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Slovak Journal of Animal Science (ISSN 1337-9984) is an international scientific journal that publishes original scientific papers, reviews, short communications, chronicles of important jubilees, reports of participation in important international conferences on animal science in English language.
Topic of the journal are problems of animal production, mainly in the sphere of genetics, breeding, nutrition and feeding, physiological processes of digestion, conservation and treatment of feeds, biotechnology, reproduction, ethology, ecologization of breeding, biology and breeding technology in farm animals, quality of meat, milk, wool, economy of breeding and production of farm animals, mainly: cattle, pigs, sheep, goats, horses, poultry, small farm animals and farm game. There are published also articles from the sphere of biochemistry, genetics, embryology, applied mathematical statistics as well as economy of animal production. There can be published also articles from the sphere of veterinary medicine concerning the themes of the journal.

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Faculty of Biotechnology and Food Science
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The 1st International Scientific Conference
Biotechnology of Farm Animals

Topic: Biotechnology in Animal Biodiversity

December 5th, 2013

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Dear Participants and Colleagues,

We are very pleased to welcome you in the 1st International Scientific Conference „Animal Biotechnology" focused on **„biotechnology in animal biodiversity protection"**. The conference is organized by the Animal Production Research Centre Nitra and Faculty of Biotechnology and Food Science, Slovak University of Agriculture Nitra.

The aim of the conference is presentation of current research results and legislative information regarding farm animal biotechnology, with actual point on Biotechnology in Animal Biodiversity. Moreover, this conference will provide an opportunity to gather researchers engaged in this field of research in order to exchange their skill and experience as well as to establish potential collaboration in the given points at issue. We would appreciate participation of colleagues from various research institutions and universities at this conference.

We would like to wish you nice atmosphere for the presentation and creative discussion and stimulating ideas during your stay in Nitra.

Nitra, December 5th 2013

Peter Chrenek

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ANIMAL GENETIC RESOURCES IN POLAND

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ABSTRACT

The evaluation of the breed structure in the most common livestock species kept in Poland suggests that in the case of the majority of species, the current structure is relevant and addresses the needs of specific sectors (dairy and beef cattle, horses, sheep and goats, fur animals, fish and honey bees). The domestic breeding stock available for pig fattening units does not fully reflect the needs of the sector. Therefore, the domestic breeding material is complemented by a growing import of replacement stock from international breeding companies. Moreover, in 2011, already 2.8 millions of piglets were imported to commercial fattening farms managed in open systems, and in 2012 this number increased up to 3.5 millions. As was already indicated here, the fast growing poultry production sector, both for meat and for eggs, is based, either solely or in the majority, on imported genetics of high productive potential.

Key words: animal genetic resources; livestock

INTRODUCTION

Conservation of genetic resources for food and agriculture requires maintaining the biological wealth of agricultural lands, including diversity of breeds and varieties of farm animals and of cultivated crops, but also their utilisation in such a way that it will be sustainable, and will provide a solid basis for the future needs of plant and animal breeding.

Therefore, conservation of animal genetic resources (AnGR) cannot be restricted or focused on rare breeds only, it has to address all populations of farm animals, and be based on improved understanding of their biology and status of their populations to prevent potential threats, especially genetic erosion. It is necessary to fully utilise in AnGR conservation approaches both *in-situ* and *ex-situ* methods, supported by the theory of small populations' management (Filistowicz and Zwolińska-Bartczak, 1995; Filistowicz, 2011; Zwolińska-Bartczak *et al.*, 1995; Żuk *et al.*, 1995).

National legislative framework relevant to AnGR conservation and sustainable will include: the law on organization of animal breeding and reproduction (animal breeding acts of 1997 and 2007 with further amendments) and the law on measures to support rural development (2004, 2007), as well as the law on organic agriculture (2001).

In 1996, the Minister of Agriculture and Food Economy formally established the National Focal Point for Animal Genetic Resources, and appointed the Central Animal Breeding Office as the host institution. At that time, the Central Animal Breeding Office was responsible for performance recording, breeding value evaluation and artificial insemination in most of farm animal species. The key achievement during this initial period was development of conservation programmes for populations that were covered by the state support measures. In May 2000, the Minister accepted 32 conservation programmes to be implemented for 75 breeds, varieties and lines of farm animals. These

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programmes covered all major livestock species that were kept in Poland, included all native breeds; and also a small number of imported populations (mainly poultry) that were bred in Poland for more than 30-40 generations.

Due to the ongoing transformation in organization of animal breeding, the National Focal Point was transferred in 2010 to the National Animal Breeding Centre and since January 2002 to the National Research Institute of Animal Production. The current structure to support AnGR conservation is provided in Figure 1.

