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PROTECTION AND SUSTAINABILITY OF SLOVAK ANIMAL GENETIC RESOURCES IN ORDER TO ENSURE THE SELF-SUFFICIENCY IN QUALITY FOOD IN SLOVAKIA

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ABSTRACT

Protection of the animal genetic resources has an impact on the present and also future life quality and important effect on the food safety. An effective cryopreservation of biological material from certain species of livestock is not yet fully mastered. The related issues are reduced viability and quality of frozen/thawed livestock sperm, embryos and stem cells. Animal genetic resources can be protected under *in situ* and *ex situ* conditions. In the field of modern biotechnology, preservation strategies are focused mainly on *in vitro ex situ* diversity conservation. This form of protection is represented by long-term storage of biological material (spermatozoa, embryos or stem cells) at temperatures well below freezing point.
In our gene bank we stored the samples of DNA, semen, embryos and stem cells from Slovak breeds which are registered in EFABIS database. We monitored, analyzed and cryopreserve biological material from breeds of cattle, rabbits, poultry and sheep. The monitoring includes also molecular characterization (DNA and specific genes analyses) of samples which are registered in a Cryoweb database.

Keywords: animal, livestock, breed, biodiversity, climatic changes

INTRODUCTION

The Convention on Biological Diversity (CBD) defines genetic resources as "genetic material of actual or potential value." Genetic material is defined as "any material of plant, animal, microbial or other origin containing functional units of heredity"(**Convention on Biodiversity, 1992**).
Farm Animal Genetic Resources (AnGR) are those animal species that are used, or may be used, for the production of food and agriculture, and the populations within each of them. These populations within each species can be classified as wild and feral populations, landraces and primary populations, standardized breeds, selected lines, varieties, strains and any conserved genetic material; all of which are currently categorized as Breeds **(FAO, 2005).**
Livestock systems have both positive and negative effects on the natural resource base, public health, social equity and economic growth. Currently, livestock is one of the fastest growing agricultural subsectors in developing countries. Its share of agricultural gross domestic product (GDP) is already 33 % and is quickly increasing. This growth is driven by the rapidly increasing demand for livestock products, this demand being driven by population growth, urbanization and increasing incomes in developing countries (**Delgado, 2005**).
Global diversity in domestic animals is considered to be under threat. A large number of domestic animal breeds are endangered worldwide, in a critical status or already extinct.
Despite enormous potential contribution to sustainable development and to reducing hunger and poverty, animal genetic resources for food and agriculture are underutilized and underconserved. At present, a substantial proportion of the world's livestock breeds are at risk of extinction.
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.
The IUCN Red List of Threatened Species significantly contributed to guiding global preservation action. The goal of the IUCN Red List of Threatened Species is to provide information and analyses on the status, trends and threats to species in order to inform and catalyse action for biodiversity conservation.
Currently, there are 69,903 species described on The IUCN Red List, and more than 8,490 are threatened, including mammals, birds, reptiles, amphibians and fishes. The statistics shows that during the last 21 years (from 1996) the number of threatened vertebrate species increased from 3,314 to 8,490 (Table 1).

Table 1 *Number of threatened vertebrate species*

	No of described species	No of species evaluated by 2019	Number of threatened species in 1996/98	Number of threatened species in 2019	Species evaluated in 2019 as % of species described
Mammals	5,792	5,792	1,096	1,223	100
Birds	11,126	11,126	1,107	1,492	100
Reptiles	10,793	7,199	253	1,311	67
Amphibians	7,962	6,756	124	2,123	85
Fishes	34,200	17,228	734	2,341	50
Total	69,903	48,101	3,314	8,490	69

Adopted from IUCN Red List 2019

The European Red List is a review of the status of European species according to IUCN Regional Red Listing guidelines. It identifies those species that are threatened with extinction at the European level (Pan-Europe and the European Union) so that appropriate conservation action can be taken to improve their status. To date 10,810 species have been assessed on the European Red List and regional assessments have been completed for all mammals, reptiles, amphibians, butterflies, dragonflies, freshwater fishes and freshwater molluscs and a selection of saproxylic beetles, terrestrial molluscs and vascular plants (http://ec.europa.eu/environment/nature/conservation/species/redlist/).
Among European 1.000 species of native mammals, birds, reptiles and amphibians, 155 are classified as threatened. Over the years, the European Red List has become a powerful tool to inform political leaders on biodiversity conservation and the protection of European natural resources. It is an instrument to measure progress towards achieving the EU 2020 Biodiversity Strategy and the Strategic Plan of the Convention on Biological Diversity as well as to guide the allocation of financial resources and support priority setting for conservation actions.

