to verify the normality of residuals, and Levene test for the homogeneity of variances. Comparisons of the results were done using the Kruskal-Wallis test. When the effects were significant they were tested with the Wilcoxon nonparametric rank test within procedure NPAR1WAY. To counteract the problem of multiple comparisons, all statements of significance for ordinal variables were based upon P < 0.016 (Bonferroni correction).

3 RESULTS AND DISCUSSION

The severity scores of FPD and HB and feathering scores on the breast and on the thighs are presented in Table 1.

Wood shavings were ranked as the litter material with the lowest FPD severity. Chopped wheat straw had the worst FPD score (Table 1). It was observed that feathering scores on the breast were significantly (P < 0.016) higher in birds on chopped wheat straw in comparison with birds od shredded paper and wood shavings. The presence of breast blisters was not observed in any experimental group. Feathering scores on the thighs were not affected by the litter materials (P > 0.016).

Initial (at 24 days) and final (at 72 days) FPD scores were similar among birds placed on the shredded paper and chopped straw (Table 2). At 47 days of age, FPD scores were higher (P < 0.016) among birds

Table 2: *Effect of litter type on the severity of foot pad dermatitis and hock burn at different ages*
Preglednica 2: *Vpliv vrste nastila na obseg poškodb kože na blazinicah stopal in kože na področju skočnih sklepov pri različnih starostih*

		FPD scores			HB scores		
Age of chickens	Experimental group	Number of animals	Mean value	P value	Number of animals	Mean value	P value
24 days	Wood shavings	55	1.00	0.0001	55	1.00[a]	1.000
	Shredded paper	57	1.19[a]		57	1.00[a]	
	Chopped straw	52	1.32[a]		52	1.00[a]	
47 days	Wood shavings	58	1.00[a]	0.0001	58	1.00[a]	1.000
	Shredded paper	58	1.36[b]		58	1.00[a]	
	Chopped straw	58	2.62[c]		58	1.00[a]	
72 days	Wood shavings	130	1.00	0.032	130	1.00[a]	1.000
	Shredded paper	139	1.05[a]		139	1.00[a]	
	Chopped straw	135	1.05[a]		135	1.00[a]	

[a,b,c] Means followed by the same letters in the same column and within the same age are not significantly different (P > 0.016)

Table 3: *Effect of litter type on the feather coverage of the breast and thighs at different ages*
Preglednica 3: *Vpliv vrste nastila na operjenost področja prsi oziroma beder pri različnih starostih*

Age of chickens	Experimental group	Feathering scores on the breast			Feathering scores on the thighs		
		Number of animals	Mean value	P value	Number of animals	Mean value	P value
24 days	Wood shavings	55	2.85[a]	0.0001	55	3.07[a]	0.0001
	Shredded paper	57	3.57[b]		57	3.22[a]	
	Chopped straw	52	3.86[c]		52	3.71	
47 days	Wood shavings	58	1.18[a]	0.0001	58	1.00[a]	1.000
	Shredded paper	58	1.25[a]		58	1.00[a]	
	Chopped straw	58	2.58		58	1.00[a]	
72 days	Wood shavings	130	1.30[a]	0.0001	130	1.00[a]	0.369
	Shredded paper	139	1.31[a]		139	1.00[a]	
	Chopped straw	135	2.31		135	1.00[a]	

[a,b,c] Means followed by the same letters in the same column and within the same age are not significantly different (P > 0.016)

raised on chopped straw than those raised on shredded paper. Throughout the whole trial period FPD scores were significantly (P < 0.016) lower in those animals that were placed on wood shavings. Greater values of breast and thighs feather scores were present at 24 days of age. Beyond this age, the values lowered in all experimental groups. This may be attributed to the fact that chicks moult the natal plumage formed in embryonic development into juvenile feathers between 21–35 days of age. Breast feather cover as indicated by feather score was significantly (P < 0.016) better in chicks placed on wood shavings compared with birds placed on chooped straw at every age (Table 3). At the ages of 47 days and 72 days all of the evaluated birds achieved a thighs feather score of 1, or full thighs feather cover. Today reduced feathering is considered beneficial when broilers are reared in hot climates, as it increases heat dissipation; however, it impairs carcass quality (Garcia *et al.*, 2012). Characteristics of litters are summarized in Table 4.

Chopped straw on one hand contained the highest percentage of moisture, on the other hand chicks grown on the chopped straw showed the most severe foot pad lesions. This is not surprising because it is well known that excessive contact with wet and ammoniacal litter is generally considered to be the primary cause of foot and hock burn (Tucker and Walker, 1992). The water holding capacity of litter is a fundamental factor in preserving the foot in a good state (Meluzzi and Sirri, 2009). Foot pad burn scores were lowest in the pen littered with wood shavings. Wood shavings as a litter material with the highest water-holding capacity and consisted of small particles resulted in lower moisture content, minimizing the incidence of lesions in foot pads and breast. Oliveira *et al.* (2004) found the similar results. Meluzzi *et al.* (2007) raised birds on chopped straw or wood shavings both in winter and in summer seasons and observed that birds kept on wood shavings exhibited a reduction of 35 % in foot pad dermatitis than those kept on straw (Meluzzi and Sirri, 2009). Tucker and Walker (1992) found lower hock burn scores in birds reared on wood shavings rather than straw, but this effect was not seen in the study of Su *et al.* (2000).

Table 4: *Chemical and physical characteristics of three litter materials*
Preglednica 4: *Kemijske in fizikalne lastnosti treh vrst nastila*

Litter material / Experimental group	Characteristics of litter materials			
	Moisture content (%)	Water absorbing capacity (%)	Bulk density (kg/m^3)	pH of litter material at 72 days
Shredded paper	9.41 ± 0.17	37.32 ± 4.10	350.2 ± 18.24	8.51 ± 0.64
Chopped straw	21.93 ± 3.67	56.84 ± 2.41	368.5 ± 42.67	9.02 ± 0.82
Wood shavings	7.62 ± 0.14	86.36 ± 7.23	317.8 ± 11.53	8.71 ± 0.34

[a,b,c] Means followed by the same letters in the same column and within the same age are not significantly different (P > 0.016)

4 CONCLUSIONS

From all collected data we can conclude that:

- Of the three litter materials tested, wood shavings showed the lowest FPD severity and the highest feather coverage ob breast, whereas chopped wheat straw showed the highest FPD severity and the lowest feather coverage of breast.
- Type of litter material had no significant effect on HB and feathering score on the thighs.
- Wood shavings as litter substrate have been found to be better than chopped wheat straw and shredded paper with regard to moisture content and water retention capacity.