Global contribution

Poland actively participated in the preparation of the first Global State of the World`s AnGR Report (SoW-AnGR) initiated by the Food and Agriculture Organization of the United Nations (FAO), including by preparing a national report on AnGR. The Country report (2002) contained: a) an analysis of the state of biodiversity in the livestock sector as well as a review of production systems and evaluation of the utilization of animal genetic resources; b) analysis of the changing demands and trends in livestock production; c) a review of the state of national capacities to manage AnGR; d) ten overall national priorities for the conservation and sustainable utilization of AnGR and e) recommendations for enhanced international co-operation in the field of farm animal biodiversity. The agreed national priorities for action were gradually implemented over the last ten years, which resulted, among other things, in increased farmers' participation in *in-situ* programmes, introduction of new breeds into conservation programmes and

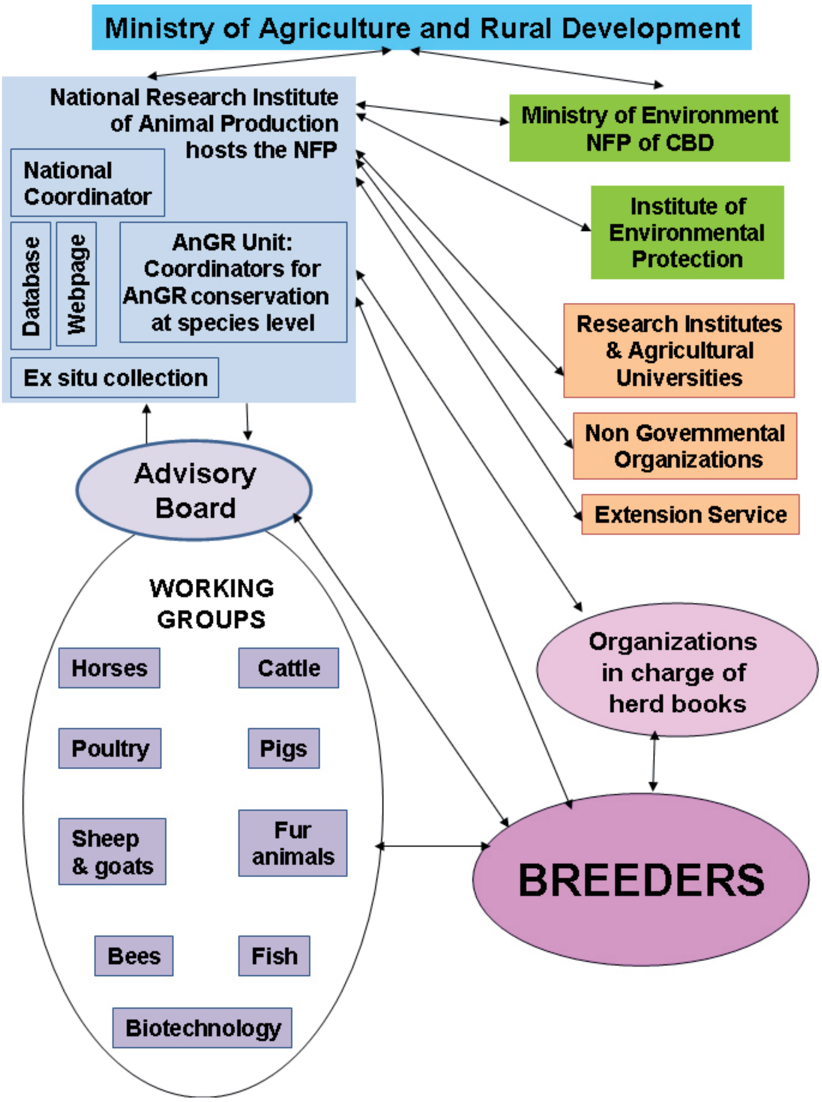

Fig. 1: The national network on animal genetic resources in Poland

enhancing *ex-situ* conservation measures.

The report on the State of the World's Animal Genetic Resources (FAO, 2007a) resulted in compilation of existing knowledge and information, and provided the first ever evaluation of the state of the resources themselves, the state of efforts and actions to enhance their sustainable management, and the state of art in development of both *ex-situ* and *in-situ* methods aimed at improved conservation and sustainable use of AnGR.

The Global Plan of Action for animal genetic resources (GPA) was adopted in Interlaken, Switzerland, in 2007, and later endorsed both by the FAO Council and the FAO Conference, demonstrating commitment by all FAO member countries to its implementation.

