

comparison with meat of commercial pig breeds. Since the Mangalitsa pigs are adapted to an extensive way of keeping and have a high disease resistance, this makes them ideal for organic production. A good example of the organic raising of Mangalitsa pigs is applied in the special nature reserve "Zasavica". Currently, only *in vivo* conservation is carried out in Serbia. Another reason for increasing the number of Mangalitsa are subsidies provided to the farmers by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. According to the data from the Main Breeding Organization for Vojvodina, the number of Mangalitsa pigs under control during the period 2013-2017 is on the rise, both for males and females. Implemented selection measures include the control of the productivity of sows and boars. The main goal of the breeding is to preserve and increase the population as well as to improve the genetic basis of the Mangalitsa population in the Republic of Serbia. In the future, new modern methods of conservation and biotechnological methods should be introduced in order to preserve the purity of the breed and increase the number of Mangalitsa pigs.

**Key words:** mangalitsa; autochthonous pig breed; *in vivo* conservation; productivity control

#### RAPID GENOTYPING OF THE SHORT AND HIGHLY VARIABLE REGION IN MTDNA OF HERBIVORES

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Mitochondrial DNA is found in nearly all eukaryotic organisms. mtDNA is a short, circular and relatively conserved DNA molecule transmitted maternally. It is one of the most useful tools in population genetics or phylogenetics for maternal inheritance. Screening of polymorphisms of mtDNA is suitable for comparisons among individuals from the same population as well as among distantly related species. mtDNA has an extremely variable region acquired by 2 billion years of mtDNA evolution, and is more variable than the nuclear genome itself. Furthermore, it is an ideal genetic marker for analysis of problematic, low quality and low quantity materials i.e. faeces. Here we present the application of universal primers for rapid genotyping of the highly variable region of mtDNA in cattle, chamois, deer, goat and sheep. DNAs of cattle, deer, chamois, goat and sheep from our DNA bank were used for validation of the method. DNA was extracted from hair roots or blood using Wizard Genomic DNA purification Kit (Promega). PCR reactions were performed with 20-40 ng DNA, 35 cycles and 53°C annealing temperature (Thermo-Start PCR Master Mix, Thermo Scientific). PCR fragments were cycle-sequenced using BigDye Terminator Cycle sequencing Kit version 1.1 and were run on an Avant 3100 Genetic Analyser (Applied Biosystems). Sequences were aligned using Geneious software (Biomatters) and BLAST (NCBI). In summary, we have validated a method for rapid genetic screening of the highly variable region in mtDNA of cattle, chamois, deer, goat and sheep, which can be used for species determination and population studies among even wider variety of herbivore species.

**Key words:** mtDNA; genotyping; herbivores

#### ANTIBIOTIC RESISTANCE OF ENTEROBACTERIACEAE SPECIES ISOLATED FROM RECTAL SWABS OF SHEEP

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The main objective of this study was to determine antibiotic resistance of *Enterobacteriaceae* species isolated from sheep rectal swabs and identification of isolated species as potential resistant gene vectors. Sixty nine sheep rectal swabs were obtained from different farms of Slovakia. Isolation of *Enterobacteriaceae* species was done on MacConkey agar during 24 h at 37 ± 1 °C under air condition. Cultures were purified by four-way streak plate method. Pure cultures were identified by MALDI TOF Mass Spectrometry using MALDI Biotyper 3.1 software (Bruker Daltonics, Germany). Antibiotic susceptibility testing was performed by disk diffusion methodology according to EUCAST. Ampicillin resistance was confirmed by indirect method using MALDI TOF MS, where beta-lactamase hydrolysis of ampicillin was assessed and resistance mechanism was detected. The following antibiotics were used in this study: streptomycin (10 µg/disc), tetracycline (30 µg/disc), chloramphenicol (30 µg/disc), oxacillin (1 µg/disc), ampicillin (10 µg/disc), nalixidic acid (30 µg/disc), amikacin (30 µg/disc), gentamicin (30 µg/disc), levofloxacin (5 µg/disc), piperacillin (30 µg/disc) and tigecycline (15 µg/disc). Overall, from 69 samples the resistance was revealed in following: 5 strains against tetracycline, 2 strains against tetracycline, 4 strains against chloramphenicol, 14 strains against oxacillin, 7 strains against ampicillin and 9 strains against levofloxacin. Antibiotic resistances against nalixidic acid, amikacin, gentamicin, piperacillin and tigecycline were not found in this study. Seven strains were purified by four-way streak plate method and identified by MALDI TOF MS as *Escherichia coli*, *Serratia odorifera* bv.1, *Enterobacter aerogenes*, *Citrobacter farmeri*, *Proteus vulgaris*, *Klebsiella* spp. and *Yersinia* spp. Disk diffusion method showed resistance of *Escherichia coli* against three antibiotics: chloramphenicol, ampicillin and levofloxacin. *Serratia odorifera* bv. 1 was resistant against streptomycin and tetracycline. Oxacillin resistance was detected in *Enterobacter aerogenes* and resistance against chloramphenicol in *Klebsiella* spp. Other identified bacteria isolated from rectal swabs of sheep were identified as susceptible to antibiotics, which were used in this study. MALDI TOF MS analysis showed that ampicillin was hydrolysed by beta-lactamases produced by *Escherichia coli*, and its decay products as ampicillin with disrupted amide bound (366 ± 0.6 m/z), its monosodium salt (389 ± 0.6 m/z),

