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PRODUCTION NITRA

# Slovak Journal of Animal Science

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# ANIMAL 2017

## BIOTECHNOLOGY



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Slovak Academy of Agricultural Sciences

Main topic of the conference: **Biodiversity – Animal Genetic Resources**  
which will involve *in situ* and *ex situ* preservation, genetics, reproduction, breeding and management

December 7<sup>th</sup>, 2017

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**Dear Participants and Dear Colleagues,**

We are very pleased to welcome you on the 5<sup>th</sup> International Scientific Conference “**Animal Biotechnology 2017**”. The conference is organized by the Research Institute for Animal Production Nitra, National Agricultural and Food Centre in cooperation with the Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture Nitra and Slovak Academy of Agricultural Sciences.

The aim of the conference is presentation of an actual research from the field of animal biotechnology, with a special focus on **Biodiversity – Animal Genetic Resources**. The topic areas of the conference will involve animal species as a cattle, horses, sheep, rabbits, poultry and bee.

Moreover, the conference will provide an opportunity to gather researchers engaged in this and adjacent fields of research in order to exchange their skill and experience as well as to establish potential collaboration in a given task. We would appreciate attendance and participation on this conference of colleagues from various research institutions and universities.

We wish you cordial and warm atmosphere at our conference for presentation, creative and fruitful discussion and inspiring ideas for future research.

Nitra, December 7<sup>th</sup>, 2017

**Peter Chrenek**



## CURRENT SITUATION IN THE GENE BANK OF ANIMAL GENETIC RESOURCES IN SLOVAKIA: A REVIEW

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### ABSTRACT

In the last fifty years, there was a significant decrease in the number of animals in the Slovak Republic, and in some species an original, (native) autochthonous breeds completely disappeared. From the last update (at the end of 2011) statuses of animal breeds in the Slovak Republic, it is obvious that three breeds of cattle and two breeds of pig are subjected to extinction. The situation with animal genetic resources in the Slovak Republic is not satisfactory due to the fact that semen doses, stored in the gene bank, are originated only from several, but not from all Slovak breeds. In particular, from the cattle breeds there are Slovak Pinzgau and Slovak Spotted, from rabbit breeds – Nitra rabbit, Zobor rabbit and Holic rabbit and from chicken breed – Oravka. In the near future we plan to store embryos from Slovak cattle, sheep and rabbit breeds. Stem cells appear to be a perspective tool for preservation of endangered species (breeds). Moreover, we also collect for storage stem cells from various sources (bone marrow, amniotic fluid, blood and fat tissues) of different Slovak animal breeds. For this reason animal genetic resource *ex situ* (gene bank) is important for solution of problems of animal genetic resource in Slovakia.

**Key words:** gene bank; animal genetic resources; Slovak animal; cryopreservation

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### INTRODUCTION

Every week the world loses two breeds of its valuable domestic animal diversity, according to estimates just published in the 3<sup>rd</sup> edition of the World Watch List for Domestic Animal Diversity. Despite enormous potential contribution to sustainable development and to reducing hunger and poverty, animal genetic resources for food and agriculture are underutilized and underconserved. In the past 100 years, we have already lost about 1 000 breeds. New findings show that domestic animal breeds continue to be in danger: one third are currently at risk of extinction (FAO, 1993).

Throughout 2014, the international conservation community celebrated the 50<sup>th</sup> anniversary of the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and its significant contribution to guiding global preservation action.

Currently there are more than 79,800 species on the IUCN Red List, and more than 23,000 are threatened with extinction, including 41 % of amphibians, 34 % of conifers, 33 % of reef building corals, 25 % of mammals and 13 % of birds. The statistics shows that in 2016, around 67,050 species of vertebrates in the world were known and 7,967 were endangered to be extinct.

For conservation of Animal Genetic Resources (AnGR) various methods can be used and conservation activities can be categorized according to whether they involve the maintenance of genetic material *in vivo* or *in vitro*. *In vivo* conservation can, in turn, be classified according to whether it takes place *in situ* or *ex situ*.

*In situ* conditions were defined in the Convention on Biological Diversity as “conditions and natural habitats, and, in the case of domesticated or cultivated species, in the surroundings where they have developed their Distinctive properties” (CBD, 1992).

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**Table 1: Current activities for *ex situ* conservation of AnGR in the European countries**

Country	Institutions	Starting date of genebank „ <i>In vivo</i> “	Starting date of sample collection „ <i>In vitro</i> “
Albania	Ministry of Agriculture	2008	
Austria	Institute of Organic Farming and Biodiversity of Animals	1997	1997
Belgium	Université catholique de Louvain	2010	2010
Bulgaria	Executive Agency on Selection and Reproduction in Animal Breeding (EASRAB)	1969	
Croatia	Croatian Agricultural Agency	2012	
Czech Republic	Institute of Animal Science	2006	2006
Denmark	Ministry of Agriculture	1987	1987
Finland	MTT Agrifood Research	1984	
France	Group Council (12 institutions)	1999	
Germany	Several institutions	planned 2013	
Hungary	Centre for Farm Animal Gene Conservation	2009	2010
Italy	Several institutions	1980	1980
Latvia	Latvia University of Agriculture	2007	2007
Lithuania	Institute of Animal Science	1998	
Netherlands	Center for Genetic Resources, Wageningen	2000	
Norway	Breeding Association	1960	
Poland	National Research Institute of Animal Production, Krakow	1968	1990
Portugal	Ministry of Agriculture and Sea	2004	
Serbia	Livestock Veterinary Center for Reproduction and Artificial Insemination Velika Plana	2003	
Slovakia	Ministry of Agriculture, NPPC, Research Institute for Animal Production, Nitra	planned 2013	2015
Slovenia	University of Ljubljana	2010	
Spain	Ministry of Agriculture, Food and Environment	1998	1999
Sweden	Swedish University of Agriculture	1990	1990
Switzerland	Several institutions	2009	2009
Ukraine	Institute of Animal Breeding and Genetics	2002	
United Kindom	Rare Breeds Survival Trust		

*In situ* conservation is undertaken in the traditional production system of the conserved AnGR. However, *in situ* conservation is not without risks. For example, a population maintained *in situ* may be struck by a disease outbreak or other disaster or may be affected by inbreeding, genetic drift or introgression from another breed.

*Ex situ* conservation (Table 1) decreases these risks by providing a backup that can be drawn upon if required. However, if the population is also maintained *in situ*, regularly collecting and conserving new samples *in vitro* can help to maintain the potential

for future adaptation. As described above, *ex situ* conservation can be undertaken either *in vivo* or *in vitro*. While in many circumstances maintaining a live *ex situ* population adds little to the conservation strategy that already includes *in situ* and *in vitro* components, it can have some advantages.

The situation with animal genetic resources in the Slovak Republic is not satisfactory due to the fact that semen doses stored in the gene bank are originated only from several, but not from all Slovak breeds. In particular, from the cattle breeds there are Slovak Pinzgau cattle and Slovak Spotted cattle, from rabbit breeds



**Table 2: Selected samples from animal genetic resources in the Slovak Republic in 2016**

Breed	Animals (N)	Females in herd book (N)	DNA*	ID** (N)	Frozen embryos	Frozen stem cells	DNA samples
Pinzgauer cattle	9883	1583	yes	200	yes	no	no
Slovak spotted cattle	161 550	30 545	yes	250	no	no	no
Walachian sheep	2437	553	yes	no	no	no	120
Tsigaja	123 660	7066	no	no	no	no	no
Improved Valachian	131 573	7732	no	no	no	no	no
Nitra rabbit	1500	-	no	155	yes	yes	yes
Zobor rabbit	100-120	-	no	267	yes	yes	yes
Holic blue rabbit	260-390	-	no	154	no	no	yes
Slovak pastel Rex	80	-	no	42	no	no	yes
Slovak greyish blue Rex	700-900	-	no	17	no	no	yes
Oravka hen	1800	-	no	100	no	no	yes
White short-haired goat	8036	1062	no	no	no	no	no
Brown short-haired goat	1627	116	no	no	no	no	no
Slovak carniolan honeybee	262 000	-	yes	no	no	no	170

dad.fao.org \* DNA microsatellites; \*\*ID - Insemination doses

– Nitra rabbit, Zobor rabbit and Holic rabbit (Kuliková *et al.*, 2015) and from chicken breed – Oravka. We have verified methods of embryo freezing and vitrification and in the near future we plan to store embryos from Slovak cattle, sheep and rabbit breeds (Kubovičová *et al.*, 1998, 1999; Popelkova *et al.*, 2009). Stem cells appear to be a prospective tool for preservation of endangered species (breeds). Moreover, we also collect for storage stem cells from various sources (bone marrow, amniotic fluid, blood and fat tissues) of different Slovak animal breeds such as chicken blastodermal stem cells (Svoradova *et al.* 2016), which were derived from X stage embryos (blastoderm stage) of Oravka breed and rabbit amniotic fluid stem cells (Kováč *et al.*, 2016). At present there are samples kept and registered at NPPC-RIAP Nitra and partner insemination center (Table 2).

## CONCLUSION

Generally, Animal Gene Bank plays an important role in agricultural production globally for the present and the future, and in sustaining most production systems and community livelihoods. At national level, collaboration between institutions has to be improved for increasing the animal resource conservation efficacy and implementation. For the gene bank development, new tools related to genomic large-scale studies and reproductive technologies are required.

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## FORMATION AND CHARACTERIZATION OF SLOVAK DAIRY COMPOSITE SHEEP BREED: DESCRIPTION OF THE PROCESS: A REVIEW

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### ABSTRACT

In the beginning of 1990s, the programme of formation of synthetic population of Slovak Dairy sheep (SD) was launched. Some flocks were involved into performance testing; the establishment of the breed was divided into two periods: first, crosses of local breeds (Improved Valachian, Tsigai and Merino) with improving breeds (Lacaune, East Friesian) of various proportions were formed; second, a close (*inter se*) breeding scheme within the crossbred population was applied. Parents of the next generation were chosen on the basis of breeding values for milk yield and litter size. At present, five thousands female SD individuals are included in the performance testing; 35 % yearling females enter the breeding scheme each year; a total, 50 thousands SD ewes are kept in commercial flocks. Milk and reproduction traits of SD were analyzed using mixed model methodology that included fixed and random effects. In similar way growth traits were analyzed. Milk traits analyses included 20,511 milk records belonging to 11,026 ewes kept in 52 flocks during the period between 1995 and 2015; reproduction traits analyses included 30,034 litter size records belonging to 9,671 ewes kept in 26 flocks during the period between 1997 and 2015. The standardized milk yield (SMY) and average daily milk yield (ADMY) increased more than twice:  $79.8 \pm 5.36$  l (1995) vs.  $164.0 \pm 2.04$  l (2015) and  $495.1 \pm 33.5$  ml (1995) vs.  $1035.3 \pm 12.8$  ml (2015), respectively. In ten percent of the most productive ewes, SMY was equal to 257.6 l, ADMY was equal to 1604 ml and dry matter was equal to 26.6 kg. Fat and protein contents decreased from  $7.82 \pm 0.101$  % (1995) to  $7.27 \pm 0.040$  % (2015) and from  $5.83 \pm 0.043$  % (1995) to  $5.69 \pm 0.017$  % (2015). Ewes of SD have good udder traits. Litter size in top 50 % flocks was above 150 %. Average daily gains from birth to weaning were 0.26 kg (female lambs) and 0.30 kg (male lambs). The population of SD was recognized as a breed in 2017.

**Key words:** dairy ewes; composite breed; milk traits; reproduction; growth

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### STATE OF THE ART

Following breeder's goals oriented towards high production, reproduction and functional traits of dairy sheep in Slovakia, the improving programme with intention of forming improved sheep population fitting semi-extensive production system was launched in Slovakia in 1990s. This was programme of forming Slovak Dairy sheep (SD). The breeding scheme was divided into two periods (Margetín and Čapistrák 1994; Margetín *et al.*, 2000 a,b,c). First, crosses of local breeds (Improved Valachian (IV), Tsigai (TS) and Merino

(M) with improving specialized breeds (Lacaune (LC), East Friesian (EF) of various proportions were formed in some flocks involved into performance testing. Preliminary evaluations of crossbreds with proportion of LC and EF ranged from 12.5 to 87.5 % were aimed at analyses of milk traits and litter size that are considered the most economically important traits (Apolen *et al.*, 2000; Čapistrák *et al.* 2000, 2002, 2005; Margetín *et al.*, 1993, 1999 a,b). Further analyses showed that the population with good udder shape, of appropriate cistern size and milkability was formed (Čapistrák *et al.*, 2006 a,b; Mačuhová *et al.*, 2008; 2009; Margetín *et al.*, 2005

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b,c; Margetín *et al.*, 2011 a,b; Milerski *et al.*, 2005, 2006; Tančín *et al.*, 2011). Crossbreds with various proportions of LC and EF were also confirmed to be of good growth traits both till and after weaning (Margetín *et al.*, 2004 a,b). In addition, genetic parameters and variance components of udder morphology and milkability traits were preliminarily studied (Margetín *et al.* 2005a, 2008).

Second, a close (*inter se*), breeding scheme within the crossbred population was proposed (Margetín *et al.*, 2010, 2011c, 2012b) and applied taking into account results of analyses during the first period of the programme. The aim was to stabilize composite population using *inter se* (i.e. purebred) mating scheme that included dam and sire animals used as parents of the next generation that were selected according to their breeding values for milk and litter size traits. During this period, a lot of analyses were also done. Performance testing data of flocks involved in the programme were used for evaluation. These included analyses of udder morphology and milkability traits, since one goal was to form population of ewes that fit machine milking (Margetín *et al.*, 2013b), have good udder morphology and functional traits (Margetín *et al.*, 2012a; Makovický *et al.*, 2013, 2014, 2015a, 2017a), are of appropriate cistern size (Margetín *et al.*, 2011d; Makovický *et al.*, 2015b,c) and of minimal mastitis occurrence (Margetín *et al.*, 2013a). Growth curves of lambs of various proportions of LC and EF within IV and TS genotypes were compared (Makovický *et al.*, 2017b).

In 2016, a complex analysis of SD synthetic population involving period from 1995 to 2015 was done. Mixed model methodology (SAS, ver. 9.2) was employed. When milk yield during milking period (MY), standardized (SMY) and daily milk yield (DMY) as well as fat % (F %), protein % (P %), dry matter % (DM %), free of fat dry matter (DM-F %) and usable dry matter (UDM; kg) were investigated, following fixed effects: flock (52 levels), year (21 levels), parity (3 levels: first, second and third+ parity), litter size (4 levels: one lamb, two, three + lambs born and missing information about number of lambs born), covariates: milking period (in days) and interval between lambing and first test-day measurement (days) and random effect of ewe were considered. A total, 20,511 records that belonged to 11,026 ewes (1.86 records per ewe) entered the model for analyses of MY, SMY, DMY and 18,081 records entered the model for analyses of F %, P %, DM-F % and UDM. When litter size was investigated, the following fixed effects: flock (26 levels), year (19 levels) and age of dam (8 levels, one year old up to eight+ years old) and random effect of ewe were considered. A total, 30,034 litter size records that belonged to 9,671 ewes entered the model.

Study of Margetín (2016) confirmed that improving programme that resulted in acknowledgement of SD sheep with more favourable traits than those of local breeds was fulfilled. During the period of the last 21 years (from 1995 to 2015), SMY increased about twice (from 79.8 l in 1995 to 167.0 l in 2015). Similarly, DMY and production of usable dry matter increased by 109 and 79 %. Most productive flocks reached MY ranging from 170 to 190 l. Top 10 % ewes reached SMY above 250 l, DMY reached more than 1,600 ml and production of usable dry matter was above 26 kg. Average F % and P % slightly decreased (7 and 2.4 %, respectively). Also, ewes of SD population were of good udder morphology and functional udder traits. Prolificacy did not fulfill expectations and was 141.1 %. Nevertheless, it was about 160 % in top flocks. Average daily gain till weaning was about 300 g in male lambs and about 260 g in female lambs. The frequency of ARR allele of the prion gene ranged from 0.711 to 0.789 (Margetín *et al.*, 2016).

Results of SD sheep flocks resulted in fact that this population was recognized as a native breed on May 26, 2017. Detailed information about history of breeding this population can be found in the study of Margetín *et al.* (2017). Moreover, the importance of development of this population from social and breeders' point of view is described in the reports of Margetín (2017a,b).