Poland made substantial efforts to implement the GPA and has provided its first Country progress report on AnGR, which was due in 2011. We have already initiated preparation of our country contribution to the second Global Report SoW-AnGR, which is scheduled to be presented to the Commission on Genetic Resources for Food and Agriculture in 2015. Preparation of the second Country progress report on the implementation of the GPA (FAO, 2013a) is also underway.

Implementation of the GPA is conducted through the national plans and programmes; development of National Strategies and Action Plans for AnGR (NSAP-AnGR) as recommended by the FAO (2009). Till 2013, a number of countries have already developed NSAP-AnGR (in Europe: Armenia, Austria, Croatia, Czech Republic, Denmark, Finland, Germany, Italy, Montenegro, Norway, Slovenia, Spain, Sweden and UK) (FAO, 2013b). Other countries are conducting preparatory work.

The National Research Institute of Animal Production was entrusted by the Ministry of Agriculture and Rural Development with the preparation of the NASP-AnGR, with the process based on the broad participation of breeding and research communities and NGOs, and extensive consultations during three national level conferences organized in Balice. The NSAP-AnGR has to fulfill an overall goal: "Effective utilisation and conservation of genetic resources of farm animals to contribute towards development of sustainable agriculture"; there are also six specific objectives to address various aspects of the overall goal. The structure of NSAP-AnGR follows the GPA structure with four strategic priority areas (characterisation, utilisation, conservation and capacity building). The Polish NSPA-AnGR contains in total 15 national strategic priorities (4+4+3+4) and 52 actions. In September 2013 the NSAP-AnGR was at the final stage of editing (Martyniuk and Krupinski, 2013).

AnGR conservation

The *in-situ* conservation programmes cover at present 88 genotypes (breeds, varieties and lines) kept at 3010 herds. The total number of females is over 90 thousands, as presented in Table 1.

The *ex-situ in vitro* conservation measures are important components of the overall AnGR conservation efforts. Cryopreservation activities were included in the NSAP-AnGR, with the aim to collect sufficient biological material that will allow the recovery of lost diversity within livestock breeds, if needed. To support *ex-situ* conservation activities, the National Research Institute of Animal Production has established the National Bank for Biological Material (*Krajowy Bank Materiałów Biologicznych, KBMB*). The National Bank for Biological Material is located in a separate building in the headquarters of the Institute, in Balice. Due to the veterinary requirements it consists of a complex of four independent divisions (gene banks for: cattle, horses, pigs, and sheep and goats - jointly). The National Bank will store biological material of breeds belonging to all five major livestock species.

In the National Bank for Biological Material, with financial support from a number of research projects, a large quantity of biological material is already deposited: 4400 semen samples of Swiniarka, Wrzosowka and Olkuska sheep (Krupiński and Martyniuk, 2009), 51 500 samples of semen from bulls belonging to four Polish native breeds: Polish Red, Polish Red and White, Polish Black and White and Polish Whitebacked cattle, as well as 1900 embryos of Polish Red cattle (Szczęśniak-Fabiańczyk, 2012).

The state of the livestock production in Poland

Livestock production in Poland is based on utilisation of a few key species: cattle, pigs, chicken and turkeys, that provide the majority of animal products for the domestic market as well as for export. In the last ten years, livestock production output and intensity have systematically increased, e.g. from 230.9 kg of warm carcass weight per 1 ha of agricultural land in 2000 to 342.2 kg in 2011; and from 648 l of milk /ha in 2000 to 780 l of milk /ha in 2011 (GUS, 2012).

Cow milk production in 2011 was 12052.2 million litres (GUS, 2012). Slaughter animal production, on the basis of warm carcass weight from six major species, reached in 2011, in total, 3757 thousands ton, with pigs contributing 1876 thousands ton, poultry 1427 thousands ton, and cattle, calves, horses and sheep respectively: 384; 28.0; 17.8 and 1.7 thousands ton (GUS, 2012).

The highest share in meat production belongs to pork (49.9 % of total production) followed by poultry meat (38.0 %) and beef and veal (10.2 % and 0.7 %).

Table 1: Number of herds/flocks and number of animals included in the *in-situ* conservation programme (data for 30th June, 2013 - National Research Institute of Animal Production)

Species	Number of breeds/ varieties/ lines	Number of herds/flocks	Number of females
Horses	7	1271	5295
Cattle	4	893	7498
Sheep	13	674	46014
Goats	1	2	29
Pigs	3	84	1764
Laying hens	19	19	19 570
Geese	14	14	4 820
Ducks	10	10	3 961
Rabbits	1	8	-
Fur animals (foxes, chinchilla, polecats, nutria)	12	24	1219
Total	84	3 010	90460
Local bee linies	4	-	699 (1282*) colonies
TOTAL	88	-	-

* jointly with the Kampinowska bee breeding region and Augustowska bee breeding zone

The remaining meats share (horsemeat and mutton/ lamb) is only 1.2 %. In the structure of poultry meat, the dominant position is held by chicken meat (72.1 %) and turkey (18.4 %). The contribution of waterfowl meat accounts for 9.5 % only. In the last period, egg production increased from 7621 million in 2000 to 10373 million in 2011 (GUS 2012).