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ANALIZA LASTNOSTI ZUNANJOSTI PRI PLEMENSKIH BIKIH CIKASTEGA GOVEDA [1]

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Analiza lastnosti zunanjosti pri plemenskih bikih cikastega goveda

V raziskavi smo analizirali lastnosti zunanjosti pri 330 plemenskih bikih cikastega goveda. Sistem ocenjevanja zunanjosti pri cikastem govedu vključuje merjene, posamezne ocenjevane in sestavljene ocenjevane lastnosti. Posamezne ocenjevane lastnosti so razdeljene v dva sklopa, in sicer avtohtonost in telesne oblike. Tri sestavljene ocenjevane lastnosti so: avtohtonost, omišičenost in telesne oblike. Sistematski del statističnega modela smo analizirali s proceduro GLM v programskem paketu SAS in je vključeval vpliv leta ocenjevanja in starost živali ob ocenjevanju kot linearno regresijo. V povprečju so bili 14,6 mesecev stari plemenski biki v vihru visoki 117,1 cm. Na podlagi višine vihra in posameznih ocenjevanih lastnosti iz sklopa avtohtonost so bili potencialni plemenski biki neposredno ob koncu ocenjevanja razvrščeni v cikasti, delni cikasti in pincgavski tip. Tak način razvrščanja ne upošteva vplivov okolja na izraženost lastnosti zunanjosti. Na lastnosti zunanjosti in posledično na razvrstitev plemenskih bikov v tip pomembneje vplivata vsaj leto ocenjevanja in starost ob ocenjevanju. Razvrstitev živali v ustrezen tip bi bilo zaradi tega potrebno opraviti po izvrednotenju ocen za lastnosti zunanjosti ob upoštevanju vplivov okolja.

Ključne besede: govedo / pasme / cikasto govedo / plemenski biki / tipi / lastnosti zunanjosti / ocenjevanje

Analysis of type traits of cika sires

The aim of the study was to analyse type traits in 330 Cika sires. Scoring system of Cika cattle includes measured, individual scored and composite scored traits. Individual scored traits are divided into two groups, autochthonousness and form. Three composite traits are autochthonousness, muscularity and form. Fixed part of the model was analysed by GLM procedure in SAS software package, which included the effect of the year and animal age on the scoring day as linear regression. On average, 14.6 months old sires were 117.1 cm high at withers. Immediately, at the end of the scoring procedure all sires were classified into Cika, Semi-Cika and Pinzgauer type, based on the height at withers and individual scored traits from the autochthonous group. This method of classification does not take into account the environmental effects. We found significant effects of the year of scoring and animal age on type traits and therefore on the classification into the type. Sires classification should be carried out after the type traits data evaluation and after the exclusion of environmental effects.

Key words: cattle / breeds / Cika / sires / type traits / scoring

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1 UVOD

Cikasto govedo je slovenska avtohtona kombinirana pasma goveda s poudarkom na prireji mleka. Rejski cilj je ohranjanje pasme v prvotnem tipu in preprečevanje parjenja v sorodstvu. Cikasto govedo je razširjeno skoraj po vsej Sloveniji, vendar je številčno najbolj zastopano na območju, od koder izvira (Bohinj, okolica Kamnika in okolica Kobarida). Barvni vzorec je tipičen in značilen za to pasmo ter se zelo razlikuje od drugih pasem goveda v Sloveniji. Nekatere živali cikastega goveda so po barvnem vzorcu bolj podobne pincgavskemu govedu, druge pa tux-zillertaler govedu iz Avstrije (Sambraus, 1999). Osnovna barva plašča je rumenordeča do temno rdeče rjava, največkrat kostanjevo rjava, ki mora prevladovati nad belo barvo. Glava mora biti osnovne barve. Po hrbtu imajo živali širši ali ožji bel pas, ki se nadaljuje pod repom do vimena ali mod in po trebuhu naprej do prsi. Rep je bele barve. Ožji ali širši beli pasovi so prisotni tudi čez zgornji del prednjih nog (v predelu komolčnega sklepa) in zadnjih nog (v predelu kolenskega sklepa). Barva vseh sluznic je rožnata. Barva rogov ob korenu je belo rumena, na gornji tretjini rjavkasta, konice pa so svetlejše. Parklji so temne barve (Navodila za presojo …, 1935; Žan Lotrič in sod., 2010). Zunanji znak pasemske pripadnosti je bela ožja ali širša podolžna lisa po hrbtu in trebuhu (Navodila za presojo …, 1935).

Selekcija cikastega goveda temelji na lastnostih zunanjosti, ki so bile pri tej pasmi prvič ocenjene v letih 2002 in 2003, ko so ugotovili veliko fenotipsko raznolikost med živalmi znotraj populacije. Poleg tega je prvo ocenjevanje cikastega goveda potekalo po sistemu ocenjevanja, ki je bil takrat v uporabi za tri pasme goveda (rjavo, lisasto, črno-belo) za prirejo mleka v Sloveniji (Pogačar in sod., 1995; Pogačar in Potočnik, 1997). Na podlagi ugotovljene večje raznolikosti med živalmi so v letu 2006 vse plemenske živali ocenili še enkrat. Ocenjevanje je takrat potekalo na podlagi posebej prirejenega obrazca za cikasto govedo.

Posebnost sistema ocenjevanja cikastega goveda je, da so v ocenjevanje lastnosti zunanjosti vključeni tudi vsi potencialni plemenski biki, ki jih ocenimo v starosti od 12 do 20 mesecev. Za cikasto govedo je bil leta 2005 sprejet in potrjen rejski program (Žan in sod., 2005). Leta 2010 je bil rejski program nekoliko dopolnjen in ponovno sprejet (Žan Lotrič in sod., 2010). V okviru tega ocenjevanje zunanjosti vključuje merjene in ocenjevane lastnosti. Pri bikih je merjenih lastnosti sedem. Na podlagi obsega prsi se določi (odčita z merilnega traku) tudi telesna masa. Posebnost ocenjevanja v primerjavi z drugimi pasmami je dvanajst tako imenovanih posameznih lastnosti za avtohtonost, ki opisujejo pasemske značilnosti. Poleg tega ocenimo tudi šest posameznih lastnosti za telesne oblike in tri sestavljene lastnosti. Na podlagi posameznih ocen za avtohtonost, ocene za izraženost skočnega sklepa in višine vihra so potencialni plemenski biki na koncu ocenjevanja razvrščeni v tri tipe (cikasti, delni cikasti, pincgavski). Na podlagi ocene za tip je žival odbrana za pleme. Čas ocenjevanja je prilagojen tehnologiji reje, zato živali ocenjujemo izven pašne sezone. Velikost populacije cikastega goveda je v primerjavi z ostalimi pasmami v Sloveniji majhna, zato vse plemenske živali ocenjuje samo en ocenjevalec.