Status of AGR in Slovakia

Slovakia hosts a large proportion of the species that are threatened at the European level, and has the important responsibility for protecting these species within its territory. Species in Slovakia require greater action to improve their status. While many species already receive some conservation attention, others do not. Species can be saved from extinction but this requires a combination of sound research and carefully coordinated efforts. Slovakia as an EU Member State has committed to halting biodiversity loss by 2020, but urgent action is needed to meet this target and better monitoring capacity is required to measure if the target is met.

Considerable conservation investment is needed from Slovakia to ensure that the status of European species improves in the long term.

In Slovakia, the different natural conditions of the individual regions find their expression in the different conditions for plant and animal production. There are intensive lowland regions as well as extensive mountainous and sub-mountainous regions in Slovakia. In the lowland regions, farms with combined plant and animal production prevail. On the contrary, farmers in regions with a lower proportion of arable land (mountainous and sub-mountainous regions) mainly deal with cattle, sheep or goat breeding. Since the rural regions of Slovakia are afflicted with high unemployment rates, a return to self-supplying poultry, sheep, goat, pig and rabbit husbandry has become evident.

Numbers of breeds of cattle, sheep, goats, horses, rabbits and poultry belonging to the farm animal genetic resources in Slovakia monitored in 2016 are shown in Table 2.

Table 2 Status of farm animal genetic resources in Slovakia

Breeds	According to EFABIS (registered)	Monitored in 2016
Cattle	16	15
Goose	4	4
Duck	3	2
Goat	5	8
Rabbit	43	46
Chicken	20	17
Horse	11	11
Turkey	1	1
Sheep	14	23
Pig	10	6

Cattle

The Slovak spotted and Slovak Pinzgau breeds belong to the cattle breeds of national interest (Kasarda et al., 2015). Although decreasing in numbers, the population of cattle is of great interest with respect to the food needs of people as well as its landscape influence. Since 1989 the numbers of cattle have tended to decrease. The highest proportion of animals belong to crosses of the Slovak Spotted and Slovak Pinzgau cattle with the Holstein breed.

Of purebred animals, the Slovak Spotted cattle represents the most widespread breed. In the purebred form about 50 000 cows are being bred, followed by the Holstein (35 000 cows) and the Slovak Pinzgau cattle (5000 cows). Mainly with the Slovak Pinzgau breed a drastic decrease in the number of purebred animals can be observed.

It is necessary to emphasize that the state of cattle (livestock) is a stable basis of animal husbandry not only in Slovakia but in all European Union.

Sheep

Before 1989 wool production was the main commodity in sheep. After that date, as a consequence of transformation and a decrease of the market price of wool, sheep milk and meat production became essential.

Between 1990 and 1999 the number of sheep in Slovakia decreased from 600 000 to 340 000, i.e. by 260 000 heads, which is 43 %. Since 1999, a reverse tendency could be seen. This trend accompanied by a slight improvement of the production and reproduction traits in sheep has been the result of increased care and mainly of economic measures. The economically most important sheep breeds are autochthonous –

the Improved Valachian and Tsigai, which are widely used for milk production.

Goat

Goat breeding is a marginal branch of animal production. Most animals can be found in the hands of small keepers (there are only 10 larger flocks in Slovakia) mainly under more difficult agro-ecological conditions i.e. in the poorer sub-mountainous and mountainous regions. In Slovakia, local breeds are bred, like the White Shorthaired and Brown Shorthaired.

The White Shorthaired goats are predominant among the goat population of Slovakia. Goats represent the livestock sector of minor importance, mainly farmed for milk.

Poultry

The global poultry sector is divided into a large-scale commercial sub-sector dominated by international, vertically integrated companies and a small-scale subsector that provides up to 90 percent of total poultry production in some of the least developed countries.