In general, small ruminant production is at a very low level. In 2011, sheep wool production was 775 tons only. Goat meat production was marginal, around 497 tons. Also, goat and sheep milk production was scarce, 35 thousand tons and 500 tons respectively (GUS, 2012).

Poland still maintains a very high level of mink skin production (5.5 million) while other carnivore species (fox, raccoon and polecat) skin production is at a much lower level: 180, 3 and 1.2 thousands respectively. Rapid decreases in demand for the herbivore species skin products resulted in drastic decrease in the use of rabbits and nutria with a rapid decrease of meat provided by these two species (5764 and 30 ton respectively in 2011) (GUS, 2012).

In Poland, utilisation of honey bee solely for pollination is rare. The main product is honey, and three breeds are used mostly: Carniolan bee (*Apis mellifera carnica*), Caucasian (*Apis mellifera caucasica*) and Central European (*Apis mellifera mellifera*). The sector is covering 1 246 633 bee colonies, which are kept in about 50,000 apiaries, that produced in 2011, in total, 23 thousands kg of honey, with average of 18.4 kg per colony.

Both aquaculture tradition in Poland and the market share indicate that the key commercial fish species in Poland are carp and trout. At present, annual carp production is at the level of 17.000 tons and trout of 18.000 tons, providing about 20 % of total fish and 90 % of freshwater fish for the domestic market.

Livestock number

Dairy cattle numbers dropped during the period 2000-2011, from 6082.6 thousands to 5760.6 thousands. In December 2012, the total population size was even smaller: 5520.3 thousands (GUS, 2013a). The dairy cow population in 2010 counted 2 528.8 individuals but in the next two years decreased further to 2 468.7 (GUS 2013a). The active population consists mainly of the Polish Holstein-Friesian Black and White (88.64 %) and Red and White varieties (3.09 %), Simmental (1.49 %) and in 4.84 % of dairy crosses.

The remaining breeds' share in the active population is only 1.95 %, and this includes mainly four breeds under the conservation programme Polish Red (around 4000), Polish Red and White (3500), Polish Black and White (2500) and Polish White-backed cattle (350 cows) as well as imported dairy breeds, such as Jersey, Montbeliarde or Ayrshire. All cattle breeds included in the conservation programme are dual purpose, milk and meat type. There is a growing interest among smallholders to keep native breeds and participate in the conservation programme.

The development of beef cattle breeding in Poland, initiated through the national programme in 1994, was solely based on imported genetics from countries with long traditions in the utilisation and development of beef cattle breeds. In 2011, the population of purebred and crossbred beef cattle was about 25 000 animals, with 16 216 purebred and 7 459 crossbred cows. These relatively small populations however provide a valuable basis for further development of the sector with the expected abolition of milk quotas. In beef cattle population, Limousine has the highest share (69.7 %), followed by Charolaise (14.4 %); Hereford (4.7 %) and Simental (3.7 %). In backcrossed population, the position of Limousine is even higher (89.2 %), indicating strong interest of beef farmers in this genotype.

In the years 2000-2007, the pig population was about 17-18 millions animals. By the end of 2012, pig number were only 11.1 million, lower by 14.8 % from the year before (GUS, 2013b). In 2011, only about 15 000 sows were under performance recording including 6317 Polish Landrace, 5149 Polish Yorkshire, 736 Duroc, 513 Pietrain and 67 Hampshire. Among native breeds included in the conservation programmes, performance recording was carried out for 804 Pulawy sows, 826 Zlotniki Spotted and for 661 Zlotniki White sows.

In the period of 2000-2010, the horse population decreased, with only 254 000 horses in 2011. In 2012, the number of mares registered in the stud books was 22 000 and the number of stallions was 4.5 thousands. Thus, the active population accounts for about 8.8 % of the total horse population. The majority of commercial population belongs to the Polish Coldblooded horse. In this species, the diversity of breeds kept in the country is tremendous, among them, seven native breeds are included in horse genetic resource conservation programmes (Hutcul, Polish Konik (since 80thies), Maloposki, Slaski (since 2004) Wielkopolski Sokolski and Sztumski (since 2007).