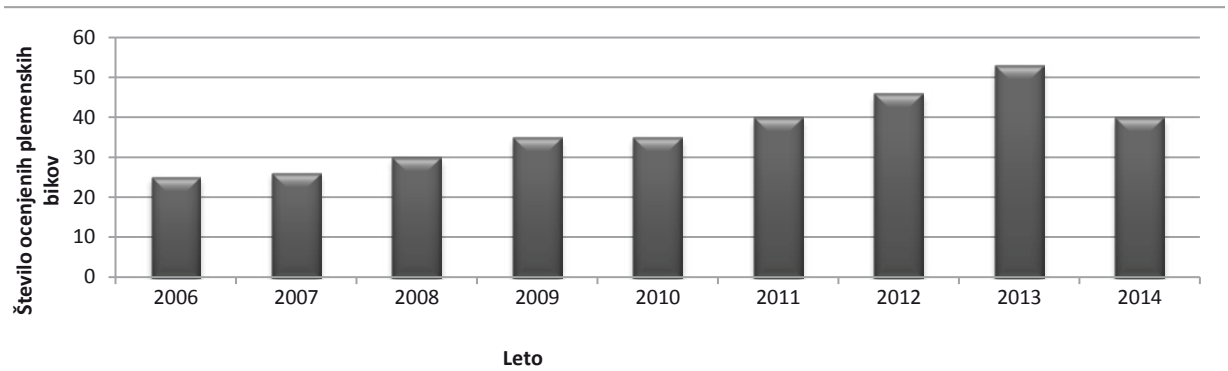
Analiza lastnosti zunanjosti je namenjena spremljanju in ugotavljanju primernosti porazdelitve ocen posameznih lastnostih in je hkrati podlaga za oceno genetskih parametrov, ki so potrebni za napovedovanje plemenskih vrednosti. Namen te raziskave je bil analizirati lastnosti zunanjosti za merjene in ocenjevane lastnosti ter poiskati vplive okolja, ki imajo neposreden vpliv na te lastnosti.

2 MATERIAL IN METODE

Podatke smo pridobili iz Centralne podatkovne zbirke (CPZ) Govedo (Logar in sod., 2005), ki jo vodijo na Kmetijskem inštitutu Slovenije (KIS), ki je druga priznana organizacija v živinoreji in pooblaščena tudi za arhiviranje podatkov. Pridobljeni podatki, zabeleženi v okviru ocenjevanja lastnosti zunanjosti, so vključevali 375 plemenskih bikov cikastega goveda, ocenjenih v letih od 2002 do 2014.

V nadaljnjo analizo smo vključili samo živali, ki so bile ocenjene po novem načinu ocenjevanja. Živali, ki so bile ocenjene pred letom 2006, smo iz obdelave izključili, tako pri analizi nismo uporabili sistematskega vpliva načina ocenjevanja. V skladu z omejitvami v rejskem programu glede starosti ob ocenjevanju bi v analizo vključili zelo majhno število plemenskih bikov, saj v praksi ocenjujejo mlajše bike, kot je priporočeno. Da bi dosegli boljšo ocenljivost posameznih vplivov, smo v analizo vključili vse plemenske bike, ki so bili na dan ocenjevanja stari od 10 do 20 mesecev. Navedenim zahtevam je ustrezalo 330 plemenskih bikov cikastega goveda, zato smo jih vključili v končno analizo. Plemenski biki so bili v povprečju stari 446,2 ± 81,3 dni. Najmlajši je bil star 307 dni, najstarejši pa 850 dni. Vsi so bili ocenjeni v letih od 2006 do 2014 (slika 1).

Sistem ocenjevanja zunanjosti vključuje sklop merjenih lastnosti (7), sklop posameznih ocenjevanih lastnosti za avtohtonost (12), sklop posameznih ocenjevanih lastnosti za telesne oblike (6) in sklop sestavljenih lastnosti (3). Istočasno na podlagi izjave rejca ocenjujejo še temperament bika in zabeležijo napake posameznih lastnosti zunanjosti. Ocenjevane napake v sklopu za avtohtonost so: temen gobec, neustrezna barva plašča, beli znaki na



Slika 1: *Število ocenjenih plemenskih bikov cikastega goveda po letih*
Figure 1: *The number of scored Cika sires by years*

glavi, beli znaki na nogah, pikasto pisana barva plašča, prekinjena hrbtna lisa in hrbtna lisa na križu. Ocenjevane napake v sklopu za telesne oblike so: razplečenost, visoko nasajen rep, vdolbina med sednicama, kravja stoja in razprti parklji. Sestavljene lastnosti ocenjujejo avtohtonost (pasemske značilnosti), omišičenost in telesne oblike. Merjene lastnosti so podane v centimetrih (cm), ocenjevane pa na linearni lestvici od 1 do 9.

Merjene lastnosti za velikost okvira merijo z Lydtinovo palico in so višina vihra, višina križa, dolžina telesa, širina prsi, globina prsi in širina križa ter z merilnim trakom obseg prsi, na podlagi katerega s pomočjo lestvice na merilnem traku določijo tudi telesno maso (kg).

Posamezne lastnosti za avtohtonost ocenjujejo z ocenami 1 do 9. Ocena 1 pomeni nezaželeno oceno za lastnost, ocena 5 povprečno in ocena 9 zelo zaželeno oceno. Ocena 1 ne pomeni pri vseh lastnosti najmanj izražene lastnosti, kakor tudi ne pomeni ocena 9 najbolj izražene lastnosti, kot jo poznano pri linearnem načinu ocenjevanja zunanjosti v skladu s pravili Mednarodnega komiteja za kontrolo proizvodnje živali – ICAR (International agreement …, 2012; Klopčič in Hamoen, 2010). V preglednici 2 za vsako posamezno lastnost za avtohtonost navajamo, kaj pomenita ekstremni oceni 1 in 9. Posamezne lastnosti za telesne oblike so ocenjene z ocenami od 1 do 9 v skladu s pravili ICAR (International agreement …, 2012).