## CONCLUSION

Population of Slovak Dairy sheep is fairly large (more than 5 ths. females in performance testing, 35 % yearling females enter mating schemes), so it can be successfully bred in Slovakia in the future. Breeders are satisfied with the level of production and reproduction traits reached (Pavlík *et al.*, 2017). With respect to appropriate effective population size, it is important that more than 300 Slovak Dairy sires are produced each year. In the future, not only the numbers of Slovak Dairy population, its production and reproduction traits, but also its exterior traits, including wool characteristics, need to be continuously stabilized (Margetín, 2017c).

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## CHARACTERIZATION OF NATIVE SLOVAK CHICKEN AND GOOSE: A REVIEW

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### ABSTRACT

Nowadays, there are four local poultry breeds originating from different parts of Slovakia. At present time, there are registered low numbers of native breeds of poultry on the territory of Slovakia. In the last century, four poultry breeds were created - chickens (Oravka, Bantam Oravka) and goose (Suchovska goose and Slovak goose). In this article we collected and analyzed the data about the origin, breed characteristics and population size of native poultry breeds.

**Key words:** chicken; goose; Oravka breed; Slovak goose; Suchovska goose

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### INTRODUCTION

The current economic situation causes an interest in specialized poultry lines in advanced countries in the world, focusing on egg-laying or meat-based performance. Due to the very intense breeding, many genes are lost and they may be missing in future. Therefore, the reasons for keeping poultry genetic resources are the same as for all species of farm animals. Characteristics of poultry genetic resources include the collection of population size data, its structure and geographic spread, the production systems in which the breed is involved, its phenotypic attributes as well as the historical development of the breed (crossing, selection). These data should be available at standardizing the breeds and experimental lines. However, it is difficult to get the data from either commercial line breeders or small farmers. Molecular markers, which also contribute to the identification of the breed's identity, can provide important information on genetic variability either within or among populations. Current data provide a fairly comprehensive picture of genetic variability and population structure

in domestic poultry breeds. In the last century four poultry breeds were bred in Slovakia – Oravka and Bantam Oravka chickens, Suchovska goose and Slovak goose.

### CHICKEN

The domestic chicken is descended primarily from the red jungle fowl (*Gallus gallus*) and is scientifically classified as the same species. As such it can and does freely interbreed with populations of red jungle fowl (Wong *et al.*, 2004). Recent genetic analysis has revealed that at least the gene for yellow skin was incorporated into domestic birds through hybridization with the grey jungle fowl (*G. sonneratii*) (Eriksson *et al.*, 2008). The traditional poultry farming view is that chickens were first domesticated for cockfighting in Asia, Africa and Europe, rather than for egg or meat production. In the last decade there have been a number of genetic studies to clarify the origins. According to one study, a single domestication event occurring in the region of modern Thailand created the modern chicken

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with minor transitions separating the modern breeds (Fumihito *et al.*, 1994).

## ORAVKA CHICKEN

Oravka chicken is a dual purpose breed. It is of Slovakia provenience and is classified as a Slovak native breeds.

### Development

The development of Oravka chicken started in 1950s under the guidance of Ing. V. Babushkin at the Research Institute for Poultry by combinatorial crossing of regional breeds with breeds of Rhode Island Red, New Hampshire and Wyandotte White (Chmelničná, 2004). The goal was to develop a breed suitable for harsh climatic conditions of northern Slovakia which can be kept in free range. It was adapted for egg and meat production. In 1990 the yellow-brownish Oravka and in 2008 white Oravka were recognized as an independent Slovak national breeds.

Bantam Oravka breed was developed in the 1980s as the result of crossbreeding of Oravka with Bantam Dresdner and Bantam Sumavanka. Bantam Oravka should follow the large fowl standard in all respects, however, the feathering color is more intense compared with large Oravka. Recently Bantam Oravka is keeping only in original yellow-brownish colour.

### Characteristics

The yellow brownish Oravka chicken according to breed standard has hard rectangular frame, comb is rosette; medium size; the mandrel follows the header line; legs are medium length; without feather; yellow; red tinted strip (lampas) on the sides of the bows is wanted; fingers are straight; well stretched; feather is rich; well-fitting; solid structure. Body weight of males is between 2.8 and 3.3 kg, body weight of females is between 2.2 and 2.7 kg, egg laying ranges from

180 to 200 pcs per year; eggs are of a brownish shell, their average weight is about 55 g.

Weight of Bantam Oravka cock is from 1.0 to 1.3 kg, hens from 0.9 to 1.1 kg. Egg yield is 140 eggs with a brownish shell, the minimum hatching egg weight is 40 g.

The National Agriculture and Food Centre – Research Institute for Animal Production (NPPC – RIAP) Nitra, Department of Small Farm Animals kept the conservation flock of yellow brownish Oravka chicken. The laboratory of the Department of Genetics and Reproduction of NPPC – RIAP Nitra is focused on the isolation, culture and cryopreservation of blastodermal and primordial germ cells in chicken. The quality of blastodermal and primordial germ cells before and after cryopreservation is evaluated by fluorescent microscopy, flow cytometry and transmission electron microscopy. We also cryopreserve rooster semen of endangered Oravka breed and assess their quality by methods mentioned above. Semen samples of individual roosters (n = 6) are stored in the gene bank at NPPC – RIAP Nitra.

Table 1 shows the basic growth characteristics of three lines of Oravka, while the table 2 shows numbers of laid eggs in these lines. Hatchability from fertilized eggs of Oravka breed in NPPC – RIAP Nitra was higher than 83 % (Hanusová *et al.*, 2016).

### Current status

The number of breeds of both forms of Oravka in Slovakia was counted by Hrnčár and Weis (2007). The development of breeds in 2007-2008, controlled by the Slovak Breeder Association, was monitored by Oravcová *et al.* (2010). In Slovakia in 2008, ten controlled flocks of Bantam Oravka with 11 males and 61 females were registered. Ten controlled flocks of Bantam Oravka with 11 males and 61 females were registered in Slovakia in 2008. Peak in frequency of Bantam Oravka controlled flocks was noted in 2005. In years 2006-2008 a tendency of decrease in the number of controlled flocks

**Table 1: Live weight of Oravka chickens at NPPC – RIAP Nitra by sex**

Line	5 weeks old (g)				12 weeks old (g)				20 weeks old (g)			
	♀		♂		♀		♂		♀		♂	
	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$
OR1	75	573.1 ± 62.2	64	645.3 ± 68.4	71	1052.0 ± 149.1	59	1295.4 ± 208.1	30	2020.0 ± 281.0	15	2337.3 ± 201.0
OR2	60	548.3 ± 75.1	81	608.6 ± 61.1	57	1044.0 ± 179.0	77	1251.4 ± 183.2	33	1955.5 ± 251.0	25	2226.9 ± 287.2
OR3	79	527.7 ± 58.3	61	624.4 ± 78.7	76	1054.6 ± 121.1	58	1364.8 ± 169.9	47	1996.4 ± 138.3	24	2335.8 ± 260.5

**Table 2: Number of laid eggs of Oravka chickens at NPPC – RIAP Nitra**

Traits	OR1	OR2	OR3
Number of days	260	260	260
Number of eggs	1948	2206	2106
Average laying (pcs)	146.08	157.60	161.98
Laying intensity (%)	60.00	60.90	62.62

of this hen’s breed was observed (Weis and Hrnčár, 2007). At present, there are 20 registered breeding farms of Oravka and 7 registered breeding farms of Bantam Oravka in Slovakia.

## GOOSE

Geese are one of the oldest species of domestic poultry. There are 181 recognized breeds of domestic goose in the world with 158 local populations distributed mainly in Europe and Asia (Mindek *et al.*, 2014). Native goose breeds have better adaptability to extensive management, better disease resistance, higher reproduction rates and better meat quality, which are based on the natural gene pool and good original material of crossbreed predominance and high performance. Such excellent native breeds may contain the gene and alleles permanent to the adaptation to particular environments and local breeding goals and needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and a source of research materials (Hrnčár *et al.*, 2012, Romanov *et al.*, 1996).

## SLOVAK GOOSE

Slovak white goose is also known as the Slovak goose of the Danube area. It belongs to moderate breeds of a solid constitution. It is characterized by a compact, slightly sloping body holding and a noble look.

The Suchovska and Slovak goose were categorized as endangered breeds (Weis *et al.*, 2010).

## Development

Slovak goose was established in 1940s on the basis of regional breeds from South-Eastern part of Slovakia (from Nitra and Levice areas). The aim of breeding was to create a medium weight triple purpose (meat, liver, feather) goose suitable for corn areas, a strong resistant goose with a good pasturing ability and with preserved clucking instinct. The interest in this type breed was renewed upon the initiative of the Slovak Union of Breeders in the 1960s (Weis and Hrnčár, 2007). The first framework standard of the Slovak goose was published by Malík (1966). Literary sources do not indicate the year of recognition and initial number of birds because the Slovak goose is a native Slovak breed, it was not necessary to recognize it as a breed (Kadlečík *et al.*, 2004).

## Characteristics

Slovak goose is middle-heavy breed of goose noble appearance and firm constitution. It uses very good pasture and has preserved quiltiness. By the first year of breeding, male reaches the weight of 7 kg, the goose female after the end of the first fertilization cycle reaches weight of 6 kg. Laying is 12 and more eggs with white egg shell. Hatching eggs weight 140 g. Feather is pure white, of medium quality. The volume fraction of the punch exceeds the volume of the cover feather. It is hardy, good for grazing. It has a preserved quondary instinct that is itself hatch and inferred by the young.

**Table 3: Genetic diversity of the Slovak goose**

Group of breeds	Sample size	Mean no. of allele	Mean no. of effect. allele	No of private allele	Mean expect. heterozyg.	Mean observed heterozyg.	LD
Suchovska	32	4.00	2.01	9/7	0.38	0.33	7
Slovak	18	3.67	2.16	7/3	0.45	0.39	3
Total	50	5.17	2.09	16/10	0.43	0.56	6

It has a good disposition for cooking and creating delicious livers. The risk factor is a low number of controlled flocks and this creates more pressure on the breeders and judges at the specialized exhibitions and the recognition of breeding flocks. A high risk for the reproduction is a tendency of gander, male goose, to be monogamic and the mutual refusal, which exist in both sexes (Kadlečík *et al.*, 2004).

#### **Current status**

The lowest population of Slovak goose was in 2001 (34 birds: 10 males and 24 females), the highest in 2005 (83 birds: 24 males and 59 females). The effective population size of Slovak goose varied widely from 28.235 (in 2001) to 76.861 (in 2008) with an average of 56.261. At present there are 6 registered breeding farms of Slovak goose in Slovakia.

### **SUCHOVSKA GOOSE**

The goose is suitable for pasture and also for small farming, because of the preservation of the clucking instinct of the goose. The risk factor is that this goose is bred in a small breeding area.

#### **Development**

The Suchovska goose is a result of crossbreeding of local yellow fathering goose with French (Toulouse, Landes) and German (Pomorany, Steinbach) goose. This breed of goose originated at the end of the 1980's in the village of Suchá nad Parnou, and was recognized as a breed in 1995 with a number of 45 birds: 21 males and 24 females (Kadlečík *et al.*, 2004, Hrnčár *et al.*, 2008). It was created seeking to breed goose of bigger body frame, firm constitution and of compact and solid body.

#### **Characteristics**

The Suchovska goose has bigger and hard body frame. The body is long, compact, muscled, firm, almost horizontally held, body width is equal to its height. The neck is medium long and coarser. Breasts are full, broad, well rounded. Body is deeper and a double-lobed. The goose is saddling and pasture type. Body weight of males is between 6.5 and 7.5 kg, body weight of females is between 5.5 and 6.5 kg, egg laying ranges from 14 to 16 egg. Color of egg shell is white. Hatching eggs weight 140 g. Feather is smoother, with more dust. Color is yellow-wild. Female is similarly colored as a male with a larger amount of gray admixture, overall appears darker.

The characterization of the genetic diversity and structure of both native Slovak goose breeds was based on six microsatellite loci analysis in a total population

of 50 birds (Mindek *et al.*, 2014). The results are given in Table 3.

#### **Current status**

The lowest population of Suchovska goose occurred in 2003 (67 birds: 26 males and 41 females) and the highest in 2005 (143 birds: 43 males and 100 females). Minimal detected effective population size of the Suchovska goose was 57.778 (year 2001), the maximum being 125.333 (year 2006) with an average of the 96.717 (Weis *et al.*, 2010). At present there are 7 registered breeding farms of Suchovska goose in Slovakia.

### **CONCLUSION**

The Oravka and Bantam Oravka chickens, Slovak and Suchovska goose are among the native Slovak breeds, reared especially under conditions that are natural for the species. They have good welfare, the possibility for pasture, moreover, goose also has access to water. Animals are fed healthily, they have food without the addition of chemical preparations, medicines and antibiotics. The products of such animal (meat and eggs) are healthy. At present, with the growing demand for poultry products from extensive systems, it is important to raise native chicken breeds suitable for free-range and organic farming because of their good adaptation to the local conditions. This is confirmed by the experience of many countries, where native breeds of slow-growing chickens provide good-quality meat. Native poultry breeds play an important social role among farmers and have a positive impact on maintaining rural society and traditional form of agriculture as well as gratify local traditions. They represent a gene source for future breeding strategies and research. An alternative native breeds in poultry production system have an important advantage of biodiversity and sustainable agricultural production.

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## HONEY BEES IN SLOVAKIA: A REVIEW

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### ABSTRACT

Beekeeping in the territory of Slovakia has a long tradition beginning in the late 18<sup>th</sup> century. Nowadays, six honey bee breeding stations, 58 reproduction farms and more than 15,000 registered beekeepers represent the apiculture sector in Slovakia. The population of indigenous Carniolan bee (*A. m. carnica*), that is the most common honey bee race in Slovakia is endangered due to crossbreeding with imported bees of unknown origin. The aim of our study was to characterize Carniolan honey bee population using molecular markers. A total of 79 Slovak Carniolan workers collected from breeding and reproduction farms in Slovakia were analysed and compared with 85 reference bees of *A. mellifera mellifera*, *A. mellifera macedonica*, *A. mellifera ligustica* and *Buckfast* using 10 microsatellite markers. Bayesian and frequency-based methods have been used to successfully assign 95 % of individuals to tested populations. Genetic structure analysis using the Bayesian clustering method incorporated in STRUCTURE package showed two distinct clusters in the Slovak population of *A. m. carnica* and its reasonable admixture with *A. m. macedonica* and *Buckfast* species. The tRNA<sup>leu</sup>-cox2 intergenic region of mitochondrial DNA was sequenced in 66 Carniolan workers. We found that 71.2 % of samples correspond to 5 known haplotypes belonging to C lineage (C2c, C1a, C2d, C2e and C2z) and 28.8 % of samples have novel mutations in the sequenced region of mtDNA.

**Key words:** Carniolan honey bee; genetic diversity; individual-population assignment; molecular markers; mtDNA

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### INTRODUCTION

Beekeeping is an ancient tradition and honey bees have been kept in Europe for several millennia. Bees are critically important for the environment, sustaining biodiversity by providing essential pollination for a wide range of crops and wild plants. The majority of crops grown in the European Union depend on insect pollination, so the apiculture sector is an important part of the EU agriculture. Indeed, insect pollination, which is currently under threat in Europe, has an estimated economic value of € 22 billion per year in the EU alone (Nieto *et al.*, 2014). Bees contribute to human benefits also directly through the production of honey and other apiculture products such as pollen, wax for food processing, propolis in food technology, and royal jelly used as a dietary supplement and as an ingredient to food. There are around 630,000 beekeepers and 16 millions of hives

in the EU, producing 268,000 tons of honey in 2015 ([https://ec.europa.eu/agriculture/honey\\_en](https://ec.europa.eu/agriculture/honey_en)). In recent years beekeepers have been reporting unusual weakening of bee numbers and colony losses, particularly in the USA and Western European countries (van Engelsdorp and Meixner, 2010). Since no single cause of declining bee colonies has been identified, several possible contributing factors have been suggested, acting in combination or separately. These include the effects of intensive agriculture and pesticide use, starvation and poor bee nutrition, viruses, environmental changes and attacks by pathogens or invasive species. The European Commission has taken important steps to diagnose and prevent bees' mortality, including actions on certain pesticides and a comprehensive study on honey bee colony mortality. There are many international research projects funded by the EU, like SMARTBEES, SWARMONITOR, SUPER-B, BEE DOC, COLOSS, EPILOBEE, etc.,

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as well as a strong support of beekeeping through national apiculture programs which aim at improving the general conditions for the production and marketing of honey and other apiculture products in the EU. In view of the important ecological and economic value of bees, there is a need to monitor and maintain healthy bee stocks, not just nationally but globally.