During the period of 1995-2010, sheep number dropped from 713 000 to 268 000. In December 2012, the sheep population was only 218.5 thousands, including 134.7 thousands of breeding ewes (GUS, 2013a). In 2010, 57.3 % of active population consisted of Polish Merino, Polish Lowland and Polish Longwool sheep. In this species, the number of native breeds included in the conservation programme is high (13) and includes: Polish Heath sheep; Swiniarka sheep; Olkuska sheep; Polish Cakiel in the old type; Polish Mountain sheep colored variety; Polish Merino in the old type; Polish Merino colored variety; Wielkopolska; Uhruska; Żelezna; Korideil; Pomorska and Kamieniecka sheep.

In 2002, there were 193 000 goats in Poland, including 111 000 females. In 2010, there were only 117 268 goats, and in 2011 the number further dropped to 111 800 (GUS, 2012). The majority of the goat population is represented by Polish White Upgraded goat (39.9 %) and Polish Coloured Upgraded goat (21.1 %). Other breeds under performance recording include: Saanen, Alpine, Anglonubian, Toggenburg and Boer goats.

The breeding stock of meat chicken and turkeys is imported in the form of grandparental stock and parental sets. In the case of laying hens, 95 % of breeding material comes from international breeding companies, while 5 % are from three Polish pedigree farms that specialise in dual purpose breeding stock. The genotypes under the domestic breeding programme include: Rhode Island Red (4 lines), Rhode Island White - New Hampshire, Barred Rock (3 lines each), Barred Plymouth Rock and Sussex (2 each) and one line of Leghorn and of Green-legged Partridge chicken. Chicken genetic resources conservation programmes are carried out for 10 lines of the following breeds: Green-legged Partridge, Yellow-legged Partridge, Polbar, Rhode Island Red, Rhode Island White, Leghorn and Sussex.

The goose genetic improvement programme is implemented on the basis of two lines: male (W-33) and female (W-11) of the White Italian goose. Beyond these two lines, and their crosses, there are 14 breeds and varieties of geese under the *in-situ* conservation programme. In case of ducks, only two Pekin lines (A-55 i F-11) and Dworka synthetic breed (line D-11), developed through crossing of cayuga males with females of A-44 i A-55 Pekin lines, are under genetic improvement programme. Duck genetic resource conservation programme covers 10 lines, with 8 of them belonging to Pekin breed.

In 2010, the performance recording and breeding value estimation was conducted in 375 flocks of fur animals managing a total population of 44 766 breeding females. The genetic resource conservation programme under *in-situ* conditions is only for the following native breeds: Popielno rabbit, Beige Polish chinchilla, common fox of Pastel and White neck varieties, Lemon polecat and six varieties of nutria representing different coat colour mutations.

CONCLUSION

The evaluation of the breed structure in the most common livestock species kept in Poland suggests that in case of the majority of species, the current structure is relevant and addresses the needs of the specific sectors (dairy and beef cattle, horses, sheep and goats, fur animals, fish and honey bees).

Further intensification of livestock production underlines the need for effective implementation of genetic resource conservation programmes, both for local breeds and commercial breeds and using as appropriate *in-situ* and *ex-situ* measures.

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MANAGEMENT OF FARM ANIMAL GENETIC RESOURCES IN THE CZECH REPUBLIC

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ABSTRACT

The paper describes the development of management of farm animal genetic resources in the Czech Republic, its development, achievements and experience. It mentions also recent policy of access to and exchange of animal genetic resources.

Management of genetic resources for food and agriculture in the Czech (Czechoslovak) Republic developed under very various conditions over time. For decades, like the economy generally, it has been under state administration and planning. In 1950, according to the law the control over breeding was taken by the state and the system of the State Breeding Authority was fixed up by the Breeding Act in 1959. A list of authorized breeds was specified with designated breeding regions for each of them, according to production and natural conditions; except these any import was allowed only for testing in our local conditions and for experimental and scientific purposes.

Key words: farm animal genetic resources; local breeds; conservation program; access and exchange policy

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INTRODUCTION

After 1990 this system was gradually decomposed. Free access to markets, privatization of breeding services and the invasion of foreign breeding companies resulted in huge expansion of foreign genetics, not something what could be called "responsible imports". In 2013, there we have for example 35 horse breeds and about 40 sheep breeds, while most of them are low-numerous, several tens or hundreds of heads.

The Breeding Act No. 240/1991 first ever declared a support to preservation of gene pools and forming gene reserves in low-numbered and endangered original breeds. A list of 29 national breeds was agreed and the first National Program on Conservation and Utilization of Farm Animal Genetic Resources established (1995).