Sestavljeno oceno za avtohtonost ocenijo z upoštevanjem optimalnih vrednosti nekaterih posameznih lastnosti v sklopih avtohtonost in telesne oblike (izraženost skočnega sklepa) ter za merjeno lastnost telesnega okvira (višina vihra), kot je navedeno v rejskem programu (pregl. 1; Žan Lotrič in sod., 2010).

Sestavljeno oceno za omišičenost predstavlja predvsem ocena zunanje linije stegna pri pogledu od zadaj z ocenami 1–9. Sestavljeno oceno za telesne oblike (1–9) ocenijo na podlagi posameznih lastnosti za telesne oblike in z upoštevanjem napak, zabeleženih v tem sklopu.

Glede na priporočila ICAR-ja (International agreement …, 2012) naj bi izračunano srednjo vrednost in standardni odklon analizirali zaradi spremljanja dela ocenjevalcev. Teoretična srednja vrednost je ocena 5 in predstavlja povprečje ekstremnih vrednosti, teoretični

Preglednica 1: *Optimalne ocene za lastnosti, ki se upoštevajo v sestavljeni lastnosti za avtohtonost*
Table 1: *Optimal scores for traits considered in the composite traits for autochthonousness*

Sklop	Lastnost	Optimalna vrednost
Lastnosti za avtohtonost	Dolžina glave	6–8
	Izraženost oči	6–8
	Debelina rogov	6–8
	Dolžina rogov	6–9
	Usmerjenost rogov	6–9
	Vrat	7–9
	Izraženost podgrline	6–9
Lastnosti za telesne oblike	Izraženost skočnega sklepa	6–9
Merjene lastnosti telesnega okvira	Višina vihra	≤ 115 cm

standardni odklon pa znaša 1,5 ocene, ker predvidevamo, da 9 točkovna lestvica obsega 6 standardnih odklonov.

Za analizo variance lastnosti zunanjosti smo uporabili proceduro GLM iz statističnega paketa SAS/STAT (SAS Institute Inc., 2001) in statistični model 1.

$$y_{ij} = \mu + L_i + b_I \left(x_{ij} - \overline{x} \right) + e_{ij} \quad (1)$$

kjer y_{ij} predstavlja posamezno lastnost zunanjosti, μ ocenjeno povprečje populacije, L_i sistematski vpliv leta ocenjevanja (i = 1, …, 9), b_I linearni regresijski koeficient za starost na dan ocenjevanja, x_{ij} je starost na dan ocenjevanja (dnevi) in e_{ij} naključni ostanek.

3 REZULTATI IN RAZPRAVA

Pričujoča raziskava je podrobna analiza lastnosti zunanjosti plemenskih bikov cikastega goveda. Ocenjevanje zunanjosti v skladu z Rejskim programom, ki velja od leta 2010 dalje (Žan Lotrič in sod., 2010), je vključevalo dve lastnosti več (plemenitost glave, dolžina rogov) v primerjavi z Rejskim programom iz leta 2005 (Žan in sod., 2005). Za analizo variance lastnosti zunanjosti živali nismo razvrstili glede na tip, temveč smo zajeli celotno populacijo, ne glede na tip.

3.1 OPISNA STATISTIKA

Opisna statistika je prikazana za plemenske bike (pregl. 2) skupaj z opisi lastnosti. Plemenski biki cikaste pasme so bili v povprečju v vihru visoki 117,09 ± 4,63 cm in nekoliko nadgrajeni, saj so bili v križu v povprečju visoki 120,20 ± 4,71 cm, kar je pričakovano glede na starost. Povprečna dolžina telesa je bila 116,15 ± 6,71 cm, kar kaže na kvadraten telesni okvir. Povprečna telesna masa je bila 319,42 ± 61,57 kg, s koeficentom variabilnosti 19,28 %. Sestavljene lastnosti so bile v povprečju za avtohtonost 6,36 ± 1,18, za omišičenost 5,73 ± 1,13 in za telesne oblike 6,38 ± 1,01. Koeficient variabilnosti je bil pri merjenih lastnostih od 3,92 % do 9,72 %. Pri analiziranju ocen smo opazili tudi zelo majhno zastopanost obeh ekstremnih ocen na lestvici, kar potrjujejo tudi majhni standardni odkloni pri nekaterih lastnostih. Nekatere lastnosti niso bile ocenjene z ekstremnima ocenama, čeprav bi lestvica morala pokriti biološke ekstreme ocenjevane populacije (Klopčič in Hamoen, 2010).

Polc (1958) je v diplomski nalogi obdelal telesne mere pri bikih bohinjskega (cikastega) goveda v Bohinju. Vključil je 14 bikov različnih starosti. Povprečna višina vihra pri enoletnih bikih je bila 105,6 cm (n = 3), pri dvoletnih 115,3 cm (n = 6) in pri triletnih 121,6 cm (n = 5). Dve leti stari biki bohinjskega goveda so bili manjši od bikov sedanje populacije cikastega goveda v povprečni starosti 14,6 mesecev.

Primerjava lastnosti zunanjosti s plemenskimi biki drugih pasem je pomanjkljiva, saj so pri večini populacij v ocenjevanje zunanjosti vključene le prvesnice. Plemenski biki cikastega goveda so bili ob starosti 14,6 mesecev v povprečju v vihru primerljivo visoki (117,09 ± 4,63 cm) kot 12 mesecev stari biki španskega pirenaica (117,6 cm), italijanskega piemontese (118,8 cm) in francoskega limuzin (118,7 cm) goveda (Alberti in sod., 2008), ki spadajo med pasme z velikim telesnim okvirom (Felius in sod., 1995). Širina križa in telesna masa cikastih bikov (40,15 ± 3,54 cm; 319,42 ± 61,57 kg) sta bili podobni kot pri 12 mesecev starih bikih španskega goveda casina (40,9 cm; 321,4 kg) (Alberti in sod., 2008), ki spada med pasme z majhnim telesnim okvirom (Felius in sod., 1995).

Razvrščanje plemenskih bikov v ustrezen tip poteka na osnovi sestavljene ocene za avtohtonost, ki zajema lastnosti zunanjosti, kot prikazujemo v preglednici 3. Razvrščanje živali v tip navadno izvajajo neposredno na koncu ocenjevanja brez upoštevanja morebitnih vplivov okolja.