## BEEKEEPING IN SLOVAKIA

In the territory of Slovakia, rational beekeeping started in the late 18<sup>th</sup> century, while new hive designs and movable frames were adopted in the second half of the 19<sup>th</sup> century. The first Union of Slovak beekeepers was founded in 1869, but regional organizations were active much earlier (Chlebo, 2010). The current structure of apiculture was formed 40 years ago by establishing 6 certified breeding stations and 58 reproduction stations (apiaries). Every breeding station keeps one of the main official lineages of the Slovak Carniolan honeybee (sometimes recognized as Carpathian Carnica sub-population). The main Slovakian lineages of the Carniolan bee include Košičanka, Carnica Sokol, Vojničanka, Tatranka, Sitnianska and Mošovčanka. In 2016 around 278,000 honey bee colonies and more than 15,500 beekeepers were registered in Central Registry of Beehives.

The Carniolan bee (*A. m. carnica*) is the most common and the only legal honey bee breed in Slovakia, originating from the mountainous north-western region of Slovenia (Kransko). Carnica's nearest relatives are the Italian bee (*A. mellifera ligustica*) and Macedonian bee (*A. mellifera macedonica*). These three breeds belong to C haplogroup (Ruttner, 1988). Carniolan bees are darker and larger than Italian bees. The characteristic yellow rings of *A. m. ligustica* being replaced by dark bands in *A. m. carnica*. Macedonian bee is also a dark one but with more yellow coloration at tergites and scutellum. The characteristic brood rhythm of *A. m. carnica* is a rapid build-up in spring, followed by a slow decline and an early cessation of brood rearing in the autumn (Ruttner, 1988; Milner, 2011). Carniolan bees are able to survive colder winters with a small winter cluster, they are very well adapted to the geographic and climatic conditions in the Central Europe and show high endurance, resistance to brood diseases and high honey yield (Chlebo and Kopernický, 2004).

The National Program of Stabilization and Development of Slovak Apiculture for 2010–2013 and other legislative documents justify the importance of beekeeping in Slovakia. The National Agricultural and Food Centre – Research Institute for Animal Production Nitra (NPPC – RIAP) is authorized as bee breeding

station and it manages the Central Registry of Hives in Slovakia. In addition, this institute has been approved as an artificial insemination service for all six breeding stations and offers morphometric control of breed purity and breed assignment. NPPC–RIAP has been involved in the COLOSS project aimed at monitoring of bee colony losses in the EU.

## ANALYSIS OF BEE POPULATION USING MICROSATELLITES

Carniolan honeybee (*Apis mellifera carnica*) is considered an endangered subspecies of *Apis mellifera* in Slovakia due to its illegal crossbreeding with allochthonous subspecies. Morphometric studies have provided a large amount of information on the structure of *Apis mellifera* species, and morphometric parameters are still frequently used to determine the origin of bees. Measurements of morphometric characters are commonly taken according to Ruttner (1988), but this method cannot provide detailed information about the genetic structure of the population. Microsatellites are useful for testing of genetic variability on the level of subspecies and populations. During the last two decades, microsatellites have been used to analyze genetic diversity of honey bee populations in many countries (Garnery *et al.*, 1998; Dall'Olio *et al.*, 2007; Uzunov *et al.*, 2014; Francis *et al.*, 2014; Nedec *et al.*, 2014). Despite more than a century-long tradition of beekeeping in Slovakia, genetic research of Slovak honeybees has not yet been done. Recently we started activities to adopt a molecular methods for characterization of Carniolan honeybee population in Slovakia using polymorphic microsatellite markers (Gasper *et al.*, 2013; Paál *et al.*, 2013; Šťastný *et al.*, 2014). A total of 79 samples of Slovak Carniolan honeybee from 19 regions of Slovakia, mostly collected at breeding and reproduction farms, were analyzed together with 85 reference samples of *Apis mellifera mellifera*, *Apis mellifera macedonica*, *Apis mellifera ligustica* and *Apis mellifera hybrid (Buckfast)*. Ten microsatellite markers showing polymorphic information content higher than 0.5 were used for molecular analysis of bees. Bayesian and frequency-based methods implemented in a GeneClass software have been used to successfully assign 95 % of individuals to tested populations. After simulating 100,000 individuals using Bayesian Markov chain Monte-Carlo resampling, we assigned 87 % individuals to correct population. Population genetic structure was tested by the Bayesian clustering method incorporated in a Structure software (v 2.3.4). Overall, 92 % of the individuals were assigned to originally declared subspecies. According to Structure analysis, Slovak population of *A. m. carnica* seems to be

separated into two distinct clusters, showing the relatively high level of gene flow from *A. m. hybrid* (*Buckfast*) and *A. m. macedonica* subspecies. It might be caused by rebuilding the Slovak bee population after colony losses using queens of non-Carniolan origin.

## ANALYSIS OF BEE POPULATION USING MITOCHONDRIAL DNA

Mitochondrial DNA is a small (about 16,000 bp), circular molecule that is transmitted intact by the queen to her offspring (workers and drones). The non-recombining maternal inheritance renders interpretation of mtDNA data straightforward, which in combination with relatively simple and inexpensive assays has made mtDNA one of the most popular markers in honey bee genetic studies. Based on morphological data 29 honeybee subspecies have been recognised (Engel, 1999; Sheppard and Meixner, 2003) and grouped in four morphological branches - C, M, A and O. The C lineage comprises seven subspecies: *A. m. carnica*, *A. m. ligustica*, *A. m. macedonica*, *A. m. siciliana*, *A. m. cecropia*, *A. m. cypria* and *A. m. adami*. Upon the inclusion of mitochondrial molecular data, the O lineage has recently been identified as a sub-lineage of the African lineage A and been renamed to Z (Alburaki *et al.*, 2011). Finally, yet another mitochondrial lineage, named Y, has been identified in north-eastern Africa (Franck *et al.*, 2001), which also belongs to the context of the African lineages (Meixner *et al.*, 2013).

PCR-RFLP method based on amplification of tRNA<sup>leu</sup>-cox2 region of mtDNA, followed by *Dra*I digestion, known as *Dra*I test (Garnery *et al.*, 1993) has been widely used in honey bee maternal identification, phylogeographical studies, understanding the complexities underlying natural hybrid zones, detecting introgression of foreign queens, among others (Meixner *et al.*, 2013). The combination of length and restriction site polymorphisms produced by the *Dra*I test has resolved over 100 haplotypes, nevertheless this method is unable to identify honey bees at the subspecies level. Therefore sequencing is the ultimate method for assessing mtDNA variation. In our study started in 2015, the tRNA<sup>leu</sup>-cox2 intergenic region of about 570 bp was sequenced in 66 *A. m. carnica* workers, collected from 6 breeding farms and 21 reproduction farms in Slovakia. The sequence data were compared with complete mtDNA sequence of *A. m. ligustica* (L06178.1; Crozier and Crozier, 1993), as well as deposited sequences in GenBank using BLAST. We found that 71.2 % of samples correspond to 5 known haplotypes belonging to C lineage (C2c, C1a, C2d, C2e and C2z) and 28.8 % of samples have novel mutations in the sequenced region of mtDNA. The most frequent

haplotype C2c in Slovak bee population (36.4 %) is also typical and dominant for Slovenian *A. m. carnica* population (Sušnik *et al.*, 2004). Haplotype C2d, detected in 6.1 % of samples from Slovakia has been observed in Croatia, Canary Islands and in *A. m. macedonica* (Munoz *et al.*, 2009). Our group found this haplotype in several *Buckfast* bees from Slovakia and Denmark (unpublished data). Haplotypes C2e (4.5 %) and C2z (1.5 %) were detected in bees from Romania and deposited to GenBank (accession no. JQ977702.1, JQ754648.1). Interestingly, haplotype C1a, typical for *A. m. ligustica* was observed in 22.7 % bees from Slovakia. Čapek (2015) reported even higher occurrence of C1a haplotype (34.4 %) in 32 Slovak Carniolan honey bee samples. Six novel haplotypes are derived from C2c (11 bees, 16.6 %), C2v (4 bees, 6.1 %) and C2y (4 bees, 6.1 %).

## CONCLUSION

To efficiently preserve Carniolan honey bee as a genetic resource in Slovakia it is necessary to know genetic characteristics of the population. Molecular markers like microsatellites and SNP in mitochondrial DNA could be useful for honey bee genetic studies. From our data we can conclude that honey bee population in Slovakia comprises predominantly Carniolan race (*A. m. carnica*). However, an apparent admixture of *A. m. ligustica*, *A. m. macedonica* and *Buckfast* bees has been observed. Therefore, simple and inexpensive genetic methods should be adopted and combined with standard morphometric tools to perform reliable bee race assignment, especially in case of breeding stations.

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## MANAGEMENT OF ANIMAL GENETIC RESOURCES IN SERBIA - CURRENT STATUS AND PERSPECTIVE: A REVIEW

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### ABSTRACT

Serbian agro biodiversity conservation program was prospering in last 15 years in great extent. From the situation when majority of locally adapted breeds and strains for domestic animals were on the edge of extinction, number of all animals was drastically increased. After Ministry of Agriculture, Forestry and Water Management introduced regular compensation measure for farmers growing endangered old breeds, increase of the number of farmers involved and number of animals conserved was fast. The following rehabilitation of some artisan food productions and introduction of few novel value chains as a fundament of the new conservation approach, animal genetic resources in Serbia got a new role regarding satisfying demand of population for high quality food (production in marginal areas, organic production) landscape conservation and integrated development of rural areas. The paper presents the status of animal genetic resources and their production systems.

**Key words:** animal; genetic; breeds; conservation

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### INTRODUCTION

The Republic of Serbia is located in the South-Eastern part of Europe, landlocked in Balkan Peninsula. It occupies an area of 88,509 km<sup>2</sup>. The Northern part of the Republic occupies the plains (Pannonia Basin) and in the Southern parts are hills and mountains. According to the most recent census, the Republic of Serbia had population of 7,186,862 inhabitants. The average population density is 93 inhabitants per km<sup>2</sup>. According to a separate census of Kosovo and Metohia in the 2011 year 1,733,872 residents lived there.

The Republic of Serbia has a total of 631,552 agriculture households and 3,437,423 ha of agricultural land, out of which 73.1 % are arable land and gardens, 20.7 % meadows and pastures, 4.8 % orchards, 0.6 % vineyards, 0.7 % crofts and 0.1 % are other permanent crops.

### THE STATE OF PRODUCTION SYSTEMS

In the Republic of Serbia, in all the branches of animal production, there are two basic types of production systems: (1) Highly specialized high input production systems and (2) Extensive low input production systems based on the combined crop/animal or mixed production systems.

Livestock production is present in all regions, however locally adapted breeds last refuges are mainly marginal mountain, partly wetland areas in lowlands with predominant extensive low input production systems. Trend of abandonment of marginal mountain areas, and concentration of livestock husbandry in intensively cultivated lowlands, have serious consequences on survival of locally adapted breeds as much as survival of traditional farming practices. Majority of locally adapted breeds in these areas are attached to semi-natural

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grasslands, which used to make essence of traditional pastoral systems with characteristics of High Nature Value Farming (HNVF). This farming was basis for production of high quality typical products from locally adapted breeds and maintaining high biodiversity value of grasslands, however not properly positioned on the market to be valorized adequately to justify lower productivity (Cooper *et al.*, 2010).

The most significant livestock products in Serbia are: milk and dairy products of cows, sheep, goat and buffalo milk, pork, beef and beef products, poultry meat, eggs, lamb, manure, skin, giblets, manufactured products and wool. Interesting for conservation of animal genetic resources (AnGR) are typical artisan products such as hard sheep and sheep/cow milk yellow cheese, curd, peppers in sour cream, kaymak cream, diverse kinds of smoked meat, sausages and bacon etc. as much as more exotic row donkey milk and cheese which are part of precious value chains mostly related to traditional pastoral systems.

## THE MONITORING OF ANIMAL GENETIC RESOURCES

The Republic of Serbia does not have national livestock information system and therefore monitoring of AnGR is still inconsistent, incomplete and superficial. Basically, flow of information regarding AnGR is carried out through network of the national agricultural service and university centers, yet more information is flowing in to the system *ad-hoc*, thanks to annual call for subsidizing animals of locally adapted breeds. Updating of data bases is carried out on the annual basis. Data on the number of animals and production parameters of major breeding organizations are submitted to the Ministry of Agriculture, Forestry and Water Management (MAFWM), and forwarded to European Farm Animal Biodiversity Information System (EFABIS)/Domestic Animal Diversity-Information System (DAD-IS). Updating of the Global database for AnGR is conducted based on Agreement which was signed on 2013 with European Regional Focal Point (ERFP). Herd books for all breeds of economic interest as well as breeding programs exist. For locally adapted breeds there are no breeding programs.

## THE CURRENT STATUS OF ANIMAL GENETIC RESOURCES

Activities related to management of AnGR are organised and financed by the state, through MAFWM. Relations between government institutions, various non-

governmental organisations, breeders, local communities and stakeholders are therefore good (Stojanović, 2003).

Locally adapted breeds which are endangered, due to the fact that they have no significant contribution to neither nutrition nor agriculture, and therefore under conservation in Serbia are:

Big ruminants: Podolian cattle (225 animals), Busha cattle (776) and Domestic Buffalo (598), primitive breeds which had combined purpose (milk–meat–work) are not profitable due to low productivity and lost the role of working animals.

Horse: Domestic-mountain Pony (113) and *Nonius* horse (106) their basic role of working animals in majority of regions ceased to exist. However, in some hilly-mountainous regions, where horse power is still needed in forestry, attempt to preserve Domestic-mountain Pony with the support of the State.

Donkey: Domestic donkey (383) In many regions, practical application of pulling role of donkey disappeared, which led to a decrease of interest for breeding. New possibility appeared with reintroduction of the idea to use donkey milk as auxiliary medicinal product. In Special Nature Reserve „Zasavica“ even some new products were introduced (sausages, cheese, cosmetics-face creams, soaps, liquor, etc.) to provide economic viability to conservation of this breed.

Pig: Mangalitsa pig (753), Morava pig (58) and Resava pig (13). These fatty breeds have lost the battle with more productive hybrids. Long-term promotion of Mangalitsa pig and its products have led to a growing interest in this breed. Some products have been found their path even to big supermarket chains after 15 years of being on the edge of extinction.

Sheep: Vlashko-vitoroga sheep (1243), Lipa sheep (991), Bardoka (130), Pirot sheep (178), Krivi vir sheep (733), Karakachan sheep (234) and Chokan Tsigai (503). Besides partially Bardoka, all mentioned breeds and strains lost importance with the introduction of more productive breeds, but even more due to the depopulation of marginal rural regions where sheep were raised in extensive production systems on mountain pastures.

Goat: Domestic Balkan goat (582). Under the influence of extension services (also media), Alpine and Saanen breeds were promoted, so Domestic Balkan goat vanished from almost every area where better conditions for housing and nutrition could be provided.

Poultry: Naked Neck (1938), Sombor Crested (478), Kosovo Singer, Svrljig hen (200), Domestic geese, Domestic turkey. These breeds are present in extremely small number hence their contribution for livestock production and food is not significant. In general, there is no economic interest for production of any of mentioned breeds; therefore they are raised

purely for hobby and cultural reasons (Stojanović and Đorđević-Milošević, 2003; Stojanović, 2009).