Later amendments (No. 154/2000) respecting the EU framework recognized breeder‘s associations as carriers of stud books and charged them to formulate and warrant breeding programmes. The management of genetic resources of the mainstream breeds is since therefore completely dependent on the decision of farmers. But this is largely influenced by the market situation, trade policy and interests of breeding companies most of which are in foreign hands. In the utilisation of mainstream breeds, awareness of the importance of intra-breed diversity and of agricultural biodiversity generally is still low and setting of different measures and subsidy rules mostly ignores it.

Where the law there does mention preservation of animal genetic resources, definition „genetic resource“ shall apply to animals and genetic material of autochthonous or locally adapted breeds only, which are filed into the National Programme. The program has been upgraded every 5 years, most recently in 2012. Since 1995 in the National Program there were gradually included 11 local livestocks and 2 poultry breeds, 10 rabbit and nutria breeds, 9 freshwater fish breeds and one honey-bee population.

In 2005, 34 source-lines of laying hen and water fowl used in hybridization programs and special experimental inbred populations of poultry for the

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Table 1: Breeds included in the National Program

Species	Breeds
Cattle	Czech Red and Czech Spotted cattle
Horses	Old Kladruby Horse, Bohemio-Moravian Belgian horse, Silesian Noriker Horse, Hutsul horse
Pigs	Black-Spotted Prestice pig
Goats	White Shorthaired goat and Brown Shorthaired goat
Sheep	Sumavska sheep, Valachian sheep
Poultry	Czech gold brindled hen, Czech goose

purposes of scientific research were also included into the National Program.

National Program is open to all breeders and the membership means an agreement between the program participant and the State. It establishes rules for breeding animals, i.e. respecting specific breeding schemes, keeping records and providing data, evaluation performance and utility traits upon the agreed method and providing samples and genetic material for a gene bank. The participant is to grant the material; costs of material collection, transport, testing and storage are covered by the State.

Early warning system and inclusion into protection mode

Data for breeds, as provided by breeders associations, are evaluated annually and breeds concerned are included into the various conservation modes, which confer specific measures and also affect the amount of support provided. The value of Critical population size Nek (limit for inclusion in protection mode) is based on the current size of the active population (Na) with regard to other indicators, like the generation interval, the average number of offspring, the length of productive life and the inbreeding coefficient of the concerned breed and is determined by the respective Breed Project.

The essence of the National Programme is the conservation of existing intra-breed diversity and the development and support of possibilities for its exploitation and in accordance with the globally-accepted procedures, both in-situ and ex-situ methods of conservation are applied. The *in situ* conservation is consisted in the regulation of breeding concerned individuals or groups (virtual nucleus) which are kept under purebred mating scheme according to given plan with the purpose to include their off-springs into the genetic nucleus.

The conservation *ex situ in vitro* is secured by conserving reproduction material (sperm doses,

Table 2: Setting conservation provisions by actual population size (Na)

Na	Categories of endangerment	Conservation provisions
Na > 2 Nk	not endangered	regular monitoring, occasional collection samples for gene bank
Na = 1,2 to 2 Nk	vulnerable	random preservation of genetic material (semen doses, embryos, somatic cells)
Na < 1,2 Nk		inclusion of breed (population) into protection mode
Na = 0,8 to 1 Nk	endangered	controlled mating system *in situ*, systematic cryopreservation of reproductive material *ex situ*
Na = 0,8 to 0,5 Nk	critically endangered	use of embryo transfer event. other biotechnology
Na < 0,5 Nk	not sustainable	considering the effectiveness of breed reconstruction

embryos, somatic cells) in gene bank. Populations are continuously sampled for building a genomic collection (DNA).

Access to and exchange of animal genetic resources

Access to genetic resources is not specifically regulated by domestic legislation. A fundamental for this topic is that private persons, e.g. farmers and breeders, own the individual animals. This property right implies a right to use and sell it for propagation purposes. The access to genetic resources is then based on and regulated by private (commercial) law agreements and a common understanding among breeders of the rights associated with the material, and is functional. Export of live individuals claimed as genetic resources has to be reported and confirmed by the Ministry and in some cases access to animals which have been supported within the NP therefore might be limited. Usually, if the animal concerned left progeny qualified as breeding stock, there are no obstacles to confirm the export.

Ownership and disposition rights to the samples collected and stored in gene banks is governed by contracts between the provider of sample and gene bank; samples obtained with a financial support from the state are owned by the state. Genetic material from gene banks is provided after approval by a designated person. Provision of genetic material to regenerate/reconstruct breed has the obligation to return back the same number and type of samples corresponding to biological and reproductive capabilities of the respective breed. Providing samples of genetic material for other purposes, for example for non-commercial research and education, is limited by the amount of disposable material, i.e. material can be granted if the stock will remain above the minimum values given in Table 3.