Pri ocenjevanju sestavljene lastnosti za avtohtonost se poleg posameznih ocen v sklopu za avtohtonost in zabeleženih napak upoštevajo tudi »Navodila za presojo barvnih znakov cikastega goveda«, ki so bila objavljena leta 1935 v Službenem listu kraljevske banske uprave dravske banovine kot Banova uredba. Bistvene napake, opisane v navodilih, ki žival »izključujejo«, so črna ali presvetla (žemljasta ali rumenkasta) barva plašča, očitna pomešanost osnovne rjave barve z belo barvo, beli znaki na glavi in na nogah, pri bikih pa še prevladujoča bela barva nad rjavo, bele lise v biclju, brezbarvni rogovi in popolnoma modrosiv gobec (Navodila za presojo …, 1935).

V preglednici 4 so deleži plemenskih bikov z napakami lastnosti zunanjosti. Največ plemenskih bikov (35,76 %) je imelo prekinjeno hrbtno liso, ki se upošteva kot napaka v sklopu avtohtonih lastnosti. V sklopu napak telesnih oblik je bil največji delež plemenskih bikov (14,24 %) z visoko nasajenim repom. Vse druge zabeležene napake v vseh sklopih so bile prisotne pri manjšem deležu živali v populaciji.

Plemenski biki, vključeni v analizo (pregl. 5), so bili razvrščeni v cikasti tip (186), delni cikasti tip (142) in v pincgavski tip (2). Biki v pincgavskem tipu niso predvideni za pleme, zato bomo v nadaljevanju primerjali le plemenske bike v cikastem in delnem cikastem tipu. Plemenski biki v cikastem tipu so bili v povprečju nižji za 3,6 cm v vihru in 3,7 cm v križu od bikov v delnem cikastem tipu. Prav tako so imeli 4,8 cm krajšo dolžino telesa, 2

Preglednica 2: *Opisna statistika za lastnosti zunanjosti pri plemenskih bikih cikastega goveda*
Table 2: *Descriptive statistics for type traits of Cika sires*

Lastnosti	n	Povprečje ± SD	Min	Max	KV (%)	Optimalna vrednost	Opis ocen 1–9
Starost (meseci)	330	14,60 ± 2,68	10,0	28,0	18,38		
Merjene lastnosti za telesni okvir							
Višina vihra (cm)	330	117,09 ± 4,63	103	131	3,95	≤115 cm	
Višina križa (cm)	330	120,20 ± 4,71	108	135	3,92		
Dolžina telesa (cm)	330	116,15 ± 6,71	85	135	5,78		
Obseg prsi (cm)	330	157,89 ± 10,49	134	190	6,64		
Širina prsi (cm)	330	38,75 ± 3,77	30	55	9,72		
Globina prsi (cm)	330	60,10 ± 4,19	49	72	6,98		
Širina križa (cm)	330	40,15 ± 3,54	30	54	8,82		
Telesna masa (kg)	330	319,42 ± 61,57	202	540	19,28		
Posamezne lastnosti za avtohtonost (1–9)							
Dolžina glave	330	6,15 ± 1,25	2	9	20,40	6–8	dolga – kratka
Plemenitost glave	179	5,78 ± 1,13	3	8	19,60		groba – plemenita
Izraženost oči	330	5,64 ± 0,92	3	9	16,30	6–8	slaba – močna
Debelina rogov	330	4,94 ± 1,29	1	9	26,02	6–8	debeli – tanki
Dolžina rogov	305	5,84 ± 1,28	3	9	21,90	6–9	dolgi – kratki
Usmerjenost rogov	330	5,25 ± 1,39	2	9	26,51	6–9	navzven – naprej
Vrat	330	5,02 ± 1,45	2	8	28,94	7–9	grob – plemenit
Izraženost podgrline	330	4,70 ± 1,43	2	8	30,41	6–9	močna – slaba
Barva plašča	330	5,39 ± 1,40	2	8	25,96		zelo temna – zelo svetla
Izraženost hrbtne lise	330	6,21 ± 1,39	1	9	22,40		močna – slaba
Izraženost pasov na zadnjih nogah	330	6,61 ± 1,71	1	9	25,90		močna – slaba
Izraženost pasov na prednjih nogah	330	7,40 ± 1,33	2	9	18,03		močna – slaba
Posamezne lastnosti za telesne oblike (1–9)							
Hrbet	330	4,66 ± 0,58	3	7	12,52	4–6	uleknjen – izbočen
Nagib križa	330	5,04 ± 0,57	3	7	11,40	4–6	nadgrajen – pobit
Kot skočnega sklepa	330	5,19 ± 0,70	3	8	13,56	4–6	strm – sabljast
Izraženost skočnega sklepa	330	6,21 ± 1,15	3	9	18,59	6–9	zadebeljen – tanek
Biclji	330	6,33 ± 0,97	3	8	15,26	6–8	mehki – strmi
Parklji	329	6,00 ± 0,99	3	9	16,46	6–9	nizki – visoki
Izjava rejca (1–5)							
Temperament	329	4,22 ± 0,78	2	5	18,43		nervozen – miren
Sestavljene lastnosti (1–9)							
Avtohtonost	329	6,36 ± 1,18	3	8	18,49		pincgavski – cikasti tip
Omišičenost	329	5,73 ± 1,13	2	9	19,80		
Telesne oblike	329	6,38 ± 1,01	3	8	15,80		

SD – standardni odklon, KV – koeficient variabilnosti

Preglednica 3: *Delež plemenskih bikov z napakami za lastnosti zunanjosti*
Table 3: *The proportion of sires with type defects*

Tip	Sestavljena ocena za avtohtonost	Opis zunanjosti
Cikasti tip	7, 8, 9	fina konstitucija, manjši okvir, kratka glava, širok gobec, izražene velike oči, tanki in kratki rogovi usmerjeni naprej in navzgor, tanka nagubana koža na vratu, neizrazita podgrlina, tanke noge, obsežen vamp
Delni cikasti tip	4, 5, 6	srednje velik telesni okvir, združuje lastnosti zunanjosti cikastega in pincgavskega tipa, določene lastnosti zunanjosti so značilne za druge pasme (primesi)
Pincgavski tip	1, 2, 3	večji okvir, dolga glava, ozek gobec, neizrazite majhne oči, debeli in navzven usmerjeni rogovi, debela koža na vratu, obsežna podgrlina, debele noge, povit trup

cm ožje prsi, 2,3 cm ožji križ in za 2,8 cm manjšo globino prsi. Plemenski biki v cikastem tipu so imeli 7,5 cm manjši obseg prsi in so bili zato pri podobni starosti povprečno kar za 43 kg lažji od plemenskih bikov v delnem cikastem tipu. Prav vse posamezne lastnosti za avtohtonost so bile v povprečju ocenjene kot bolj zaželene pri plemenskih bikih v cikastem tipu. Sestavljena lastnost za avtohtonost je bila za 1,9 ocene večja in s tem bolj zaželena pri bikih v cikastem tipu. Posamezne lastnosti za telesne oblike so bile zelo podobne pri obeh tipih cikastih bikov z izjemo izraženosti skočnega sklepa, ki je bil pri bikih v cikastem tipu v povprečju ocenjen z eno oceno bolje v primerjavi z delnim cikastim tipom. Sestavljena lastnost za telesne oblike je bila za 0,7 ocene boljša pri bikih v cikastem tipu. Biki v delnem cikastem tipu pa so imeli za 0,4 ocene boljšo omišičenost.