## PROGRAMME AND CONSERVATION STRATEGY

In the proposed „Strategy for the development of agriculture and rural areas of the Republic of Serbia for the period of 2014-2024“ in the part referring to the “Environment and Natural Resources”, a series of measures specific to AnGR was designed, with aim of:

- Stabilizing & increasing size of the population & prevention of genetic diversity loss;
- Keeping records of AnGR;
- Development of protocols for monitoring of movement of AnGR and risk;
- Characterization, inventory and monitoring of trends and risks and setting standards for phenotypic and molecular characterization of AnGR;
- Definition of breeding objectives and development of breeding programs for AnGR;
- AnGR sustainable use in traditional agro-ecological systems, fair sharing of benefits;
- Assessing the value and importance of AnGR and production systems;
- Market development of traditional products from AnGR and increase of the participation of public in conservation of AnGR;
- Strengthening the national capacity for sustainable management of AnGR;
- Establishing a national policy for conservation and use of AnGR;
- Establishing and/or strengthening of the capacity for *in situ* and *ex situ* conservation;
- Establishing and strengthening of education and research capacity for AnGR;
- Strengthening international cooperation on the exchange of information about AnGR;
- Provision of funding of AnGR through existing and additional funds.

## ALTERNATIVE APPROACHES FOR THE MANAGEMENT OF AnGR

The significance of AnGR within the frames of the new policy of sustainable development in Serbia is considered from the aspect of multifunctionality of agriculture. Among the most important aspects identified so far are some possibilities, which may emerge from the comprehensive utilization of AnGR with the aim of valorization of different food resources, then those related to the food safety, diversification of rural economy and development of non-agricultural activities, and finally the possibilities regarding maintaining of regional diversity

and protection of the environment (Đorđević-Milošević and Stojanović, 2005).

### 1. Production systems based on AnGR in the regions with natural constraints

Mountain marginal rural areas in the Republic of Serbia were abandoned due to the poor soil or rough climate, which was limiting intensification. Locally adapted breeds in these areas are the only option for revitalization of production of typical products required for tourism development. The production of high quality food with ecological labels and geographic indications is possible, however it requires careful modeling. Introduction of contemporary food safety and quality standards require further work for identifying gaps and introduce derogations which are necessary to save artisan production. In some of these areas, locally adapted breeds as much as artisan products are completely lost and need to be reintroduced.

### 2. Conservation of AnGR to support biodiversity conservation strategies

Modern livestock production over the last decades of the twentieth century caused extinction of number of locally adapted breeds and strains of domestic animals. After the failure of intensification in some areas, livestock production was neglected, leaving waste areas of grasslands, undermanaged or abandoned. Neglecting of livestock production in some regions led to dissipation of (natural) biodiversity. In depopulated mountain regions, for example, grassland communities which were invaded by shrubs in absence of grazing halved the number of plant species, while lowland grasslands as a consequence of ceasing of grazing have lost some small mammals and birds of prey feeding on them.

### 3. Conservation of AnGR and protection of rural regions and landscape diversity

Traditional farming in the last century maintained tens of types of rural landscapes considered nowadays valuable from ecological, social and economic standpoint. After rural environment become unattractive space for living in last few decades, outmigration have left unmanaged space and number of landscapes was degraded. However, this space is again of public interest for getting closer to the nature and awareness of need for its conservation, has triggered initiatives for conservation of locally adapted animals and their integration into profitable production systems.

### 4. Improvement of forests and water management in light of conservation of AnGR

The most sensitive relations between agriculture and the nature, environmentalists see with point source pollution related to intensive livestock farming. Pollution

with nitrates and phosphates, eutrophication, emergence of causative agents of some fifty zoonosis in water. Locally adapted breeds might contribute to mitigation of risks from pollution from agriculture in numerous ways. One of them is certainly their genetic potential to resist disease and better utilize the available natural feeds which makes them suitable for organic production.

### 5. Cultural heritage and conservation of AnGR

Traditional combined farm systems and HNMF in addition to typical artisan products are part of the cultural heritage. This includes conservation of traditional knowledge for processing, but also introduction of tourism. Loss of adequate raw material base originating from the locally adapted breeds of domestic animals is inadmissible. So for example, original Pirot sheep katchkaval cheese, or Pirot rug, is impossible to conserve if Pirot sheep isn't saved from extinction. Since typical artisan products of the kind have profitable market, income can support conservation of AnGR, and vice versa AnGR can support rural economy diversification.

### NATIONAL PRIORITIES FOR CONSERVATION AND UTILIZATION OF AnGR

The main priorities are:

1. Characterization of locally adapted breeds, as well as their potentials in the sense of achieving economic justification of conservation of *on farm* conservation systems.
2. Clear definition of the role, utilization and conservation of AnGR, that is, precise definition of current and future needs of the nation in livestock production.
3. Enhancement of capacities of communication and information systems.
4. Establishment of permanent programs for monitoring farm AnGR, which have agricultural, economic, cultural or scientific value.
5. Training of personnel, especially s about the new technologies applied in conservation.
6. Development of permanent and functional relationship with the public (media, publications, public affirmation of scientific and professional papers etc.)
7. Development of legislation and regulations.
8. The increase of the level of international communication.

### ANIMAL GENETIC RESOURCES CONSERVATION DEVELOPMENT IN SERBIA

Agro biodiversity conservation program was prospering in last 15 years in great extent. From the situation when majority of locally adapted breeds and

strains for domestic animals were on the edge of extinction, number of all animals was drastically increased. After MAFWM introduced regular compensation measure for farmers growing endangered old breeds, increase of the number of farmers involved and number of animals conserved was fast.

Further development was leading to strengthening capacities for introduction of new measures within innovative agrarian policy, which was provided through few projects implement with external financial support. International assistance came as a natural continuation of local efforts, yet limited national absorption capacities were obvious during implementation of all projects and some of established goals were never reached. At the same time additional effort was made by farmers and non-governmental organizations themselves to rehabilitate traditional artisan production and provide adding value to their animal products to increase economic sustainability of the conservation programs. These were promoted on the market with lots of enthusiasm and assistance of enlightened individuals in media. Clever idea led to first positive results - increase of consumer interest and increase of breeds population.

Due to intensive migration of Serbian population, locally adapted breeds surviving in economically and environmentally justified traditional low input farming systems are losing ground. Entirely new deals are needed to keep these animals alive, such as introduction of novel models of managing nature protected areas and using of ecological services.

Economic crisis which caused decrease of purchase power of customers prevented market to grow significantly and provoke relevant increase of the number of autochthonic animals and breeders growing locally adapted breeds. Every conservation program for endangered locally adapted animal breeds should have the component which includes education of consumers about the quality of food, healthy diets and characteristics of traditional and sustainable farming practices. Development of curricula for elementary and high school covering these issue is an ideal opportunity to start with education early enough to provide space for old breeds in the future. If consumer taste is not cultivated, the opportunity to raise his awareness about importance of locally adapted breeds is a mission impossible. Consumers have had for a few decades already very few opportunities to get familiar with food deriving from locally adapted breeds. His emotions about these animals derive more from moral and cultural teasers than scientifically proven, exact data. Knowledge introduced through advertisements is less suitable for raising awareness of population and their feelings for conservation programs.

The opportunity which was seen with protected origin for typical artisan products deriving from these products is missed in Serbia, since majority of Serbian typical artisan products were not protected in a way to include origin from defined locally adapted animal breed or a strain. Indigenous property rights for mountain or other rural communities need to be appreciated and protected also to regulate this issue. The conservation of AnGR is their chance to prosper without jeopardizing the rest of their natural and cultural heritage and right to be different. Indigenous rights are also creating emotions of consumers on wider market, which are going in favor of conservation of locally adapted breeds.

Locally adapted breeds of ruminants in Serbia are well adapted to conditions of natural and semi-natural grasslands in marginal lowland and mountain areas. Diverse breeds and strains of sheep, cattle, buffalo, goats and horses and donkeys are suitable for using grasslands, which are in these areas not possible to convert in to arable land, due to its quality and shallow profile. Rehabilitation of the line of traditional HNPF practices which include extensive grazing animals important from the aspect of conservation of AnGR, can bring multiple benefits to the local rural economy, as well as to the landscape and biodiversity conservation of wild species. This HNPF should be supported within agro environmental schemes of the axis 2 type.

AnGR in Serbia are highly vulnerable, no matter of the present status of every individual breed. This primarily because their conservation is organized *in situ*, so there is no back-up in gene banks or any other *ex situ* system if collection is exposed to disease or catastrophe of a different kind. The establishment and inclusion of the gene bank in existing and new programs for the preservation of locally adapted races is of great importance for the sustainability of total genetic resources.

For Serbian agrarian policy agro-environmental issues remain marginal. Conservation of animal genetic resources requires wide community action and should overcome narrow frames of agriculture policy and practice.

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#### TETRASPANINS EXPRESSION ON BOVINE AND PORCINE OOCYTES

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Tetraspanins are multifunctional molecules located in specific microdomains on the plasma membrane. Thanks to their ability to enter into molecular partnerships with other members of tetraspanin family or other proteins, they can form tetraspanin web and thereby affect many cellular functions. Well-described involvement of tetraspanins in somatic cell immune response, cell migration, viral infections, metastasis formation etc., definitely suggests their participation in similar processes occurring during gamete interactions. Up to this date, although an extensive study proved the fact that tetraspanins CD9 and CD81 are directly involved in gamete interaction of mammals, their precise role in the fertilization process is not clear yet. In the present study, we compared the localisation of these two tetraspanins on bovine and porcine oocytes at different stages of development. We investigated a possible role of CD9 and CD81 molecules in the fertilization process of cattle using polyclonal antibodies to CD9 and CD81 in *in vitro* fertilization assay. In our experiment, no significant reduction in the number of fertilized eggs and cleavage of zygotes after pre-treatment of oocytes with CD9 and CD81 antibodies was observed. The obtained results provide additional data on comparison of fertilization process in cattle and pigs.

**Key words:** CD9; CD81; pig; cattle; fertilization

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#### THE EFFECT OF THIACTOPRID ON PREIMPLANTATION EMBRYO DEVELOPMENT IN MOUSE AND RABBIT

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The aim of this *in vitro* study was to evaluate the potential toxicity of different doses of active neonicotinoid compound thiacloprid on developmental capacities and qualitative parameters of preimplantation embryos in mice and rabbits. Embryos isolated from superovulated dams at the 2-cell stage of development in mouse / the pronuclear stage of development

in rabbit were cultured in media supplemented with different concentrations of the insecticide until blastocysts formation. Stereomicroscopic examination of *in vitro* developed mouse embryos showed that thiacloprid, given at the highest concentration (100 µM), negatively affected development of 2-cell stage embryos and quality of obtained blastocysts, as shown by significantly decreased average cell number and increased incidence of dead cells in the pesticide-exposed mice embryos (assessed by fluorescence microscopy). Moreover, thiacloprid influenced qualitative parameters of blastocysts also at 10 µM concentration. Lower doses of tested substance had no detrimental effect on mice embryos. The presence of thiacloprid at concentration 100 µM had negative effect also on rabbit embryonic development. Thus, in the blastocysts derived from rabbit eggs, significantly decreased cell numbers and increased percentages of dead cells were recorded. Thiacloprid at 10 µM slightly increased incidence of dead cells, but it did not affect average cell numbers in the pesticide-exposed rabbit embryos. The results of the present study indicate that thiacloprid can influence development and quality of mouse and rabbit preimplantation embryos. The serious toxic effect of this neonicotinoid was observed in a dose-related manner, with significant impact at a concentration of 10 µM and above. Sensitivity of embryonic cells to thiacloprid in two evaluated species (mouse and rabbit) was similar. The neonicotinoid-induced changes during preimplantation period of development have to be considered a potential risk factor in mammalian reproduction.

**Key words:** thiacloprid; preimplantation embryo; toxicity

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#### SAFEGUARDING DIVERSITY – THE AUSTRIAN GENE BANK FOR FARM ANIMALS 1997 TO 2017

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The Austrian Genebank for Farm Animals was founded in 1997 at the former Institute of Organic Farming, now Institute of Organic Farming and Biodiversity of Farm Animals. It belongs to the Austrian Government and is funded by the research budget of the AREC Raumberg-Gumpenstein. The Genebank is a registered AI centre for cattle, goats and sheep. Originally planned as support for *in situ* conservation programs for highly endangered Austrian breeds it has developed into a comprehensive archive of Austrian animal breeding. Today the Genebank consists of semen collections of rare cattle, goat, sheep, pig and horse breeds, backup collections of cattle and pig artificial insemination centres, a collection of commercial goat breeds and the Farm Animal DNA Bank with isolated genomic DNA. The collection of rare breeds is divided into a working collection supporting the ongoing *in situ* conservation programs for highly endangered Austrian breeds and a duplicate collection for safety reasons. All collections are continually replenished and completed. Currently an extension of the Farm Animal DNA Bank to supply genomic DNA of sufficient quality

for single nucleotide polymorphism genotyping is planned. Sires of rare breeds chosen for semen collection should be as little related as possible to each other and to the breeding population. Until now, relatedness was calculated according to pedigree data, now genomic analysis is employed increasingly. The main genomic material in store is semen. To be able to re-establish breeds in case of catastrophes, a supplemental storage of embryos, ova and tissue for cloning would be advisable. The oldest material in storage is about 50 years old. To ensure the continuing use of the Genebank, a national derogation for use of old genebank material not complying with modern regulations is in preparation.

**Key words:** Genebank; rare breeds; farm animals; semen

#### MANAGEMENT AND CONSERVATION OF ANGR IN SLOVENIA

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The most important milestone in the field of Animal Genetic Resources (AnGR) conservation in Slovenia was the ratification of the Convention on Biological Diversity (CBD) in 1996 and the preparation of the Biodiversity conservation strategy, where AnGR conservation was revealed. In 2002, the Livestock Breeding Act protected the Slovenian local breeds and established the institutional framework for their conservation. Management and conservation of AnGR is carried out by the Public service for AnGR conservation (Public service) authorized by the Ministry of Agriculture, Forestry and Food (Ministry). The role of the Public service is to carry out all the necessary measures and actions to protect the AnGR in Slovenia, such as preparation of the long-term and yearly National programmes for AnGR conservation, which form a part of the global efforts to conserve AnGR. In the past, many breeds have been lost, however today we managed to conserve all the local breeds which were almost lost 30 years ago. The Public service is actively working on awareness rising amongst wider public, schools and farmers; it establishes ark farms, centres and parks as well as maintains gene bank of cryopreserved material. In addition, different mechanisms to support and conserve local breeds are used in the country, such as incentives from Rural Development Plan and dedicated “*de minimis*” aids. Despite the efforts in the past 30 years, among the twelve local Slovenian breeds, seven breeds are critically endangered, three are endangered and one is vulnerable. The outline of the past and current conservation measures and activities on the AnGR clearly shows that there is still a considerable need for the careful planning of their management and conservation. Their conservation will contribute to the global and national food security and would better respond to the climate changes, different consumer demands and modern animal breeding needs.

**Key words:** animal genetic resources; local breeds; conservation; Slovenia; endangerment

#### NEED FOR A PROGRAMME FOR ANIMAL GENETIC RESOURCES

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Biodiversity is useful to humankind for economic, cultural and ecological purposes. Not only does it give us many primary materials and draws great benefit from it, it regulates the biosphere as well. Animal genetic diversity is a part of the earth's natural heritage, and humankind depends on animals for food, for clothing and for work. Animal genetic resources allow farmers to select stocks or develop new breeds in response to changes in the environment, threats of disease, new knowledge of human nutrition requirements, changing market conditions and social needs, all of which are largely unpredictable. What is predictable is the future human demand for food. Given the above facts, domestic animal diversity is critical for food security. It is important to stop and reverse the erosion of this diversity. For thousands of years, livestock have been intimately associated with human life. They are one of the special characteristics of human culture. Livestock are essential elements of the economy at local, national and international levels. Livestock management systems respond to economic conditions. Genetic variation is the raw material for animal improvement, its conservation provides options for uncertain and unknown future economic needs. Conserved genetic variation will offer the resources to respond quickly and economically to changes in the market. Breeds with unique traits are of great scientific interest in a number of ways. Some obvious examples are the unique DNA sequences of species, breeds, strains and populations, the specialised physiological and adaptive functions and the opportunity to study animals as biological models. Investigations at the molecular level enter a new era, when society will benefit from manipulation with biological material. Genetic variation will be of increasing interest and importance to science both at the animal and molecular or sub-molecular levels in the future. Domestic animals not only share DNA as the common basis of their genetic nature with all other species, but they are integrated also in many management systems with pasture and forest species and wild animals. The overall approach to conservation of biological diversity should ideally embrace all these species as well as the animal breeds, which provide diversity within the domestic animal species. A primary goal of the management of local, national and global animal genetic resources is to ensure the sustainable development of livestock in particular and agriculture in general.