In the long term, the poultry sector in Slovakia, as a part of animal production, was stable, even though the transition to a market economy was proved also in the mentioned sector. Since 2004, Slovak poultry meat production has been increased by 2.9 %. In Slovakia, an increasing trend in the poultry meat consumption per inhabitant was observed. In 2010, in the total meat consumption poultry meat shared with 33.7 %, beef with 7.4 % and pig meat with 55.8 %. The change of market conditions due to the economic reform and afterwards an access of Slovakia to the EU in 2004 was manifested by a decrease in the animal number and the variable development of production. The average total poultry number reached 13 074 thousand pieces per annum. Relatively highest inter-annual decrease of poultry numbers was reached in 2006 (7.4 %) and in 2008 (12.8 %). The development of hen number was variable. The most significant hen decrease was marked in 2004 (7.8 %) (**Jamborova, 2011**).

To avoid crossings with imported strains in Slovakia, Poland and Hungary it was decided to collect purebred animals in one to four localisations to be bred in closed stocks. In the 1970s, the Research Institute of Animal Production in Nitra proposed creating a genetic reserve of three local strains: New Hampshire, Rhode Island Red, White Sussex and in 1994 a fourth one – Oravka (**Hanusova et al., 2014**).

Oravka breed was formed by crossbreeding of local hens in the Orava region with Rhode Island, Wyandotte and New Hampshire breeds. The breeding programme aimed at forming a dual-purpose breed with good egg production, growth ability and adaptability to harsh environment started in 1950s (**Kadlecik et al., 2004**). The breeding programme consisted of three consecutive phases **(Chmelnicna et al., 2004**), and Oravka breed was recognized in 1990. Oravka is a dual-purpose breed kept for egg and meat production, respectively. The animals are of yellowish-brown colour and of rectangular body frame. The live weight of adult females is 2.2 to 2.7 kg and that of males is 2.8 to 3.3 kg. About 180-200 eggs per female and year are produced. The egg shell is brownish. The minimum hatching egg weight is 55 g (**Hrncar and Weis, 2007**). A survey of Oravka living animals (breeding males and females) was done by **Hrncar and Weis (2007)** and **Oravcová et al. (2010),** respectively.

Geese

Geese are one of the oldest species of domestic poultry. There are two recognized national goose breeds in Slovakia - Suchovska and Slovak, as a combination of old indigenous breeds and still genetically undefined foreign introduced breeds. The **Suchovska Goose** is a result of crossbreeding of local yellow fathering geese with French (Toulouse, Landes) and German (Pomorany, Steinbach) geese. This breed of geese 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 (**Kadlecik et al., 2004**). It was produced in seeking to breed geese of bigger body frame, firm constitution and of compact and solid body. The geese are suitable for pasture. They are also suitable for small farming because of the preservation of the clucking instinct (**Weis and Hrncar, 2009 a; b**). In case of the Suchovska goose its lowest population occurred in 2003 (67 birds: 26 males and 41 females) and at present there is 150 females and 75 males.

The **Slovak White goose** is a breed of domestic goose originating in Nitra in Slovakia. The Slovak White goose is an autochthonous breed of Slovakia. It was originated from regional breeds from the South-Western Slovakia in 1940s. Regional German and Hungarian types of goose were used during the breeding process. Due to low population numbers (200 females and 100 males) the Slovak White goose is considered to be an endangered breed. Preservation of the local breed genome is important in order to save its unique genetic traits. The Suchovska and Slovak geese were categorized as endangered breeds (**Weis et al., 2010).**

Rabbit

Rabbit breeding has a long-year tradition in Slovakia. The first two rabbit breeds developed in Slovakia were Slovak grey-blue Rex and Blue of Holic rabbit. In Slovakia, rabbit husbandry was applied to the research programs at the end of the 1960s at the Research Institute for Animal Production (RIAP) Nitra. The results of the breeding efforts in the 70s and 80s were manifested in the early 90s, when the changing political and social situation caused an interest in broiler rabbit breeding as a sphere of agriculture with a great commercial potential in the creation of a national breed – "Nitra rabbit", which was introduced into The Book of Rabbit Breed Standards within Slovak Breeders Association in 1977. Twenty

years later it was recognized as a national breed under the name "Zobor rabbit" **(Rafay et al., 2014)**.

Nowadays, there are about 70 pure breeds of rabbits in Slovakia in many color types. Of this number, 12 breeds were generated by Slovakian breeders. At present, there are (fourteen) Slovak national breeds (Nitra rabbit, Zobor rabbit, Zemplin rabbit, Slovak Greyblue Rex, Holic blue, Slovak pastel Rex, **Liptov bald-spotted rabbit, Zemplin pastel rabbit, Diminutive Slovak Greyblue Rex,** Chrabrany rabbit, Gepard rabbit from Štrba, Saris giant rabbit, Slovak tricolor strakosh, Tatra baran) **which** are currently kept by breeders organized into Slovak Breeders Association. Some of breeds are represented by very small populations consisting of approximately 20 breeding females and of 10-15 males.