Razliko med povprečnimi vrednostmi pri merjenih lastnostih pri plemenskih bikih obeh tipov lahko pojasnimo z oplemenjevanjem cikastega goveda s pincgavskim govedom in s pretapljanjem z lisastim govedom v preteklosti. Pincgavsko in lisasto govedo imata večji telesni okvir, zato so živali v delnem cikastem tipu večjega okvira. Živali v delnem cikastem tipu imajo v genotipu največkrat večji delež drugih pasem govedi, kar je bilo potrjeno tudi z genetsko karakterizacijo na osnovi genetskih mikrosatelitnih označevalcev (Simčič in sod, 2013a, Simčič in sod, 2013b) in označevalcev SNP (Simčič in sod., 2015). Plemenski biki v cikastem tipu so manjšega telesnega okvira, kar je pozitivna lastnost za pasmo, katere tehnologija reje temelji na paši na strmih alpskih pašnikih. Živali manjšega telesnega okvira imajo namreč nižje postavljeno težišče, kar omogoča, da so bolj stabilne na strmih pašnikih. Poleg tega imajo pasme z manjšim okvirom tudi manjše potrebe po vzdrževalni krmi (Communod in sod., 2013).

3.2 VIRI VARIABILNOSTI

V preglednici 6 so prikazani viri variabilnosti na osnovi analize variance za lastnosti zunanjosti pri plemenskih bikih, izračunani po modelu 1. Sistematski vpliv leto ocenjevanja je bil statistično značilen pri štirih merjenih lastnostih (dolžina telesa, širina prsi, globina prsi, širina križa) in pri štirih posameznih ocenjevanih lastnostih. Leto ocenjevanja je statistično značilno vplivalo na sestavljeni lastnosti za omišičenost in za telesne oblike. Starost ob ocenjevanju je statistično značilno vplivala na vse merjene lastnosti in na šest posameznih ocenjevanih lastnosti za avtohtonost ter na sestavljeno lastnost za avtohtonost. Največji delež variabilnosti (R^2) za lastnosti zunanjosti pri plemenskih bikih cikastega goveda smo pojasnili pri merjenih lastnostih (18–30 %). Delež pojasnjene variabilnosti pri posameznih lastnostih v sklopu

Preglednica 4: *Opis treh tipov cikastega goveda (Žan Lotrič in sod., 2010)*
Table 4: *Description of three types of Cika cattle (Žan Lotrič et al., 2010)*

Napaka v sklopu avtohtonost	n	%	Napaka v sklopu telesne oblike	n	%
Temen gobec	1	0,30	Razplečenost	3	0,91
Neustrezna barva plašča	2	0,61	Visoko nasajen rep	47	14,24
Beli znaki na nogah	2	0,61	Kravja stoja	11	3,33
Pikasto pisana	1	0,30	Razprti parklji	2	0,61
Prekinjena hrbtna lisa	118	35,76			
Hrbtna lisa na križu	39	11,82			
Vseh bikov	330	100,00		330	100,00

Preglednica 5: *Opisna statistika za lastnosti zunanjosti pri plemenskih bikih treh tipov cikastega goveda*
Table 5: *Descriptive statistics for type traits of Cika sires of three different types*

Lastnosti (povprečje ± SD)	Cikasti tip (n = 186)	Delni cikasti tip (n = 142)	Pincgavski tip (n = 2)
Starost (meseci)	14,33 ± 2,67	14,96 ± 2,67	14,50 ± 3,54
Merjene lastnosti za telesni okvir			
Višina vihra (cm)	115,47 ± 4,22	119,11 ± 4,22	124,00 ± 9,90
Višina križa (cm)	118,53 ± 4,26	122,28 ± 4,29	128,00 ± 9,90
Dolžina telesa (cm)	114,05 ± 6,76	118,81 ± 5,59	123,00 ± 5,66
Obseg prsi (cm)	154,61 ± 9,64	162,06 ± 10,04	167,00 ± 12,73
Širina prsi (cm)	37,85 ± 3,51	39,92 ± 3,78	38,00 ± 5,66
Globina prsi (cm)	58,88 ± 4,00	61,66 ± 3,90	62,50 ± 6,36
Širina križa (cm)	39,14 ± 3,51	41,43 ± 3,12	44,00 ± 5,66
Telesna masa (kg)	300,83 ± 54,97	342,99 ± 61,52	375,00 ± 73,54
Posamezne lastnosti za avtohtonost (1–9)			
Dolžina glave	6,35 ± 1,19	5,87 ± 1,29	6,50 ± 0,71
Plemenitost glave	6,42 ± 0,86	5,01 ± 0,93	5,00 ± 0,00
Izraženost oči	5,92 ± 0,86	5,29 ± 0,85	4,00 ± 1,41
Debelina rogov	5,29 ± 1,25	4,47 ± 1,18	6,00 ± 0,00
Dolžina rogov	6,20 ± 1,24	5,36 ± 1,16	5,50 ± 2,12
Usmerjenost rogov	5,59 ± 1,31	4,83 ± 1,38	4,50 ± 2,12
Vrat	5,71 ± 1,20	4,15 ± 1,23	2,00 ± 0,00
Izraženost podgrline	5,26 ± 1,31	4,00 ± 1,25	3,00 ± 0,00
Pigmentacija plašča	5,80 ± 1,28	4,89 ± 1,35	2,50 ± 0,71
Izraženost hrbtne lise	6,31 ± 1,34	6,06 ± 1,44	8,00 ± 0,00
Izraženost pasov na zadnjih nogah	6,81 ± 1,70	6,31 ± 1,69	8,50 ± 0,71
Izraženost pasov na prednjih nogah	7,49 ± 1,27	7,26 ± 1,41	8,50 ± 0,71
Posamezne lastnosti za telesne oblike (1–9)			
Hrbet	4,69 ± 0,53	4,63 ± 0,65	4,50 ± 0,71
Nagib križa	4,99 ± 0,51	5,09 ± 0,64	5,50 ± 0,71
Kot skočnega sklepa	5,18 ± 0,68	5,20 ± 0,74	5,00 ± 0,00
Izraženost skočnega sklepa	6,68 ± 0,99	5,64 ± 1,03	3,00 ± 0,00
Biclji	6,44 ± 0,85	6,20 ± 1,09	6,00 ± 0,00
Parklji	5,99 ± 0,99	6,01 ± 1,00	6,00 ± 0,00
Izjava rejca (1–5)			
Temperament	4,35 ± 0,73	4,04 ±0,81	4,00 ± 0,00
Sestavljene lastnosti (1–9)			
Avtohtonost	7,22 ± 0,42	5,28 ± 0,83	3,00 ± 0,00
Omišičenost	5,56 ± 1,08	5,96 ± 1,18	6,00 ± 1,41
Telesne oblike	6,69 ± 0,85	5,99 ± 1,06	5,50 ± 0,71