Table 3: Minimum safety stock of cryopreserved material

	cattle	horse	pigs	sheep and goat
ID: minimum number of the doses remaining	>500	>50	>80	>50
Embryos: Minimum number of stored embryos	>100	> 100	>100	> 100
and minimum size of current female population	>500	> 500	>300	> 500

Achievements and lessons learned

Achievements in conservation genetic resources can be assessed by internationally accepted indicators. The FAO evaluation criteria based on estimate of effective population size (Ne) are used.

Changes in seemingly non-problematic population can occur very quickly. At the beginning of the program, the number of the Czech Spotted cattle reached nearly 250 000 and in the year 2000 there about 30 000 dams still remained. After conserving 12 000 semen doses of 22 bulls and 950 embryos and the breed remained in the monitoring mode.

Then the breeder‘s association agreed to recognize mating the Czech Spotted dams to Montbéliarde and Fleckvieh bulls as purebred and reported the progeny still as Czech Spotted. After this period of massive upgrade breeding in 2009 from the newly licensed young bulls 76 % were sons of two top-ranked and partly related German Fleckvieh sires. In female population, only last 63 cows of wholly Czech origin remained. Testing the quality of semen doses of some bulls stored previously in the gene bank showed average to very low motility and unsatisfactory conception rate. Therefore, in 2011 the last 30 suitable dams were collected to a conservation nucleus with the aim to multiply the number to 100-150, dispersed into smaller groups across farms for *in vivo* conservation.

Marketing products from local breeds are considered to be the best support of conservation projects. Establishment of goat dairy farms and building market for their products after 1990 halt rapid decline in goat numbers and allowed the conservation of two local goat breeds. During the last decade, however, goat dairy farms due to continued growth in market demand for dairy products and meat rely on use exotic breeds, like Anglo-Nubian and Boer goat in the attempt to increase their production. Total goat numbers are growing, but the proportion of the two original breeds falls with a rather disturbing trend.

Therefore, we try to compensate for this disadvantage of the local breeds by facilitating access to innovative breeding methods. We provide breeders

Table 4: Development of population parameters of breeds included in the National Program

	starting year	n fem	Ne	breed status	n fem 2013	Ne 2013	breed status
Cattle							
Czech Red cattle	1995	16	10	critical-maintained	175	24	endangered-maintained
Czech Spotted cattle	2011	30	8	critical-maintained			
Horses							
Old Kladruby horse	1995	320	79	endangered-maintained	520	144	endangered-maintained
Bohemio-Moravian Belgian horse	2003	740	106	endangered	1 012	162	not at risk
Silesian Noriker horse	2003	320	88	endangered-maintained	476	103	endangered-maintained
Hutsul horse	1995	580	57	endangered-maintained	145	45	endangered-maintained
Pig							
Black-Spotted Prestice pig	1995	1 600	221	not at risk	120	105	endangered-maintained
Goat							
White Shorthaired goat	1995	32 000	4 318	not at risk	14 000	3 564	not at risk
Brown Shorthaired goat	1995	160	55	endangered	1 100	760	not at risk
Sheep							
Sumavska	1995	1 800	45	endangered-maintained	3 800	326	not at risk
Valachian	2004	170	36	endangered-maintained	500	97	endangered-maintained

n fem = number of active females
Ne = effective population size according to the formula Ne = (4MF/(M+F)) (Wright, 1931), where M and F are respectively the number of breeding males and females, modified by the model of Santiago and Caballero (1995) which takes in account selection in populations and is implemented in a simplified way as Ne x 0.7.
breed status = according to the FAO rank

with casein genotype analysis of their breeding bucks so that they select preferred genotypes which exhibit higher levels of milk protein and higher cheese yield. At the same time, however, we must ensure that within the population also other genotypes are used in a sufficient range.

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ANIMAL GENETIC RESOURCES IN LITHUANIA