**Key words:** biodiversity; animal genetic resources; livestock; molecular and economic needs; sustainability

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#### ALTERATIONS IN HAEMATOLOGICAL PARAMETERS IN DIABETIC RATS

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The aim of this study was to determine the effect of chronic high-energy diet feeding on development of *diabetes mellitus* complications in ZDF rats focusing on haematological parameters. Male Zucker diabetic fatty (ZDF) rats (a fatty *fa/fa* mutation (-/-); n = 20) and their lean controls (ZL, non-diabetic, +/- or +/+, not display expression of *fa* phenotype, n = 10) at the age of 3 months were involved in the experiment. The animals (Breeding Facility of the Institute of Experimental Pharmacology and Toxicology Dobra Voda, Slovak Republic, SK U 25016) were housed at number of two rats per plastic cage (800 m<sup>2</sup>) and under specific pathogen free conditions at 23 ± 2 °C and 55 ± 10 % relative humidity with a 12 h light-dark cycle. Rats were provided with water and diet on *ad libitum* base. Rats were divided into three experimental groups as follows: lean untreated rats (C) fed KKZ-P/M (10 MJ.kg<sup>-1</sup>), obese rats fed KKZ-P/M (10 MJ.kg<sup>-1</sup>, E1) and obese rats fed enriched high energy diet (E2, enriched KKZ-P/M, 20 MJ.kg<sup>-1</sup>). Enriched diet was served to induce *diabetes mellitus* symptoms earlier. At the end of the experiment (after 2 months), animals were anesthetized by intraperitoneal injection with chloral hydrate. Blood samples were collected into EDTA-treated tubes. In a whole blood, chosen haematological parameters [total white blood cell count (WBC), granulocyte count (GRA), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelet count (PLT), mean platelet volume (MPV)] were measured using Abacus junior VET haematology analyser (Diatron®, Vienna, Austria). One-way ANOVA test was performed to calculate basic statistical characteristics and to determine significant differences. GRA count increased significantly (P < 0.05) in E1 against the control and E2 group. The high-energy diet caused significant decrease (P < 0.05) in MCV in comparison with the control group. Significant increase (P < 0.05) in MPV was observed in E1 group against the control. The results of other haematological parameters were without significant differences (P > 0.05) among the groups of rats. The rise in the secondary symptoms of diabetes complications by high-energy diet was accompanied by disturbed haematology parameters, which is a potential marker of angiopathy. Extended research on diabetes is needed.

**Key words:** diabetes mellitus; Zucker diabetic fatty rats; high-energy diet; haematology

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#### Invited paper

#### PRESERVATION AND CONSERVATION OF SHEEP GENETIC RESOURCES IN NORTHERN SERBIA, VOJVODINA

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Nowadays, the largest number of sheep breeds in Vojvodina belong to breeds imported from abroad, primarily oriented to the production of meat. In the past decades, the number of autochthonous sheep breeds has been declining. The main factors affecting the number of autochthonous sheep breeds are low productivity and unprofitability of their production. The areas under the pastures in Vojvodina have been significantly reduced, which further complicates the breeding of endangered sheep breeds, whose nutrition is mainly based on grazing. An additional problem is their unplanned crossing with other breeds. Endangered autochthonous breeds of sheep in Vojvodina are Wallachian sheep and Choka Tsigai sheep. Currently, in the Republic of Serbia only *in situ* conservation is being performed, while *ex situ* or cryopreservation is not implemented. *In situ* conservation includes conservation or the breeding of live animals in production systems which have arisen or in which they are located. *In situ* conservation allows the preservation and maintenance of animal population in their natural environment, leaving the evolutionary processes to shape the genetic divergence and continue the adaptability process of the population, which we preserve. Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia decided to grant subsidies to breeders involved in the breeding of endangered sheep breeds. This decision had a positive effect, to a certain extent, on the number of animals, which has been stabilized. In the future, there is a need to find new ways to achieve a sufficient number of animals by introducing new conservation technologies and additional stimulating measures for breeders.

**Key words:** Autochthonous sheep breeds; Wallachian sheep; Choka Tsigai sheep; *in situ* conservation

#### OCCURRENCE OF CHOSEN TRACE METALS DEPENDS ON THE GENDER OF FRESHWATER FISH (CYPRINUS CARPIO)

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Nowadays a significant increase in heavy metal contamination in the environment has become a worldwide problem due to anthropogenic activity such as industry, agriculture and metal processing. Chronic exposure to heavy metals may result in various alterations of animal and human health, especially when

their dietary content exceeds the permissible levels. Heavy metal toxicity may result in neurological disorders, cancer, liver and kidney damage, as well as numerous other health complications, which have significantly increased the mortality rate over the past decades. Therefore, our study was aimed to compare the concentrations of chosen heavy metals in male and female carp blood and to detect the possible associations. Blood samples were taken from males ( $n = 18$ ) and females ( $n = 24$ ) of freshwater fish (*Cyprinus carpio*). The coagulated blood was centrifuged for 20 min. at 3000 rpm, the blood serum was collected and stored at  $-20\text{ }^{\circ}\text{C}$  until analyses. The concentrations of chosen heavy metals (Mo, Al, Ba, Li) were determined from blood serum by inductively-coupled plasma optical emission spectrometry (ICP-OES). Basic statistical analysis as well as analysis of variance (one-way ANOVA) following Tukey's HSD procedure and Pearson's correlations were performed using STATGRAPHICS Centurion. Our results showed significant negative correlation between Ba and Li ( $r = -0.588$ ;  $P < 0.05$ ) concentrations in male carp blood samples. Moreover, in the female blood samples significant correlation between Mo and Li content ( $r = -0.631$ ;  $P < 0.001$ ) was detected. However, no noticeable differences in concentrations of chosen heavy metals between genders were observed. Obtained data indicate possible correlations between selected heavy metals and their increased content in blood, which may have significant impact on physiological processes in animal organism. Therefore, it is highly recommended to monitor the content of environmental contaminants in organisms.

**Key words:** heavy metals; fish; gender; blood

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## SLOVAK BREEDS OF HORSES

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Horse breeding has undergone significant changes in recent years. From the basic element of agricultural production, horse breeding has become only an additional part of landscaping, forestry and usage of agricultural products. For these reasons, breeding of traditional breeds of horses belongs to a marginal area and is seriously endangered. In Slovakia, this group includes Norik of Muran, Hucul horse breeds and Slovak sport pony. Norik horse is the most typical representative of a horse western breed group. This breed has undergone long-term development due to external conditions and now it differs from eastern type of horses with a more robust body structure. Today the Norik breed is fixed and clearly defined. One of the important breeders of coldblood horses not only within Slovakia is the "Forests of the Slovak Republic", s.e. Horse breeding is realized since 1950 in Forest enterprise Revúca of Horse breeding resort Dobšiná. An incentive for its establishment was the need for horses for forestry.

The importance of coldblood horses will also increase during approaching timber from the protected areas and national parks. The Hucul horse is the typical representative of a mountain horse breed. The home of this horse is Hucul - a distinctive region in the Eastern Carpathians of Romania-Ukraine border. Skeletal remains and hoofs print of the right horseshoe in the underground area of about 700,000 to 245,000 years old allow us to mark a Hucul horse as the autochthonous breed of the Carpathians. The base of Hucul horse breeding in Slovakia has been built in 1922 in the National Stud Farm Topoľčianky. Hucul horse breeding in Europe is currently concentrated mainly in Romania, Poland, the Czech Republic, Slovakia and, to a lesser extent, in Hungary and Austria. There are 7 lines kept in the breeding and they are recognized as international lines of the Hucul horse: Gurgul, Goral, Oušor, Hroby, Prislop, Pietros, Polan. For the rescue and development of the Hucul horse, the HIF (Hucul International Federation) was established in 1994. The Slovak sport pony is a product of a systematic interbreeding crossing of mares of the noble warmblood breeds of horse breed in Slovakia. In particular, there are breeds of Arabian and Slovak warmblood with stallions of pony breeds: the Welsh pony, the German riding pony, and in the second phase of the English halfblood horses. The Slovak sport pony is a multipurpose utility, driving-riding type, with a predominance of riding type. It is designed for riding training for children and teenagers aged 8 to 16, sports, recreational riding and hiporehabilitation. Pony breeding in Slovakia has been carried out since 1980 and currently the breeding core consists of 55 breeding mares and 4 breeding stallions.

**Key words:** gene reserves; Hucul horse; Norik of Muran; Slovak sports pony

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## THE EFFECT OF CONSERVATION MEDIUM ON STALLION SPERMATOZOA MOTILITY

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Horse breeding in the last decades is mainly related to leisure and sporting activities. Horses are in this respect related to great international quantities. Breeders invest significant funds to obtain the most powerful individuals. Top individuals are used for long time in sport and at the same time, application of biotechnological methods is implemented in their reproduction. The objective of this study was to evaluate and compare qualitative differences in motility parameters of ejaculate with the addition of commercial preservation medium (at the ratio 1:3) and ejaculate diluted with physiological solution (NaCl 0.9 %, Braun, B. Braun Melsungen AG, Germany) at the ratio 1:3. Fresh semen was obtained from 12 breeding stallions at the National Stud Farm in Topoľčianky at age of 5–26 years composed of following breeds: Lipican, Arab

thoroughbred, Hucul, Selle française, Holsteiner, Shagya-arab. Semen was collected by lubricated pre-warmed artificial vagina at the start of the breeding season in February. CASA analysis (Sperm Vision; Minitube, Tiefenbach, Germany) was used for determination of spermatozoa motility. Analysis was performed at seven time periods of sampling, after the 0, 1, 2, 3, 6, 24 and 48 hours. The ejaculate diluted with physiological solution of selected stallions showed rapid reduction of spermatozoa activity in all measured parameters and viability 6 hours after the collection. Analysis of spermatozoa with an added preservation medium showed that motility achieved significantly higher values. From these results, it can be concluded that the addition of preservation medium helps to prolong the viability of stallion spermatozoa and increase the reproductive quality of breeding stallions.

**Key words:** stallion; spermatozoa; preservation medium; CASA

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## PRESERVATION OF ANIMAL GENETIC RESOURCES IN CROATIA

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Animal genetic resources are Croatian heritage with values visible on the economic, social, natural and cultural level. Local breeds include within genome numerous events that occurred in their environment, with or without human influence. They are a living monument to past times and they preserve the identity of the area. Their genes contain a possible potential for safe food production in future times when the importance of their resistance and adaptability will reach its full extent. Main reasons for the disappearance of a part of local breeds in Croatia are: globalisation, industrialisation and intensification of agricultural production, market liberalisation, import of "more productive" exotic breeds, depopulation and urbanisation of rural areas. Program for protection of autochthonous breeds in the Republic of Croatia started three decades ago. Protection is mainly performed through *in situ* models, in which local breeds present a part of the agro-ecosystem, economy and culture of the local community. Six years ago the Croatian government adopted "National programme for the protection of autochthonous breeds". This programme includes three cattle, nine sheep, three goat, three donkey, four horse, two pig and two poultry breeds. The Croatian "Gene bank" was established four years ago. Programs for preservation of Istrian and Slavonian Strymian Podolian cattle started twenty-five years ago, while Busa cattle is included in a protection programme since 2003. Sheep production in the Republic of Croatia is based on native and protected breeds and, therefore, in a better position in terms of sustainability. Croatian spotted and Croatian white goats are economically active, while Istrian

goat has been included in protection program since 2013. Local pig breeds (Black Slavonian and Turopolje pig) are protected primarily with *in situ* program as well as local horse and donkey breeds. Breeding structure ensures sustainability for the Lipican, Posavina and Croatian Coldblood horse, while the Murinsulaner horse is critically endangered breed. Population of the Littoral Dinaric donkey is the most numerous, while Istrian and North Adriatic donkey belong to the endangered group. Two local breeds of poultry are protected through *in situ* programs.

**Key words:** local breeds; protection programme; genetic resources; Croatia

## GENETIC SCREENING FOR THE MUTATION C.1332 C>T IN THE SLC37A2 GENE ASSOCIATED WITH CANINE CRANIOMANDIBULAR OSTEOPATHY

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Canine craniomandibular osteopathy (CMO) is a non-neoplastic and non-cancerous, proliferative disease in dogs causing extensive developmental changes in the bones of the jaw and skull. It manifests between 4 to 8 months of age with typical clinical signs including lack of appetite, pain, swelling of the jaw, periodical fever, difficulty opening the mouth and dysphagia (Hytönen *et al.*, 2016). CMO corresponds to an infantile cortical hyperostosis (Caffey disease) in humans. It is an autosomal incompletely dominant disease caused by the mutation c.1332 C>T in the SLC37A2 gene in terriers, particularly Scottish, West Highland White and Cairn terriers. SLC37A2 is a glucose-phosphate transporter in osteoclasts and the mutant variant (T allele) of SLC37A2 eliminates a potential binding site for the splicing factor ASF/SF-2. DNA from our canine DNA bank was used for the validation method. DNA was extracted from buccal swabs using Wizard Genomic DNA purification Kit (Promega). PCR reactions were performed in 10 µl volume consisting of 1 µl of extracted DNA (~40 ng), 0.5 mM of each primer, 1x Thermo-Start PCR Master Mix (Thermo Scientific) following PCR amplification protocol 15 min. at 95 °C, 30 s. at 95 °C, 30 s. at 55 °C and 1.30 min. at 72 °C for 35 cycles and final extension 10 min. at 72 °C. Primer sequences were designed according to the reference sequence JH373163.1 from NCBI. The PCR fragments were cycle sequenced using the BigDye Terminator Cycle sequencing Kit version 1.1 and were run on an Avant 3100 Genetic Analyser (Applied Biosystems). Sequences were aligned using the Geneious software (Biomatters). In summary, we validated the method of rapid genetic screening for the mutant allele c.1332 C>T by targeted sequencing of the SLC37A2 gene. This method of genetic identification of the mutant T-allele should help to reduce the prevalence of the causative mutation and decrease the risk of CMO in the three related breeds: West Highland White Terriers, Cairn Terriers and Scottish Terriers.

**Key words:** canine; SLC37A2; CMO; sequencing

### CD81 TETRASPANIN EXPRESSION ON BOVINE GAMETES

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Sperm-egg interaction and fusion represents a key moment of fertilization, and it would not happen in mammals without the interaction of the tetraspanin superfamily members including protein CD81. A detailed immunohistochemical localization of CD81 was monitored on bovine oocytes at different maturation stages, as well as during early embryogenesis. A CD81 protein was also characterized on bovine sperm. On bovine eggs, CD81 was detected on the plasma membrane of the germinal vesicle, metaphase I and metaphase II oocytes. During fertilization, an accumulation of CD81 molecules in the perivitelline space of fertilized oocytes, appeared as vesicles associated to plasma membrane, was observed. In majority of bull ejaculated sperm and caput, corpus and cauda epididymal sperm, CD81 was found on the plasma membrane covering the apical acrosome. Although the process of capacitation did not influence the localization of CD81, it was lost from the surface of the acrosome-reacted spermatozoa in bull. Presented results document certain aspects of CD81 expression in bovine gametes suggesting their possible importance in fertilization process in cattle.

**Key words:** sperm; egg; fertilization; cluster of differentiation 81; tetraspanin

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### ANIMAL BREEDING IN LATVIA

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In 2016, 153,927 dairy cows were registered in the Latvian animal register. The number of dairy farms as well as the number of dairy cows is decreasing in recent years. There are quite many small farms in Latvia – the average number of dairy cows in farm is 8.6 cows. However, the average milk yield is increasing steadily and reaches 6,182 kg per cow per year. The development of the pig breeding sector is negatively affected by the various trade restrictions posed by the outbreak of the African swine fever (from 2014) and by the Russian embargo. Beef cattle breeding is developing quite fast. The number of beef cattle and suckling cows is increasing. In 2016, in animal register 412,084 animals were registered, including 64,332 beef cattle. The poultry sector in Latvia is developing steadily, because this sector has a perspective and is profitable. In Latvia the demand for poultry meat and eggs, especially demand for free-range kept poultry meat and eggs, exceeds the supply. Sheep breeding sector is developing – we can see rapid increase in sheep number as well as live sheep export to other EU countries. Sheep are kept for meat production. Last year a number of sheep in farms increased by 4.2 % compared with 2015. Goats are kept mainly for milk production. In this year, one milk processing

company started to process goat milk and produce semi-hard cheese, so it is possible that this will be a good incentive for goat breeding industry. State aid is currently granted for the conservation of six native breeds – Latvian Brown cow, Latvian Blue cow, Latvian Darkheaded sheep, Latvian Native goat, Latvian White pig and driving type of Latvian horse breed. The number of all native breeds is decreasing, with the exception of the Latvian darkheaded sheep breed. The Latvian white pig is in critical situation. A gene bank is established at the Latvian University of Agriculture to store material from native breeds; also 60 local bee colonies are kept at the Latvian University of Agriculture.