State of utilization of genetic resources

Pursuant to Act No. 194 of 1998 and the Regulation of the Ministry of Agriculture of 2000 on Performance testing, heredity testing, health testing, exterior evaluation of farm animals, establishment and management of herd book, breed registry, record-keeping and verification of the origin of farm animals, the following traits are monitored in charge of performance testing in farm animal species.

In **cattle,** monitoring is carried out of traits, such as milk performance (milk yield, content of fat and protein, especially in dairy and dual purpose cattle), beef performance and carcass traits (beef cattle), growth traits (weight of calves and young cattle, daily gain etc.) and other traits, like milkability, persistence of lactation, number of somatic cells in milk, length of production period, parturition course, ratio of still-born calves or exterior traits. These data represent a basis for genetic evaluation and breeding value estimation (for individual traits or aggregate selection indices) using modern genetic and statistical analyses and taking into account all accessible information on the animals.

In **pigs**, performance testing includes reproduction traits, field and station test, fattening and slaughter value or meat quality traits. Performance testing in **sheep** includes fertility traits, lamb growth intensity and milk production traits. In meat sheep, fattening and slaughter values are tested. At present, genetic evaluation of sheep using animal model is developed. Genetic evaluation of milk performance (animal model – test day model) has been introduced into routine. Genetic evaluation of other traits is under preparation.

Goat performance testing includes reproduction and milk production traits.

Tomka et al. (2016) analysed the development of 15 breeds in the Slovak Republic using the data on purebred animals registered in herdbook in years 2005, 2010 and 2015. These data are the part of the animal genetic resource monitoring, which is performed in cooperation with the Breeding Services of the Slovak Republic, s.e. and authorized breeders` organizations of respective breeds. Twelve breeds were classified as being endangered in 2015, from which 6 were classified as critically endangered. In cattle the class of endangerment of Slovak Spotted breed was not changed during the whole period. However, the effective population size was decreasing continually. Results showed an increase of purebred registered Pinzgau animals in first half of the studied period and a decrease in the second half. However, the effective population size was also decreasing continually during the whole period.

The cryobank (within the programme of biodiversity preservation) conserves available semen and embryos of cattle and horse breeds. The aim is to extend the supplies of reproductive cells and embryos also of other farm animals. Cryopreservation and storage of semen and embryos represents the basis of animal genetic resources under *ex situ* conditions.

Conservation of avian genetic resources in the gene bank is necessary for the preservation of endangered species. Cryopreserved biological material can be used for recovering the lost variation within breeds and restoring of the breeds, which have become endangered as a result of destruction of their natural conditions (**Sawicka et al., 2011).**

In our institute NPPC-RIAP Nitra we are dealing with cryopreservation and storage of the genetic material like semen from bulls (Chrenek et al., 2017 a; b; Makarevic et al., 2018; Olexikova et al., 2016; Spalekova et al., 2012); rabbits (Kulikova et al., 2014; 2017), rams (Kulikova et al., 2018; Makarevic et al., 2012) , chicken (Svoradova et al., 2018) or embryos from cattle (Makarevic et al., 2014), rabbit (Chrenek et al., 2014), stem cells from rabbits (Kovac et al., 2017; Vasicek et al., 2011) and DNA from honey bee (Bauer et al., 2017).

Molecular Characterization of Animal Genetics Resources

Characterization of animal genetic resources encompasses all activities associated with the identification, quantitative and qualitative description and documentation of breed populations, their natural habitats and production systems to which they are or are not adapted to (**FAO, 2007**). Characterization is typically differentiated into two categories: phenotypic characterization and molecular characterization.

The term „phenotypic characterization" of animal genetic resources generally refers to the process of identifying distinct breed populations and describing their external and performance characteristics within given production environments.

Molecular characterization or genetic characterization therefore, can be defined as the complementary procedures used to unravel the genetic basis of phenotypes, their patterns of inheritance from one generation to the next, within-breed genetic structure and levels of variability and relationships between breeds. The guidelines on Molecular characterization published by **FAO (2011**) include a short overview of progress in molecular characterization of Animal Genetic Resources and prospect for the future.