its disodium salt ( $412 \pm 0.6$  m/z), spontaneous decarboxylated ampicillin ( $323 \pm 0.6$  m/z) and decarboxylated ampicillin sodium salt ( $344 \pm 0.6$  m/z) were detected. Therefore, enzyme hydrolysis strategy was confirmed as main strategy of antibiotic resistance in *E. coli*. In conclusion, we determined that the resistance against several antibiotics was found in rectal swabs of sheep and it is spread within sheep breeding. Also, enzymatic resistance mechanism is a main resistant strategy within *Enterobacteriaceae* genera in sheep breeding.

**Key words:** antibiotic resistance; sheep; *Enterobacteriaceae*; MALDI TOF MS

**Acknowledgements:** The research leading to these results has received funding from the European Community under project no 26220220180 and the Building Research Centre "AgroBioTech," and the Slovak Research and Development Agency under Contract no. APVV-16-0289.

### CHICKEN STEM CELLS AS A POTENTIAL SOURCE FOR ANIMAL GENE BANK

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Biosecurity and sustainability in chicken production requires reliable germplasm conservation. Germplasm conservation in chicken is more challenging in comparison to other livestock species. Embryo cryopreservation is not feasible for egg-laying animals, and chicken semen conservation in different chicken breeds has variable success. A potential solution is the cryopreservation of the committed diploid stem cell, blastodermal cells (BCs), the precursor of gametes and primordial germ cells (PGCs). BCs and PGCs are the lineage-restricted cells found at early embryonic stages in birds. This research dealt with isolation, characterisation and cryopreservation of chicken stem cells for the animal gene bank purposes. Trypan blue, fluorescence microscopy, flow cytometry and transmission electron microscopy were used for the viability assessment and characterisation of fresh and frozen/thawed chicken stem cells. Our results showed that BCs contain lipid granules, which prevent successful freezing even though different methods of cryopreservation were used. However, in contrast, PGCs contain a smaller amount of lipid granules, and, therefore, PGCs are more suitable for cryopreservation. The present study suggests that PGCs should be considered as more preferable source for animal biobanking, and the choice of proper cell source should be done carefully.

**Key words:** chicken; blastodermal cells; primordial germ cells; cryopreservation; viability

**Acknowledgements:** The study was financially supported by the Slovak Research and Development Agency (the grant No. APVV-17-0124), VEGA 1/0611/15, VEGA 1/0160/18 and KEGA 026 SPU-4/2018.

### INBREDSATION – THE WAY FOR CREATION OF GENETICALLY UNIFORM RABBIT GROUPS

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Utilization of rabbits as experimental animals is oriented to specific populations because of user goals. Human diseases, organ transplantations, physiological experiments, feeding trials or genetic experiments are most frequent examples for exploration of rabbits. These utilizations are associated with homogenous genetic background, which can be reached by inbreeding process. Depending on the type of observed and applied traits, homozygosity can be an effective way to create appropriate populations with unique characteristics.

We used 3 panmictic rabbit populations (New Zealand White – NZW, Californian – C and Nitra rabbit – Ni) for directed selection during 5 generations. In the course of inbreeding, the animals were mated *inter se* (full siblings with each other). Initial (founder) animals were selected from a wider population and they formed the original parental groups with 20 does and 5 males of each breed. At average, the same number of selected animals was mated for next generation. Selection criteria were following: for NZW it were – increase of live weight, for C – high level of untroubled behaviour, and for Ni – long ear shell with clear blood vessel. NZW animals were selected on the basis of regular weekly measuring of live weight. Californian rabbits were tested in open field equipment for peaceful habitus as a number of movements per time unit. Animals in Nitra breed population were selected basing on the ear length and good visualisation of central ear blood vessel. In addition to the selected traits, in all three populations the standard breed characteristics were maintained.

After the 5 year selection process the results are following: in the 5<sup>th</sup> generation of NZW rabbit population an average initial live weight was increased daily from  $25.4 \pm 6.2$  g to  $30.2 \pm 3.2$  g. It represents difference in 411.6 g at slaughter age in favour of the inbred population. Panmictic Californian rabbits had  $25.6 \pm 7.5$  movements in contrast to  $14.5 \pm 5.7$  units for animals in inbred population. The length of ear in the initial Nitra rabbits was  $11.5 \pm 1.6$  cm in comparison to the inbred population ( $13.5 \pm 1.8$  cm). According to these results, inbreeding is an effective process for creating relevant populations.

**Key words:** rabbit; inbreeding; selected traits; live weight; ear length

**Acknowledgements:** This work was supported by the Slovak Research and Development Agency under the contract APVV-16-0067 and APVV-15-0229.

### DIFFERENCES BETWEEN EWES AND MOUFLONS IN SELECTED METABOLIC PARAMETERS IN RELATION TO THE YIELD AND EMBRYO QUALITY

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