**Key words:** animal breeding; animal genetic resources; native breeds; conservation

### EFFECT OF PERGA CONSUMPTION ON GLYCAEMIA OF ZUCKER DIABETIC FATTY RATS

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The aim of this study was to determine the effect of bee bread (perga) consumption on glycaemia of Zucker diabetic fatty (ZDF) rat model on high-energy diet. Male Zucker diabetic fatty (ZDF) rats (a fatty *fa/fa* mutation (-/-); n = 20) and their lean controls (ZL, non-diabetic, +/+ or +/-, not display expression of *fa* phenotype, n = 10) at the age of 3 months were involved in the experiment (Breeding Facility of the Institute of Experimental Pharmacology and Toxicology, Dobra Voda, Slovak Republic, SK U 25016). Rats were divided into three groups as follows: lean untreated rats (C) fed by KKZ-P/M, obese rats fed by enriched high energy diet (E1, enriched KKZ-P/M) and obese rats fed by enriched high energy diet (E2, enriched KKZ-P/M) and perga given orally by gastric feeding tube at a dose of 250 mg.kg<sup>-1</sup> body weight – for 30 days. A drop of blood was collected from the tail vein for blood glucose determination with digital glucose meter and test strips (FreeStyle, Abbott Diabetes Care Ltd., UK). One-way ANOVA test was performed to calculate basic statistical characteristics and to determine significant differences. High energy diet (E1 and E2 group) caused massive increase (P < 0.05) in the blood glucose level in comparison to the control. Conclusion: Perga consumption had no influence on glucose level in experimental groups when compared to the control. Further experiments with longer consumption of perga are reasonable.

**Key words:** diabetes mellitus; Zucker diabetic fatty rats; high-energy diet; haematology

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#### CHARACTERIZATION OF SLOVAK DUAL-PURPOSE CATTLE BREED DIVERSITY BASED ON GENOMIC DATA

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The aim of the study was to make a detailed insight into genetic diversity and nucleus population structure of Slovak Pinzgau and Slovak Spotted cattle and to describe the genetic diversity based on the runs of homozygosity (ROH), linkage disequilibrium (LD) and effective population size ( $N_e$ ) using genome-wide data. Moreover, Bayesian clustering algorithms and multivariate methods were used to detect the population structure, potential admixture level and relationship between breeds. BAPS is computationally superior to STRUCTURE and is suitable for the analysis of large datasets such as SNP arrays. To determine candidate markers for natural selection an alternative approach, based on principal component analysis (PCA), which uses multivariate evaluation to identify the population structure, was used. Relatively low genetic variability was observed in the population, especially in bulls. Most of the bulls clustered together except one bull and his daughters. More than half of the mating combinations were between highly related individuals. Recent genomic inbreeding reached 0.85 % in bulls and 0.94 % in cows, while historical inbreeding covers 5.69 % of genome in bulls and 6.23 % in cows. Based on the false discovery rate equal to 10 % up to 213 loci were identified as outliers. Most of them were found on BTA6 (118) in the two genomic regions within the sequence of genes encoding Toll-like receptors (TLR1, TLR6, TLR10) and immunoglobulin J chain (RBPJ). On BTA21 the strongest signal was detected directly within the sequence of immunoglobulin superfamily (ISLR, ISLR2). The lowest proportion of outlier loci was found on BTA23 (13) mostly within region controlling major histocompatibility complex (MHC). Despite the low variability, it is possible to design appropriate mating plans derived from observing the fine-scale structure of the population. Our results indicated that the signals of selection in genomic regions responsible for adaptive and innate immune response across both cattle breeds resulted mainly from the correlated selection response in a way to maintain their fitness. In the future, genetic selection for animals characterized by better immune response to environmental conditions and diseases can provide health and productivity advantages and can complement traditional health-maintenance methods.

**Key words:** cattle; autochthonous; endangered; high density data

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#### SERUM LIPIDS IN RABBIT BLOOD AFTER APPLICATION OF PATULIN AND STRAWBERRY LEAVES

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Most herbs and plants have been chemically evaluated and their activity has also been proven in clinical studies. However, the elementary mechanisms of function are still unclear. Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties. Patulin is a toxic chemical contaminant produced by several species of mold. It is the most common mycotoxin found in apples, apricots, grapes, grape fruit, peaches, pears, olives and cereals. Patulin has been reported to be a genotoxic, reprotoxic, embryotoxic, and immunosuppressive compound. The aim of the present study was to determine the effect of the inclusion of strawberry leaves and single dose of patulin to the feed mixture on levels of blood serum cholesterol and triglycerides in rabbits. Adult rabbits ( $n = 32$ ) of Californian broiler line were used in the experiment. Rabbits were obtained from an experimental farm of the NPPC, RIAP Nitra, Slovak Republic. The animals were divided into eight groups: one control group - C (0 % strawberry leaves, 0  $\mu\text{g.kg}^{-1}$  patulin) and seven experimental groups: EP ( $n = 4$ ; 10  $\mu\text{g.kg}^{-1}$  patulin, 0 % strawberry leaves), E1 ( $n = 4$ ; 0  $\mu\text{g.kg}^{-1}$  patulin, 0.5 % strawberry leaves), E2 ( $n = 4$ ; 10  $\mu\text{g.kg}^{-1}$  patulin, 0.5 % strawberry leaves), E3 ( $n = 4$ ; 0  $\mu\text{g.kg}^{-1}$  patulin, 1.0 % strawberry leaves), E4 ( $n = 4$ ; 10  $\mu\text{g.kg}^{-1}$  patulin, 1.0 % strawberry leaves), E5 ( $n = 4$ ; 0  $\mu\text{g.kg}^{-1}$  patulin, 1.5 % strawberry leaves) and E6 ( $n = 4$ ; 10  $\mu\text{g.kg}^{-1}$  patulin, 1.5 % strawberry leaves). The animals were fed with a granular feed mixture containing strawberry leaves at various doses, and some groups received patulin (Sigma Aldrich, Germany) intramuscularly in injectable form at 10  $\mu\text{g.kg}^{-1}$  two times a week for 30 days. The blood serum was separated from the whole blood by centrifugation (30 min at 3000 rpm). Cholesterol and triglycerides were determined by RX Monza automatic clinical analyser (Randox, United Kingdom). Cholesterol level was insignificantly lower in all experimental groups compared to the control group. In the groups E1, E2 and E3 non-significant decrease ( $P > 0.05$ ) of triglycerides in comparison to the control group was found. Our results are preliminary and further research is needed to verify this trend.

**Key words:** cholesterol; triglycerides; strawberry leaves; rabbits; patulin

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#### MONITORING OF SELECTED TRACE METALS AND THEIR INTERACTIONS TO INDUCE OXIDATIVE STRESS IN COMMON CARP (*CYPRINUS CARPIO*)

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Aquatic animals receive increased number of agricultural and industrial pollutants, which affect health status and free radical formation. The main pollutants are heavy metals, antibiotics and endocrine disruptors. Typical toxic metals (Cd, Pb, Hg, As) can cause hazardous effect to physiological systems of aquatic animals. Trace metals such as Mo, Al, Li, Ba and Ag are not thoroughly investigated. The induction of oxidative stress is monitored as ROS (reactive oxygen species) products. Contrariwise, total antioxidant capacity (TAC) is considered a cumulative action of all antioxidants. The aim of our study was to investigate the associations between oxidative stress markers and trace metal content in freshwater fish. Freshwater adult fishes (*Cyprinus carpio*) were used in our study (n = 36). The blood samples were taken by cardiac puncture, centrifuged for 20 min. at 3,000 rpm and blood serum was collected and stored at -20 °C until analyses. The content of trace metals (Mo, Al, Ag, Ba, Li) was determined by ICP-OES (Agilent Technologies Australia (M) Pty Ltd.). Oxidative stress markers (ROS, TAC) were assessed using luminol-based luminometry. Statistical analyses were performed using STATGRAPHICS Centurion. Concentration of Li significantly correlated with TAC (r = 0.452; P < 0.01). Mo, Al, Ba and Li were in negative association with ROS (Mo, r = -0.079; P > 0.05; Al, r = -0.107; P > 0.05; Ba, r = -0.082; P > 0.05; Li, r = -0.276; P > 0.05), and Ag was in positive association with ROS (r = 0.301; P > 0.05). The opposite tendency was observed between trace metals and TAC (Mo, r = 0.105; P > 0.05; Al, r = 0.250; P > 0.05; Ba, r = 0.052; P > 0.05; Ag, r = -0.197; P > 0.05). Analysis also showed statistically significant correlation between ROS and TAC (r = -0.742; P < 0.001) and between Ba and Li (r = -0.423; P < 0.05). Weak and nonsignificant correlations were detected between other metals. In conclusion, our results demonstrated effects of trace metals on antioxidant status under natural conditions. Further studies are necessary to test ecotoxicological interactions between other oxidative stress markers and environmental pollutants.

**Key words:** heavy metals; oxidative stress; Common Carp; biomonitoring

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#### EXPRESSION OF HORMONALLY REGULATED GLUCOSE TRANSPORTERS IN PREIMPLANTATION EMBRYOS OF MOUSE CD-1 STRAIN

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It has been well-documented that insulin stimulates glucose uptake in mouse blastocysts. Glucose is an essential energy source for preimplantation embryo especially at later developmental stages, and facilitative glucose transporters (GLUTs) are most important for glucose transport into early embryo cells. GLUTs exhibit a high degree of sequence homology but differ in their substrate specificity, kinetic characteristics, tissue distribution and responsiveness to endocrine signals. Two insulin- or adiponectin-responsive facilitative glucose transporters have been identified in mammalian tissues. Both GLUT4 (SLC2A4) and GLUT8 (SLC2A8) were found in bovine and rabbit blastocysts, but there is a controversy on their expression in mouse blastocysts. The aim of this study was to examine whether both hormone-sensitive glucose transporters, GLUT4 and GLUT8, are expressed in mouse blastocysts of CD-1 strain. We detected PCR products corresponding to both examined glucose transporters in mouse blastocysts of CD-1 strain. Sequencing the PCR products confirmed the GLUT4 and GLUT8 sequence; 100 % sequence identity for GLUT4 and 99.8 % sequence identity for GLUT8 was found between the PCR products and corresponding GenBank reference sequences. Our immunohistochemical study showed the presence of GLUT4 and GLUT8 proteins in CD-1 mouse blastocysts. The fluorescence signal produced by GLUT4 was evenly distributed in TE and ICM cells. The signal for GLUT8 was slightly stronger in mural TE cells than in other cells. Our results indicate that two insulin and/or adiponectin-sensitive facilitative glucose transporters can be expressed in mouse preimplantation embryos.

**Key words:** preimplantation embryo; GLUT-4; GLUT-8

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#### IMPLEMENTATION OF THE REGULATION 511/2014 IN ANIMAL BREEDING AND RESEARCH SECTOR

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The EU ABS Regulation (No 511/2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union) entered fully into application on 12 October 2015. The EU ABS Regulation is based on the concept of due diligence. The user, defined as a natural or legal person that utilises genetic resources or traditional knowledge associated with genetic resources, is under the obligation to make sure that genetic resources and the associated traditional knowledge have been accessed in accordance with applicable access and benefit-sharing legislation or regulatory requirements, and that benefits are fairly and equitably shared according to mutually agreed terms (Article 4 of the Regulation). The modalities of application of EU ABS Regulation regarding the register of collections, monitoring user compliance and best practices are defined in the Commission implementing Regulation (EU) 2015/1866.

In 2015, the Commission initiated a process to develop guidance documents for users to support fulfilment of their ABS obligations arising from utilisation of genetic resources and associated traditional knowledge. The set of guidance documents include horizontal guidance on the scope of application and core obligations of the EU ABS Regulation (adopted August 2016), and sectoral guidance for users for sectors such as animal breeding, biocontrol and biostimulants, biotechnology, cosmetics, food and feed, pharmaceutical and plant breeding. These sectoral guidance documents will soon be completed. In March 2017, the preparation of additional guidance for public research and for collection holders has been initiated. Guidance on animal breeding as well as guidance for public research and collection holders will provide much needed advice for users of genetic resources and traditional knowledge from the livestock research and breeding communities. The guidance provides examples of activities, which fall within the scope of the EU ABS Regulation or are considered to be outside the scope of this Regulation.

**Key words:** Access and Benefit-Sharing; EU ABS Regulation; animal breeding and research

#### TRANSFORMING GROWTH FACTOR B1 AND VIABILITY OF OVARIAN CANCER CELLS OVCAR-3 AFTER DEOXYNIVALENOL AND ZEARELENONE TREATMENT *IN VITRO*

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The mycotoxins zearalenone (ZEA) and deoxynivalenol (DON) are formed by fungi of the *Fusarium* genus. These mycotoxins are secondary metabolites found as contaminants in almost all agricultural commodities worldwide. The exposure risk to human is either directly through plant foods or indirectly through foods of animal origin. The gross toxic effects of ZEA and DON cause nutritional losses and represent a significant hazard for growth performance, endocrine regulation of the reproductive functions and fertility in females. Nowadays, ovarian cancer represents one of the most lethal of all gynaecological tumours in women. The failure to improve the prognosis for women with ovarian cancer is directly attributable to the lack of an effective screening test for early-stage disease. The objective of this *in vitro* study was to determine the metabolic activity and secretion of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) in ovarian cancer cell line (OVCAR-3) after application of ZEA and DON mycotoxins. Ovarian cancer cells were incubated without (control group) or with ZEA/DON toxins at different concentrations (0.5, 1, 2.5 and 5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) for 24 hours. The metabolic activity was determined by alamar Blue™ cell viability assay and the release of TGF- $\beta$ 1 was assayed by ELISA method. Our results showed that the cell viability significantly decreased ( $P \leq 0.05$ ) after DON addition at higher concentrations (2.5; 5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) compared with control untreated group. In contrast, ZEA at any used concentration had no effect

on the number of viable cells. Furthermore, TGF- $\beta$ 1 production did not significantly ( $P \geq 0.05$ ) differ either after DON or ZEA addition. Currently, when all possible ways to decrease cancer risk are being sought, our results showed that deoxynivalenol has ability to decrease the number of ovarian cancer cells *in vitro*. In summary, this *in vitro* study suggests that mycotoxins may affect the viability of ovarian cancer cells, and therefore, could provide important insights for searching of new types of anti-tumor drugs and a better understanding of regulatory mechanisms in ovarian cancer cells.

**Key words:** mycotoxins; ovarian cancer cells; viability; TGF- $\beta$ 1

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#### IMMUNOCYTOCHEMICAL DETECTION OF NUCLEOLAR PROTEINS IN INTERSPECIES NUCLEOLUS EXCHANGED EMBRYOS

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It is well known that nucleoli of fully-grown mammal oocytes, so-called nucleolus precursor bodies (NPBs), are essential for embryonic development and their absence leads to the developmental failure mainly at the time of embryonic genome activation (EGA). Therefore, the embryos originated from previously enucleolated oocytes can cleave only one or two times and then their development ceases. Goal of our study was to analyze the presence of nucleolar proteins, C23 and UBF, in interspecies nucleolus transferred embryos (NuTE). In our study the interspecies (mouse/pig) NuTE were produced and were analyzed using immunofluorescence (C23 and UBF staining) from 2-cell stage to blastocyst stage. We have compared the presence of nucleolar proteins (UBF and C23) in three experimental groups of embryos: 1. micromanipulated by transfer of nucleoli from pig oocytes to pig oocytes (P+P), 2. micromanipulated by transfer of nucleoli from pig oocytes to mouse oocytes (P+M) and 3. control group (intact) embryos. The number of nucleoli in P+P and P+M embryos, labeled with C23, was lower than those of control group. UBF was localized in small foci around the nucleoli of blastocysts in control group and P+P embryos, but in P+M embryos was evenly distributed in the nucleoplasm. In conclusion, our results indicate that the mouse nucleolus can partially substitute porcine nucleolus in enucleolated porcine oocytes, but the localization of some nucleolar proteins in NuTE comparing with control group indicates the aberrations occurring already during *in vitro* oocyte maturation and/or initial embryonic cleavage. We believe that our new micromanipulation approach, together with results presented in this study, will form a solid basis for further studies aimed to elucidate the role

of nucleoli in the process of regulation of mammalian oocyte maturation and early embryonic development.