It is obvious that in future special attention should be paid to establishing a gene bank of tissues, blood derivatives and DNA samples of farm animal genetic resources. Advances in molecular biology, principally in the development of polymerase chain reaction (PCR) for amplifying deoxyribonucleic acid (DNA), DNA sequencing and data analysis have resulted in powerful techniques, which are used for the screening, characterization and evaluation of genetic diversity.

Genomic tools, like SNP-genotyping and whole genome sequencing, and their analysis offer great opportunities for the conservation and utilisation of animal genetic diversity, both among and within breeds. These genomic tools can be used to detect potentially valuable rare alleles and haplotypes. They are important parts of the genetic diversity we need to conserve now for possible utilisation in the future.

Some of the recently developed molecular tools or techniques and their potential application in conservation of animal resources include DNA sequencing, conventional Sequencing Technique, next Generation Sequencing Techniques (microsatellites or simple sequence Repeats SSRs, single-nucleotide polymorphism – SNP, random amplified polymorphic DNA – RAPD, amplified fragment length polymorphism – AFLP, markers of sex-specific inheritance) (**Pareek et al., 2011**).

The management of genetic diversity with genomic tools is outlined both *in vitro* (gene banks), and *in vivo* (small populations of rare breeds or large populations with small effective population sizes).

Molecular characterization of animal genetic resource is offering unprecedented opportunities for increasing agricultural productivity and for protecting the environment through reduced use of chemicals for fumigation and control of external parasites. Molecular markers based on DNA have a high polymorphism level, and they have been successfully used for evaluation of genetic diversity and variation in breeding programmes with an impact on the level of genetic conservation schemes (Židek and Kasarda, 2010). In Slovakia, Šidlova et al. (2016) analysed genetic structure of Pinzgau cattle using autosomal SNPs. Analysis of genetic structure of populations and degree of diversity loss within horse breeds (Lipizzan, Furioso, and Nonius) that belong currently to most endangered warmblood horse populations in Slovakia was done by Kasarda et al. (2018).

The Global Plan of Action for Animal Genetic Resources

Animal development and conservation are essential for agriculture and food production, rural development and environment. In recognition of the need to develop an effective framework for managing these resources and deal with the threat of their genetic erosion in September 2007 brought together representatives of 109 countries at the First International Technical Conference in Interlaken (Switzerland). The Conference adopted the Global Plan of Action, which sets out 23 strategic priorities defining sense approach to the management of animal genetic resources. The Conference also adopted the Interlaken Declaration, which confirms the commitment of countries to implement the Global Plan of Action at the national level and ensure the use of livestock biodiversity in support of food security and their availability for future generations (**Hiemstra et al., 2014).**

Ratification of the Convention on the Biodiversity was confirmed by the Slovak Republic also in 1994 to protect biodiversity, to guarantee sustainable use of its components including also animal genetic resources.

The importance of animal genetic resource protection emerges from biological, economic, landscaping and cultural needs of each country that are realized using national gene banks (NGB). NGB should guarantee monitoring, collection and preservation of samples from genetic resources (spermatozoa, oocytes, embryos, somatic cells, tissues, DNA etc.) in original condition, deeply frozen or lyophilized, their storage, molecular-genetic characterization of samples, research-comparison of the genetic biodiversity, management of information system about stored samples and their use for original breed restoration.

National gene banks collaborate in EUGENA, the European Gene Bank Network for Animal Genetic Resources. The objective of this platform is a rational, efficient, regional, integrated conservation approach in Europe. Twenty five countries in Europe contributed to a survey to generate an overview of key characteristics of national gene banks including legal and institutional aspects, history of collections, collection objectives and documentation.

CONCLUSION

Domestic animal diversity represents a resource that is crucial to achieving food security for the rapidly growing human population, not only with respect to the local or national situation, but also because of the increasing interdependence among countries for unique animal genetic resources.

Identification of Slovak autochthonous breeds possessing unique genes in their genetic fund is of interest for the national, European and world genetic science and for livestock management practice.

The possible extinction of some breeds mean irrecoverable loss of the genetic variability and so the loss of unique gene and allele combinations that would be very useful in the future e.g. for the generation of new farm animal genotypes.
New development in cryobiology and reproductive technologies offer opportunities to store genetic material in many different forms and for regeneration of individuals from cryopreserved material. The national gene bank and the private breeding sector will mutually benefit from this development. Research is needed to analyse opportunities and risks of new technologies in the context of conservation and sustainable use of animal genetic resources.

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