SD – standardni odklon, KV – koeficient variabilnosti

Preglednica 6: *Viri variabilnosti za lastnosti zunanjosti pri plemenskih bikih cikastega goveda*
Table 6: *The source of variability for type traits of Cika sires*

	Viri variabilnosti (p-vrednosti)		
	Leto ocenjevanja	Starost ob ocenjevanju	R^2
Merjene lastnosti			
Višina vihra (cm)	ns	< 0,001	0,20
Višina križa (cm)	ns	< 0,001	0,20
Dolžina telesa (cm)	< 0,001	< 0,001	0,26
Obseg prsi (cm)	ns	< 0,001	0,27
Širina prsi (cm)	0,016	< 0,001	0,18
Globina prsi (cm)	< 0,001	< 0,001	0,30
Širina križa (cm)	0,001	< 0,001	0,25
Telesna masa (kg)	ns	< 0,001	0,28
Posamezne lastnosti za avtohtonost (1–9)			
Dolžina glave	0,001	0,003	0,13
Plemenitost glave	ns	ns	0,03
Izraženost oči	ns	0,002	0,05
Debelina rogov	ns	ns	0,03
Dolžina rogov	0,044	0,002	0,08
Usmerjenost rogov	< 0,001	ns	0,12
Vrat	ns	0,038	0,06
Izraženost podgrline	0,003	ns	0,07
Pigmentacija plašča	0,005	0,010	0,09
Izraženost hrbtne lise	ns	0,036	0,04
Izraženost pasov na zadnjih nogah	ns	ns	0,04
Izraženost pasov na prednjih nogah	ns	ns	0,03
Posamezne lastnosti za telesne oblike (1–9)			
Hrbet	ns	ns	0,04
Nagib križa	ns	ns	0,02
Kot skočnega sklepa	0,005	ns	0,07
Izraženost skočnega sklepa	ns	0,018	0,05
Biclji	ns	0,001	0,08
Parklji	0,001	ns	0,09
Sestavljene lastnosti (1–9)			
Avtohtonost	ns	0,025	0,06
Omišičenost	0,035	ns	0,05
Telesne oblike	0,002	ns	0,08

R^2 – koeficient determinacije, ns – vpliv statistično ni značilen (p > 0,05)

avtohtonost je bil med 3 in 13 %, pri posameznih lastnostih za telesne oblike pa med 2 in 9 %. Delež pojasnjene variabilnosti za sestavljeno lastnost avtohtonost je bil 6 %.

ICAR (International agreement …, 2012) priporoča analizo lastnosti zunanjosti z modelom, kjer naj bi bili vključeni vplivi starosti in sezone. Ocenjevalci naj ne bi korigirali ocen glede na starost, sezono, očeta in tehnologijo reje v času postopka ocenjevanja, ampak morajo oceniti le biološko izraženost lastnosti. Zabeležiti bi morali še način uhlevitve (prosta reja, vezana reja, reja z izpustom) in tip tal (beton, cement, les, pesek, guma, slama, pašnik), ker bi lahko vplivali na nekatere lastnosti zunanjosti.

V zadnjem obdobju so tudi pri analizah variance lastnosti zunanjosti pri prvesnicah italijanskih avtohtonih pasem valdostana (Mazza in sod., 2013), rendena (Mazza in sod., 2014) in piemontese (Mantovani in sod.,

2010) vključili vpliv starosti ob ocenjevanju. Poleg tega so vključili še interakcijo čreda-leto-ocenjevalec. Z navedenim modelom so pojasnili tudi večji delež variabilnosti (R^2 = 0,19–0,36). V model za analizo lastnosti zunanjosti španske pasme asturiana de los valles so vključili vplive črede, interakcijo ocenjevalec-leto-sezona in starost (Gutiérrez in Goyache, 2002). V našem primeru je ocenjevalec samo eden, po čredi pa je premalo meritev, da bi jo lahko obravnavali kot sistematski vpliv.

Nekateri avtorji (npr. Dal Zotto in sod., 2007, Klopčič in Hamoen, 2010) priporočajo tudi vključitev telesne kondicije v model kot vpliv pri analizi lastnosti zunanjosti. Telesna kondicija predstavlja pokritost s podkožno maščobo oziroma lojem, ki ga otipamo na predelu korena repa in ledvenih vretenc in lahko vpliva na ocene zunanjosti, še posebno, ko so živali v preskromni ali v predobri kondiciji. Ocenjevanje telesne kondicije bi zato kazalo vpeljati tudi v ocenjevanje zunanjosti plemenskih bikov cikaste pasme.

4 SKLEPI

Razvrščanje plemenskih bikov cikastega goveda v tip poteka ob zaključku ocenjevanja, kar onemogoča, da bi upoštevali morebitne vplive okolja. Pri razvrščanju se upošteva oceno za sestavljeno lastnost avtohtonost. Ugotovili smo, da na lastnosti zunanjosti in posledično na razvrstitev plemenskih bikov v tip značilno vplivata vsaj leto ocenjevanja in starost ob ocenjevanju. Razvrstitev živali v ustrezen tip bi bilo zaradi tega potrebno opraviti računsko, po izvrednotenju ocen za lastnosti zunanjosti, pri katerem se v veliki meri izključi vplive okolja. S tem bi dobili bolj pravilne razvrstitve živali v tip.