**Key words:** nucleolus; mouse; pig; embryo; immunocytochemistry

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### MC4R RABBIT POLYMORPHISM

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The melanocortin 4 receptor (MC4R) is a protein produced from the MC4R gene, which has been found in humans to be involved in feeding intake and body weight, and also has a vital role in the control of energy balance and the genetic basis of obesity. Melanocortin 4 receptor (MC4R) gene is suggested to be a promising candidate gene for weight finishing in rabbits. In the present study we focused on detection of MC4R gene polymorphism in 30 rabbits of New Zealand white inbred line. Rabbits used in this study were obtained from National Agricultural and Food Centre, Research Institute for Animal Production Nitra. Genotyping was carried out by PCR-RFLP using primers that insert a restriction site for *SpeI* (*BcuI*). The pattern of restriction analysis, confirming the presence of two fragments of 100 bp and 27 bp, was not observed. The analysis showed that only one undigested DNA fragment of 127 bp was detected, indicating that all 30 rabbits are monomorphic. Additional research exploring the variability of production traits with potential application in rabbit breeding programs is needed.

**Key words:** rabbit; polymorphism; MC4R; PCR-RFLP

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### SCREENING OF THE CZECH RED PIED CATTLE POPULATION FOR DIVERSITY IN INNATE IMMUNITY GENES

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A program aimed at the screening and evaluation of the diversity

in innate immunity genes in the Czech Red Pied cattle has been carried out since April 2016. Since the repertoire of allelic variants of disease resistance genes is supposed to reflect local infection factors and selection pressure, the results are expected to provide informative material for comparison to other world dairy breeds. The survey of innate immunity gene diversity is supposed to be helpful to the oriented breeding and can be used to counteract gene pool erosion. Comparison to the subpopulation of the breed that is conserved in the genetic resource programme since 2010 and reflects the genetic structure around 2000, will facilitate the detection of the current selection trends. Currently, the survey is based on a set of 150 bulls from the production population and 35 animals of the conserved herd. The screening is carried out with targeted re-sequencing that exploits the Pacific Biosciences platform providing reads of full amplicons up to 2000 bp, thus facilitating phasing of structural variants. The monitored set of genes includes *TLRs* coding for both anti-bacterial and anti-viral Toll-like receptors, genes for the members of their signalling pathway, as well as additional chosen innate-immunity genes with previously reported effects on animal health. The detected structural variants are subsequently validated in individual animals with developed series of genotyping assays based on the primer extension method. In order to rationalise the number of reactions, the multiplexes preferentially include diagnostic SNPs (tag SNPs) that are based on the reconstructed haplotype structure of the population. The currently used genotyping panel consists of 93 reactions. The effect of the found gene variants will be further evaluated in the daughter population of 1500 cows, for which the extensive health data are collected in a two-year span. The diagnoses used approximately follow the recommendations of ICAR (2013). The cow population is genotyped only for the relevant subset of genetic polymorphisms.

**Key words:** cattle; innate immunity; Toll-like receptors; haplotypes

### ULTRASTRUCTURE OF VITRIFICATION-INDUCED DAMAGES IN BOVINE OOCYTES

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Cryopreservation of tissue from cow's ovaries can help to conserve reproductive potential of animals. The protocol used must ensure the good quality of preserved oocytes after thawing. Ultrastructure of bovine germinal vesicle (GV)-stage oocytes, frozen in the ovarian tissue fragments by two vitrification techniques, was evaluated. For solid surface vitrification (SSV), ovarian fragments were exposed to 4 % ethylene glycol (EG) in DPBS + 10 % FBS for 15 min. and then rinsed in a vitrification solution composed of 35 % 6 M ethylene glycol and 0.4 M trehalose in DPBS + 10 % FBS. After equilibration (5 min. in ice bath), the fragments will be placed in a minimum volume of vitrification solution onto the surface of a metal plate pre-cooled by partial immersion into liquid nitrogen (LN). For



liquid vitrification (LV) ovarian fragments were equilibrated in a vitrification medium containing 40 % ethylene glycol (v/v), 30 % Ficoll 70 (w/v), 1M sucrose and 4 mg.ml<sup>-1</sup> bovine serum albumin at room temperature for 5 min. Then, the tissues in 1.8 ml cryovials, were placed into LN. Oocytes were isolated from thawed ovarian fragments by a puncture of follicles. Oocytes were then fixed in Karnovsky fixative solution, individually embedded into 2 % agar and post-fixed in 1 % osmium tetroxide and embedded into the Poly/Bed 812<sup>®</sup> Embedding Media (Polysciences, Inc.). Ultrathin sections (70 nm) were examined on a transmission electron microscope operating at 80 kV. Oocytes from control group contained mainly hood-shaped mitochondria, vacuoles, lipid droplets, endoplasmic reticulum and germinal vesicle. In both experimental groups (SSV, LV) the oocytes showed disintegrated ooplasm and high degree of vacuolization and vesiculation. Germinal vesicles showed nucleus precursor bodies and the nuclear envelope was disintegrated. Microvilli of cytoplasmic membrane were disrupted. Following vitrification *zona pellucida* acquired layer-like structure and cells of *corona radiata* showed extensive damages. In conclusion, ultrastructure of bovine GV-stage oocytes following vitrification of tissue fragments by both techniques revealed severe damages in the membrane structures (cytoplasmic membrane, nuclear envelope) and ooplasm disintegration. A protocol for cryopreservation of ovarian tissues requires further optimization to maintain good oocyte quality after thawing.

**Key words:** cow; GV-stage oocytes; vitrification; ultrastructure

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#### ASSOCIATION OF SOME METABOLIC PARAMETERS OF EWES WITH YIELD AND QUALITY OF EMBRYOS

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The aim of the study was the assessment of the effect of ovulatory response and metabolic profile (presence of cholesterol, urea and total protein before and after superovulation) of ewes on the yield and quality of embryos. Positive correlation was found between the level of cholesterol and superovulatory response ( $r = 0.54$ ), total number of embryos ( $r = 0.01$ ) and transferable embryos ( $r = 0.39$ ). Levels of urea were in negative correlation with the superovulatory response ( $r = -0.42$ ), with the total number of flushed embryos ( $r = -0.49$ ) or transferable embryos ( $r = -0.58$ ). The influence of total proteins in blood serum of donor ewes on efficiency of embryo production was not proven in our research.

**Key words:** blood; embryos; ewe; metabolic change; yield embryos

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#### REALIZATION OF GENETIC RESOURCES CONSERVATION PROGRAMS IN POLAND

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Poland is one of the precursors of animal species protection, as already in the first half of the twentieth century began the program of restoring bison and breeding of Polish konik, the descendant of wild Tarpan. In 1996, Poland signed the Convention on Biological Diversity and started the creation of programs to protect animal genetic resources. Initially (1999) there were 29 breeds (horses – 2, cattle – 1, sheep – 9, pigs – 3, fur animals – 4 and poultry – 10). In the field of protection, important roles are played by scientific centres, especially the National Institute for Animal Production, which was entrusted in 2004 by the Minister of Agriculture and Rural Development with the coordination of livestock genetic resource conservation programs. Currently, after 18 years of conservation, the number of protected populations has increased to 83 and includes the following species: cattle, horses, pigs, sheep, goats, hen, ducks, geese, fur animals (8 species) and honey bee. In 2016 the number of flocks exceeded 3.3 thousands and the number of breeding females - 102 thousands (including bee families). The objectives of the Program are: a) preserving the population and increasing its number; b) maintaining the greatest possible genetic variability and c) reconstitution/stabilization of characteristics specific to a given population.

**Key words:** conservation programs; domestic animals; coordination

#### SELECTION PROCESS IN THE BREEDING OF NITRA AND ZOBOR RABBITS

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Nitra and Zobor rabbits belong to the group of national breeds. In contrast to other national breeds, both populations were bred at the Research Institute of Animal Production Nitra as “secondary product” of the breeding process focused on creation of more efficient broiler rabbit populations. These breeds are represented at regional as well as national exhibitions. Rabbit breed “Nitra” (in Slovak – *Nitriansky králik*) was established and certified in 1977. Jaroslav Zelník was the main breeder and organizer of the selection process. The initial parent populations were French light silver rabbit and Californian breed. Main emphasis in the selection was put on traits associated with meat utility characteristics like growth intensity, carcass quality and food conversion. In the course of breeding, specific inbred lines were established and after utility tests were realized; the line FRF was selected for further exploitation in the breeding process. Blue acromelanistic animals with middle live weight were stabilized and after this the population was increased, the new zootechnical individual was called Nitra rabbit. The number of bred animals was increased after dissemination of Nitra rabbits to breeders from practice. In the late 80s,

approximately 600 breeding animals were bred in the area of former Czechoslovakia. Raising interest in broiler rabbit production stimulated more intensive use of Nitra rabbits. Their extraordinary utility characteristics and disease resistance were dominant reasons for using this animal in broiler production process. Nowadays, Nitra rabbits are bred by some dozens small animal breeders in Slovakia. Rabbits are regularly exhibited on regional and national level. “Zobor” rabbit (in Slovak - *Zoborský králik*) is a product of hybrid experiments among different New Zealand White (NZW) breed types and acromelanistic breeds (Himalaian, Californian, Nitra rabbit). In some NZW animals (growing in cold environment nest), shadow Dutch spotting phenotypes were manifested. After genetic analysis, it was determined to be a manifestation of Dutch recessive allele present in genotypes. It is a consequence of albinotic recessive allele expression. The current constellation of two active alleles from different genes (acromelanism and Dutch spotting) causes manifestation of interallelic interaction called recessive epistasis. This interaction induces changes in segregation classes during Mendelian segregation. Phenotypically, the exterior of the Zobor rabbit is characterized by NZW type and incomplete acromelanistic spotting i.e. melanin is present in ear hairs, pigment collar in the tarsals region of hind limb and shaded tail hair. Incomplete acromelanistic phenotype is under activity of many genes determining high level of spot variability. For this reason, the typical Zobor exterior is a challenge for breeders.

**Key words:** Nitra and Zobor rabbit; national breeds; selection process

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#### ACTIVITIES OF THE MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT OF THE SLOVAK REPUBLIC IN THE FIELD OF ANIMAL GENETIC RESOURCES

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The market economy and the ever-increasing request for animal proteins have brought increased demands on livestock productivity. In breeding-advanced countries, their breeding was more intense, with a higher level of specialization in a particular performance, as it was in the territory of today's Slovak Republic, thanks to research in the field of biotechnology, the different development of the farmer's property arrangement, the large livestock populations and the social and political conditions. By the end of the last century, the original breeds of farm animals reared in our country were almost entirely displaced by the breeds with higher yields, which were imported mainly from Western Europe, both in the form of live animals but mainly in the form of germ products. These imports have been intensified with the entry of the Slovak Republic into the European Union. Increasing pressure on the livestock commodity economy has forced farmers in Slovakia to refocus themselves to efficient livestock breeds, the weakness of which is a high degree of breed improvement by the high criteria on breeding conditions, nutrition and health prevention. The state has become aware in time of the importance of the genome of the original livestock

breed that has partly formed the domestic environment, as well as the need to preserve biodiversity in this area also for future generations. The role of the Ministry of Agriculture and Rural Development of the Slovak Republic in the area of animal genetic resources is to provide a legal framework for their conservation, protection and exploitation both from the point of view of the cultural heritage and from the point of view of the nutrition needs of the population. Therefore, the ministry applies the measures of the Common Agricultural Policy of the European Union to compensate the income of native breed farmers who are not financially competitive in terms of their sustainability.

**Key words:** biodiversity; support; competitiveness

#### THE RELATIONSHIP BETWEEN HEAVY METAL LEVELS AND SEASONAL PERIOD IN FRESHWATER FISH (*CYPRINUS CARPIO*)

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Heavy metal environmental contamination is a serious problem nowadays, which directly threatens human health. With increasing industrialization and population growth, the problems associated with heavy metal pollution in rivers and water areas are more and more frequent. Heavy metal pollution in rivers constitutes a threat to the consumers of fishery sources. Therefore, measuring the metal bioaccumulation in aquatic organisms is very important. The aim of the study was to determine concentrations of chosen heavy metals in the blood of European carp in different seasonal periods. Blood samples used in our study were collected from European carp (*Cyprinus carpio*) during two seasons - the spring season (n = 23) and the summer season (n = 19). Blood serum obtained from coagulated blood was used for analyses. The concentrations of chosen heavy metals – Mo (molybdenum), Al (aluminium), Ba (barium) and Li (lithium), were determined from blood serum by inductively-coupled plasma optical emission spectrometry (ICP-OES). Basic statistical analysis as well as analysis of variance (one-way ANOVA) following Tukey's HSD procedure and Pearson's correlations were performed using STATGRAPHICS Centurion. Concentrations of monitored heavy metals showed non-significant differences between spring and summer season. The concentrations of analysed metals showed following variations between seasons (expressed as spring season/summer season): Mo – 8.09/5.96 µg L<sup>-1</sup>, Al – 631.30/591.95 µg L<sup>-1</sup>, Ba – 197.43/143.03 µg L<sup>-1</sup>, Li – 4.79/5.76 µg L<sup>-1</sup>. The correlation analysis of the results in the group of spring season showed statistically significant associations only between Li and Mo (r = 0.6758; P < 0.001). In the case of summer season group, non-significant correlations between all analysed metals were detected. Aquatic organisms, especially freshwater, are a suitable subject of environmental load studies because they are in permanent contact with contaminated environment. Therefore, it is important to check regularly not only the environment but also the animals in which the substances such as heavy metals accumulate.

**Key words:** carp; blood; metals; trace elements

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#### THE USE OF STARTING FLUORESCENCE CALCULATION FOR TRANSCRIPT REAL-TIME PCR RELATIVE QUANTIFICATION

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The most widely used techniques for relative quantification of specific transcripts by real-time PCR are delta Ct method and the technique using relative standard curve. Several alternatives to these methods were developed, and we verified the usability of the technique using calculation of a PCR template starting fluorescence. It is assumed that the intensity of reporter fluorescence dye is directly proportional to the amount of PCR amplification product in fluorescent real-time PCR. Based on this assumption, theoretical starting fluorescence can be calculated from amplification efficiency and from fractional number of amplification cycles needed for reaching a selected benchmark. The starting fluorescence is then proportional to the amount of PCR template and represents the quantity of target nucleic acid sequence expressed in arbitrary fluorescence units. We amplified two adiponectin receptor transcripts (AdipoR1 and AdipoR2), isolated PCR products from agarose gel and quantified them spectrophotometrically. We put known amounts of adiponectin receptor PCR products (calculated amount of DNA copies) into PCR reactions and re-amplified these templates in a real-time PCR system. Threshold fluorescence was set in the exponential phase of amplifications and corresponding cycle number was calculated. Amplification efficiencies were measured using LinRegPCR algorithm and theoretical starting fluorescences of DNA templates were calculated. We found that calculated ratios of AdipoR1/AdipoR2 starting fluorescences were very close to ratios of DNA copies of AdipoR1 and AdipoR2 PCR templates put into reactions. These results indicate that starting fluorescence can be successfully used for calculation of the ratio of two different transcript amounts in the sample.

**Key words:** real-time PCR; starting fluorescence; relative quantification

**Acknowledgements:** This work was supported by the Slovak Research and Development Agency (project VEGA 2/0039/15).

#### SUSTAINABLE USE OF PODOLIAN COW GENETIC RESOURCES

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Biodiversity preservation is a process of genetic conservation through renewal of degraded ecosystems and natural habitats, and the preservation and recovery of breeds. Sustainable use represents utilization of biodiversity components that does not cause distortion of biodiversity, but represents a rational use of natural resources and maintenance of the potential biodiversity. Rational use of animal genetic resources for the agricultural industry and food production is a good strategy for sustainability of their production. The concept of sustainable use has a great economic importance, but also has an impact on rural development and increase of employment opportunities. Sustainable agricultural production systems are those, which allow the conversion of resources into human food and agricultural products without reducing the availability of these resources in the future or causing degradation of the environment. Sustainable livestock production systems are those in which the animal genetic resources are in accordance with other available resources. According to FAO, achieving this integration is the key to maintaining and developing production through a full range of global resources and ecological production. The aim of this work was to show that the use of Podolian cattle for meat production can be cost-effective, beside the benefits in preserving genetic diversity within an autochthonous breed, also in the social aspects, such as an employment and maintenance of the national traditions. In our work, we will present the sustainable farm production on the example of AnGR farm “Tikvicki” keeping Podolian cows. The Podolian cows are in the herd of 30 reproductively capable females and 20 animals in other categories. Because of the small number of animals there is an intake of new males every two years to prevent inbreeding. In a further analysis, it is necessary to determine the exact revenues and expenditures of the monitored production observed in certain time periods.

**Key words:** AnGR; sustainability; biodiversity; preservation; Podolian cow

#### SLOVAK RABBIT BREEDS

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Nowadays, there are about 70 purebred breeds of rabbits in Slovakia in many color types. Of this number, 12 breeds are generated by Slovakian breeders. Total numbers of breeding individuals kept in Slovakia are different according to the breed. The largest population and the highest number of breeders belong to the following breeds: Slovak grey-blue Rex (200 females and 40 males), the Rabbit of Nitra (150 females and 30 males), the Zemplin rabbit (70 females and 20 males), the Blue of Holic rabbit (60 females and 30 males), the Liptov bold-spotted rabbit (50 females and 20 males), the Slovak pastel Rex (70 females and 30 males) and the Zobor rabbit (50 females and 20 males). Rest of the breeds are represented by very small populations consisting of approximately 20 breeding females and of 10 to 15 males. It is very helpful that a portion of these breeds has farmers interested in its breeding also outside Slovakia and, for example, the population of Blue of Holic breeding rabbits in Germany is estimated to be 30 breeding females and 100 males. Slovak national breeds of rabbits belong to the cultural biological heritage, and our aim is

to preserve and raise it for future generations of breeders. However, without targeted and long-term state assistance this effort is very difficult.

**Key words:** rabbit; Slovakian breed; animal genetic resources

#### MANAGEMENT OF FARM ANIMAL GENETIC RESOURCES IN LITHUANIA

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The main purpose of the first National Programme for the conservation of farm animal genetic resources (AnGR), adopted by the Ministry of Agriculture of the Republic of Lithuania in 1996, was to collect, investigate and conserve the Lithuanian AnGR. At the beginning, only 6 indigenous breeds were included into this programme. The main purpose of the programme was collection, investigation and *in situ* conservation of AnGR. In 1993-1997, herds of indigenous breeds were formed of the animals remaining after expeditions, thus recreating mini-populations of 6 breeds at the Institute of Animal Science (LIAS). Conservation work was supported by the Ministry of Agriculture. Later, unfavourable conditions were created for other 6 breeds developed in 20<sup>th</sup> century, and these breeds were included into conservation programme. In 2008, national programme of AnGR was renewed and the Coordinating Centre for the National Farm Animal Genetic Resources was established at the LIAS. The main activities of the Centre: coordination of animal genetic resources, identification, monitoring, preparation of conservation programs, evaluation of animals, conservation of farm animals *in situ* (selection nucleus) and *ex situ* (conservation of animal genetic material in the form of semen, embryos, samples of blood or hair and DNA). The principles of conservation of AnGR and evaluation of breed status are based on the experience of animal breeding in small conserved herds and on the criteria and global strategy of FAO for the management of AnGR. The first decision in setting up conservation schemes is to carry forward the existing variability in the breeds. The status of Lithuanian AnGR was evaluated by their monitoring. In the period of 1993-2017, a population size of some breeds increased from 30 to 700 animals per breed and has a status of endangered-maintained population. However, some of them are still on the verge of extinction; effective population size is still below 50. Main reasons behind the critical status are small subsidies for breeders, diseases (African swine fever) and limited possibilities for the use of their products. In case a substantial number of animals was lost in different lines and families, the *ex situ* conservation was initiated.

**Key words:** animal genetic resources; conservation; monitoring; indigenous breed

#### PARAMETERS OF SPERM MOTILITY IN ENDANGERED ORAVKA CHICKEN

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The evaluation of semen motility parameters of poultry gives an excellent indicator of their success in cryopreservation and fertilization process. We aimed to evaluate the spermatozoa motility parameters of individual roosters of Oravka breed. Sexually mature roosters (n=6) without infections were used in the experiments. The semen samples were collected twice a week by dorso-abdominal massage during two months (from April to May 2017). The samples were diluted in a saline at the ratio of 1:100 (v:v). The concentration and motility characteristics of diluted rooster spermatozoa were analyzed using the CASA system. Average concentration (10<sup>9</sup> per ml), percentage of total motile spermatozoa (total motility > 5 µm/s), percentage of progressive motile spermatozoa (progressive movement > 20 µm/s), VCL (velocity curved line, µm/s), VSL (velocity straight line, µm/s), STR (straightness - VSL:VAP, velocity average path), LIN (linearity- VSL:VCL), BCF (beat cross frequency, Hz) were recorded. Variability in spermatozoa concentration (3.04 ± 0.36 to 4.12 ± 0.43), total motility (50.71 ± 11.31 to 77.05 ± 5.26) and progressive movement (29.54 ± 8.63 to 58.96 ± 6.82) was observed among individuals. However, no significant differences in concentration and motility parameters among analysed individuals were found. In conclusion, this study provides the first characterization of Oravka spermatozoa motility. Based on our results rooster semen can be used for cryopreservation and fertilization.

**Key words:** semen; motility; Computer assisted sperm analysis; Oravka breed

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#### EFFECT OF DIFFERENT STORAGE TEMPERATURES ON RED DEER SPERMATOZOA MOTILITY

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Intensive red deer breeding in captivity expanded in the second half of the 20<sup>th</sup> century with the purpose of meat production. Red deer breeding recorded a large development including implementation of various biotechnological methods, such as artificial insemination, MOET and *in vitro* production of embryos. Application of these methods leads to intensification of the animal production and enables the use of high quality sires. The aim of this research was to evaluate the motility of breeding bucks (*Cervus elaphus*; n = 10) spermatozoa in different storage

and assessment temperatures, specifically 10 °C and 37 °C. Semen was collected from bucks from different age groups using artificial vagina. Collected semen was extended with conservation medium at ratio 1:1. Conservation medium consisted of demineralized water, fructose, glycerol, citric acid, buffer solution, phospholipids and antibiotics. During the transportation to the laboratory, the semen was stored for about an hour at defined temperatures. The semen was evaluated by CASA system, which determined the following parameters: motility (MOT), progressive motility (PRO), velocity curved line (VCL), beat cross frequency (BCF) and amplitude of lateral head displacement (ALH). Based on the obtained results, the samples, which were incubated at 10 °C showed better motility, progressive motility, VCL, DCL, BCF and ALH than those incubated at 37 °C. In conclusion, the storage of collected semen at 10 °C maintains the spermatozoa properties, which are closely associated with successful fertilization.

**Key words:** Red deer (*Cervus elaphus*); deer farming; spermatozoa motility; CASA

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#### ANIMAL GENETIC RESOURCES IN THE SLOVAK REPUBLIC

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Slovak Republic supports the conservation of animal genetic resources through a variety of means and tools. Important part of this effort is monitoring of livestock populations. This activity is undertaken by NPPC – Research Institute for Animal Production Nitra. The monitoring of population status is based on the cooperation with Breeding Services of the Slovak Republic, s.e. and authorized breeders' organizations. The monitoring covers not only the endangered breeds, but also other main breeds of livestock. The data are stored and published in the EFABIS database. At the moment, 15 breeds of cattle, 6 breeds of pigs, 23 breeds of sheep, 8 breeds of goats, 11 breeds of horses, 46 breeds of rabbit, 17 breeds of chicken, 3 breeds of geese and 2 breeds of ducks are regularly monitored. Besides the monitoring of population data, we also try to identify the motivation factors, which influence the farmers' decision for the local breed breeding. Another important battery of activities is propagation of local breeds and raising public awareness. NPPC – Research Institute for Animal Production Nitra keeps live animals from several local breeds under *ex situ* conditions. These are used for providing breeding material but also for presenting the local breeds during national and regional exhibitions. The presentation of animals goes hand in hand with presentation of local products from these animals. The close cooperation with media helps to spread the issues of animal genetic resources among the broader public.

**Key words:** animal genetic resources; local breeds; conservation; monitoring

#### SLOW-FREEZING DOES NOT CHANGE CHROMOSOMAL COUNTS IN RABBIT AMNIOTIC FLUID STEM CELLS

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Amniotic fluid stem cells (AFSCs) are frequently used in human regenerative medicine. After collection of amniotic fluid it is important to process and store the cells in the most efficient way for the future use in cell therapy or for the conservation as gene resources. Slow-freezing method is often used for long-term storage of stem cells in many laboratories, however there is a need to control the cytogenetic state of the cells post-thawing. Therefore, the aim of this study was to determine the changes in chromosomal count in rabbit AFSCs before and after the slow-freezing. Samples of fresh and slowly frozen AFSCs at third passage were subjected to the karyotype analysis. In this study, 30 metaphase plates per sample were observed. A normal rabbit karyotype consists of 22 pairs (2n = 44) of chromosomes. Chromosomal abnormality was defined as following: hyperploidy - > 44, hypoploidy - < 44. Results showed that 60 % of fresh cells were able to maintain the stable karyotype. Abnormal karyotype was present in 40% of cells, of which 10 % were hyperploid and 30 % hypoploid cells. In slowly frozen cells, normal karyotype was monitored in 66.6 % of cells. Chromosomal aberrations were detected in 33.2 % of cells including 6.6 % hyperploid and 26.6 % hypoploid. Stem cell preservation is a necessary step from the view of the therapeutic perspective as well as the perspective of gene resource storage. However, it is strongly recommended to monitor the numeric chromosomal status following the freezing process.

**Key words:** rabbit; amniotic fluid stem cells; karyotype; slow-freezing

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#### ASSOCIATION BETWEEN SELECTED ENVIRONMENTAL POLLUTANTS AND OXIDATIVE STRESS MARKERS IN CARP SEMEN

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Aquatic contamination has become a worldwide concern as the ever-increasing presence of heavy metals, pesticides and endocrine disruptors may have detrimental effects on the natural habitat with subsequent hazard to the food chain. The male reproductive system is a highly sensitive barometer of changes in the internal or external milieu of the organism, and hence may serve as a suitable indicator of environmental pollution.

Heavy metals such as lead, cadmium or mercury are well-known contaminants of the aquatic environment with detrimental effects on the structure and function of male reproductive structures. Less attention is given to minor yet potentially dangerous chemical elements, such as aluminium (Al), lithium (Li) and barium (Ba). As a proper oxidative balance has become a crucial prerequisite for an optimal reproductive performance, the aim of this study was to assess possible relationships between the total antioxidant capacity (TAC), production of reactive oxygen species (ROS) and the content of Al, Li and Ba in carp semen. Semen samples were collected from 15 carp males and lysed using RIPA buffer and sonication. ROS and TAC were assessed using luminol-based luminometry, while the concentrations of Al, Li and Ba were determined using inductively coupled plasma mass spectrometry (ICP-MS). Basic statistical analysis as well as Pearson's correlations were performed using GraphPad Prism, version 6.0. The analysis revealed significant negative associations between ROS and TAC ( $r = -0.652$ ;  $P < 0.001$ ), indicating a direct inverse relationship between these two parameters. Weak and insignificant associations were detected between Al and Li ( $r = 0.258$ ;  $P > 0.05$ ) as well as Li and Ba ( $r = 0.069$ ;  $P > 0.05$ ), however a significant positive relationship was revealed between Al and Ba ( $r = 0.956$ ;  $P < 0.001$ ). All elements were in a positive association with ROS ( $r = 0.310$ ;  $P < 0.05$  in case of Li;  $r = 0.412$ ;  $P < 0.05$  with respect to Ba;  $r = 0.515$ ;  $P < 0.001$  in relation to Al). Inversely, negative correlations were recorded between the concentration of the selected chemical elements and TAC ( $r = -0.280$ ;  $P > 0.05$  in relation to Li;  $r = -0.423$ ;  $P < 0.05$  in case of Ba;  $r = -0.556$ ;  $P < 0.01$  with respect to Al). Our data suggest that the presence of minor chemical elements may represent a threat to the oxidative milieu of male reproductive structures and subsequently decrease male fertility in fish. As such, we may recommend that a regular monitoring system should be established to screen the occurrence of hazardous chemical elements in aquatic environment.

**Key words:** heavy metals; oxidative stress; ROS; TAC; freshwater fish

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#### NEW CD34 ANTIBODY SUB-CLONES FOR THE HEMATOPOIETIC STEM CELL PHENOTYPING IN THE RABBIT BLOOD AND BONE MARROW

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The aim of this preliminary study was to compare the antigen specificity of newly prepared antibody sub-clones against rabbit CD34 peptide for the identification of hematopoietic stem cells (HSCs) among the rabbit mononuclear cells isolated from the peripheral blood and bone marrow. Three young (four months old) and clinically health rabbits of New Zealand White (NZW) line reared in a partially air-conditioned hall of a local rabbit farm at RIAP Nitra were used in the experiment. Mononuclear cells were isolated using Biocoll solution from the rabbit peripheral blood (PBMCs) and bone marrow (BMMCs), aliquoted into prepared tubes and stained with 20 different sub-clones of mouse anti-rabbit CD34 monoclonal antibodies: IgG1 isotype sub-clones (257/77, 257/80, 274/9/76, 274/9/78, 7/1, 7/4, 7/2, 417/55, 417/58, 417/68, 485/71 and 485/72), IgG2b isotype sub-clones (465/1, 465/3 and 465/13) and IgM isotype sub-clones (63/29, 63/45, 63/47, 507/15 and 507/27). Briefly, at least  $1 \times 10^6$  cells were incubated with 100  $\mu$ l of each CD34 sub-clone supernatant that was extracted from the corresponding hybridoma cell line at 4 °C for 15 min. Subsequently, cells were incubated with a secondary antibody - goat anti-mouse Ig-APC (BD Biosciences, USA) at 4 °C for a further 15 min. After washing, cells were stained with 7-AAD (Bioscience, Austria) in order to exclude dead cells from the analysis. All labeled cells were analyzed using a flow cytometer FACSCalibur (BD Biosciences, USA). At least 50,000 events (cells) were analyzed in each sample. Modified flow cytometry method based on International Society for Hematology and Graft Engineering was used to determine CD34<sup>+</sup> (stem and progenitor) cell counts. Observed results were evaluated by a one-way ANOVA (Holm-Sidak method) using SigmaPlot software (Systat Software Inc., Germany) and expressed as the means  $\pm$  SEM. We observed significantly higher expression (%) of CD34 antigen within PBMCs using sub-clones: 465/1 ( $6.2 \pm 0.5$ ), 465/3 ( $5.2 \pm 0.6$ ), 465/13 ( $5.4 \pm 0.7$ ), 63/29 ( $3.2 \pm 0.8$ ), 63/45 ( $5.1 \pm 2.4$ ) and 63/47 ( $11.5 \pm 1.1$ ) compared to other sub-clones. Similarly, the same sub-clones identified significantly higher percentage of CD34<sup>+</sup> cells within BMMCs ( $5.5 \pm 1.6$ ,  $3.6 \pm 0.4$ ,  $3.3 \pm 0.2$ ,  $6.5 \pm 2.3$ ,  $6.8 \pm 2.0$  and  $11.7 \pm 1.7$ , respectively) compared to other sub-clones. In conclusion, some of the tested sub-clones showed increased expression of CD34 antigen within the rabbit PBMCs and BMMCs. However, further analyses are required in order to determine the true CD34 antigen specificity of newly prepared monoclonal antibodies.

**Key words:** rabbit; HSCs; CD34 sub-clones; flow cytometry

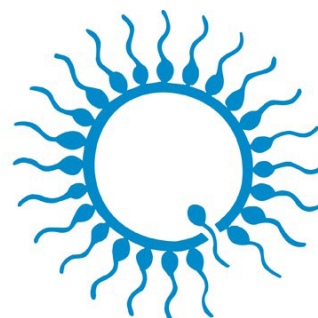
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