Analiza lastnosti zunanjosti pri plemenskih bikih cikastega goveda je bila narejena kot prvi korak pred oceno fenotipskih in genetskih parametrov, ki so osnova za napoved plemenskih vrednosti. Plemenske vrednosti bi lahko bolje služile kot osnova za selekcijo, saj predstavljajo aditivni genetski vpliv in hkrati omogočajo razvrščanje živali. Tak parameter je s selekcijskega vidika učinkovitejši od razvrstitve v tri tipe na osnovi fenotipskih vrednosti, ki poleg tega, da ne upošteva vplivov okolja, vključuje tudi tiste dele genetske vrednosti, ki se ne dedujejo.

Predlagamo, da bi ob ocenjevanju zunanjosti v celoti upoštevali pravila ICAR in zabeležili tehnologijo reje ter ocenili tudi telesno kondicijo živali, kar bi bilo mogoče vključiti kot vpliv v postopku genetskega vrednotenja. Pri ocenjevanju zunanjosti nekaterih pasem goveda v Sloveniji že nekaj let spremljajo telesno kondicijo.

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SUBJECT INDEX BY AGROVOC DESCRIPTORS
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ABECEDNO KAZALO AVTORJEV
AUTHOR'S INDEX

NAVODILA AVTORJEM

PRISPEVKI

Sprejemamo izvirne znanstvene članke, predhodne objave in raziskovalne notice s področja zootehnike (genetika, mikrobiologija, imunologija, prehrana, fiziologija, ekologija, etologija, mlekarstvo, ekonomika, bioinformatika, živalska proizvodnja in predelava živalskih proizvodov, tehnologija in dokumentalistika) v slovenskem in angleškem jeziku, pregledne znanstvene članke pa samo po poprejšnjem dogovoru. Objavljamo tudi prispevke, podane na simpozijih, ki niso bili v celoti objavljeni v zborniku simpozija. Če je prispevek del diplomskega, magistrskega ali doktorskega dela, navedemo to in tudi mentorja v sprotni opombi na dnu prve strani. Navedbe morajo biti v slovenskem in angleškem jeziku.

Pri prispevkih v slovenskem jeziku morajo biti preglednice, grafikoni, slike in priloge dvojezični, povsod je slovenščina na prvem mestu. Naslovi grafikonov in slik so pod njimi. Preglednice, slike in grafikoni so v besedilu. Grafikoni morajo biti črno-beli. Latinske izraze pišemo ležeče. V slovenščini uporabljamo decimalno vejico, v angleščini decimalno piko.

Prispevki naj bodo strnjeni, kratki, največ 12 strani, napisani z urejevalnikom besedil in oddani v doc ali rtf formatu (Windows). Izgled strani naj bo čim bolj enostaven; v besedilo ne vstavljajte glave in noge. Pisava v besedilu in preglednicah je Times New Roman, velikost črk 12, v obsežnih preglednicah je lahko 10, pisava v grafikonih in slikah je Ariel, velikost črk najmanj 8, pisava za primerjave nukleotidnih in aminokislinskih zaporedij je Courier; zunanji rob 2,0 cm, notranji 2,5 cm.

PRVA STRAN

Na prvi strani prispevka na desni strani označimo vrsto prispevka, sledi naslov prispevka, pod njim avtorji. Ime avtorjev navedemo v polni obliki (ime in priimek). Vsakemu avtorju dodamo sprotno opombo, ki je vidna na dnu strani, in vsebuje polni naslov ustanove ter znanstveni in akademski naslov; vse v jeziku prispevka. Navedemo sedež ustanove, kjer avtor dela. Če je raziskava opravljena drugje, avtor navede tudi sedež te inštitucije. Na željo avtorjev bomo navedli naslov elektronske pošte.

Pod imeni avtorjev je datum prispetja in datum sprejetja prispevka, ki ostaneta odprta. Sledi razumljiv in poveden izvleček v enem odstavku (skupaj s presledki do 1400 znakov). Vsebuje namen in metode dela, rezultate, razpravo in sklepe. Sledijo ključne besede.

Izvlečku v jeziku objave sledi naslov in izvleček s ključnimi besedami v drugem jeziku.

VIRI

V besedilu navajamo v oklepaju avtorja in leto objave: (priimek, leto). Če sta avtorja dva, pišemo: (priimek in priimek, leto), če je avtorjev več, pišemo: (priimek in sod., leto). Sekundarni vir označimo z »navedeno v« ali »cv.«.

Seznam virov je na koncu prispevka, neoštevilčen in v abecednem redu. Vire istega avtorja, objavljene v istem letu, razvrstimo kronološko z a, b, c. Primer: 1997a. Nekaj primerov navajanja virov:

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ODDAJA

Avtorji prispevke oddajo v natisnjenem in elektronskem izvodu. Priložijo tudi izjavo s podpisi vseh avtorjev, da avtorske pravice v celoti odstopajo reviji.

Prispevke recenziramo in lektoriramo. Praviloma pošljemo mnenje prvemu avtorju, po želji lahko tudi drugače. Če urednik ali recenzenti predlagajo spremembe oz. izboljšave, vrne avtor popravljeno besedilo v 10 dneh v natisnjenem in elektronskem izvodu. Ko prvi avtor vnese še lektorjeve pripombe, odda popravljeno besedilo v natisnjenem in elektronskem izvodu.

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Fraser A.F., Broom D.M. 1990. Farm animal behaviour and welfare. London, Bailliere Tindall: 437 p.

Hvelplund T. 1989. Protein evaluation of treated straws. In: Evaluation of straws in ruminant feeding. Chenost M., Reiniger, A. (eds.). London, Elsevier Applied Science: 66–74

Žgajnar J., Kermauner A., Kavčič S. 2007. Model za ocenjevanje prehranskih potreb prežvekovalcev in optimiranje krmnih obrokov. In: Slovensko kmetijstvo in podeželje v Evropi, ki se širi in spreminja. 4. konferenca DAES, Ljubljana, 8.–9. sep. 2007. Kavčič S. (ed.). Domžale, Društvo agrarnih ekonomistov Slovenije: 279–288

ISO 5534 / IDF 4. Cheese and processed cheese – Determination of the total solids content – Reference method. 2004: 1–7

Frajman P., Dovč P. 2004. Milk production in the post-genomic era. *Acta agriculturae Slovenica*, 84, 2: 109–119. http://aas.bf.uni-lj.si/zootehnika/84-2004/PDF/84-2004-2-109-119.pdf (15. mar. 2009)

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VSEBINA / CONTENTS