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ABSTRACT

The objective of this study was to analyse data of farm animal monitoring and to estimate the status and effective population size of Lithuanian farm animal breeds. The principles of conservation of Lithuanian farm animal genetic resources and evaluation of Lithuanian breed status are based on the experience of animal breeding in small conserved herds and on the criteria of global strategy of FAO for the management of farm animal genetic resources. The minimal size of the conserved population was determined according to the breeding possibility of isolated animals without considerable inbreeding increase and regarding to the evaluation standards for breeding animals. The numbers of animals from native breeds were stabilized and even had increased for some breeds. After restoration and conservation of Lithuanian old native breeds' it seems that Lithuanian animal breeds could experience bottleneck effect. Effective population size for many Lithuanian breeds is below 50 till now, there is driftless reproduction and, therefore, the survival of the population is uncertain. The inbreeding can be minimized by having a larger effective population size (more than 50) and by using special mating schemes to maintain genealogical structure. The first decision in setting up conservation schemes was to carry forward the existing variability in the breeds. This is mainly concerned with the size of available resources, which could be adjusted by choosing individuals for conservation action from different lines and by carrying out planned mating between the chosen animals. Although the establishment of isolated herds with four non-related groups based on founders generation and implementation of special mating schemes had prevented the total disappearing of Lithuanian old animal breeds, the amounts of the compensatory payments are not sufficient for successful conservation of Lithuanian farm animal breeds. Despite the numbers of animals from rare Lithuanian breeds were stabilized and even have increased for some breeds, the numbers of sires should be increased and higher requirements for pure breeding and participation in the programme must also be considered.

Key words: effective population size; animal; breeds; genetic resources

INTRODUCTION

Animal genetic resources are those animal species that are used, or may be used, for food and agriculture and the populations should be conserved within those species as well as their stored genetic material. Animal genetic resources are among the most valuable and strategically important assets a country possesses. Animal genetic resource diversity may become even more important in the future as farmers and breeders face the challenge of adapting their animals to ever-changing socio-economic demands and environmental condition, including possible adaptation to climate change (FAO, 2009).

The globalization process should take into account the increasingly diversified social requirements which tend to safeguard the specific features of different communities and cultures linked to diverse traditions and history; these specificities have proved to be determining factors of sustainable development, going beyond environment-related and socio-economic aspects (Casabianca and Matassino, 2006). Local biological resources will constitute an element of increasing importance, especially as regards the necessity to restore the widest range of genetic differentiation of livestock species. Weitzman (1993) recognized that the issue of

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biodiversity conservation is an inherently economic question, and provided a framework with the objective to distribute a limited budget among conservation efforts and so maximize the expected welfare from diversity and other conserved characteristics. The activities for conservation of Lithuanian breeds were launched respectively in 1994 and 1999 when minimal herds of Žemaitukai horses, ash-grey and white-backed cattle, indigenous wattle pigs, local coarse-woolen sheep and a flock of "Vištines" geese, and herd of old genotype of Lithuanian White pigs were formed at the Institute of Animal Science (LIAS), and thus their complete extinction has been prevented (Razmaitė and Šveistienė, 2003). In 1998-2002, National research programme "Investigations and conservation of genetic resources of cultivated plants and farm animals" was approved and implemented. Researchers of LIAS prepared the National Programme for the conservation of the native farm animal genetic resources adopted by the Ministry of Agriculture of Lithuania in 1996 and in 2008. The main purpose of this programme was collection, investigation and conservation of Lithuanian local breeds. In this programme we used FAO definition of local breeds: breeds that occur only in one country (FAO, 2007). The national Farm Animal Genetic Resources Coordinating Centre was established at the Institute of Animal Science at the end of 2008. The main activities of the Centre are coordination of animal genetic resources, identification, characterization and evaluation of animals, monitoring, and preparation of conservation programs, conservation *in-situ* and *ex-situ*.

The objective of this study was to analyse data of farm animal monitoring and estimate the status and effective population size of Lithuanian farm animal breeds.

MATERIAL AND METHODS

Lithuanian farm animal genetic resources include old breeds (Žemaitukai, Ash-Grey and White-Backed cattle, Lithuanian Coarse-Woollen sheep, Vištinės geese) and breeds that were developed in the 20th century (Lithuanian Heavy Draught horse, Large type Žemaitukai horse, Lithuanian Black-and-White cattle (old genotype), Lithuanian Red cattle old genotype, Lithuanian White pigs old genotype, Lithuanian Blackface sheep). The principles of conservation of Lithuanian farm animal genetic resources and evaluation of Lithuanian breed status are based on the experience of animal breeding in small conserved herds and on the criteria of global strategy of FAO for the management of farm animal genetic resources (Bodo, 1999; FAO, 1999; Hammond, 1998; Marx, 1990). The minimal size of the conserved population was determined according to the breeding possibility of isolated animals without considerable inbreeding increase and regarding to the evaluation standards for breeding animals. The status of Lithuanian animal breeds was evaluated by their monitoring using the data of animals which are included in the breeding system of Lithuania. Period of our investigation was 1994-2012. The effective population size was expressed as $N_e = 4N_mN_f / (N_m + N_f)$, where: N_m – number of breeding males; N_f – number of breeding females (Maijala, 1999).

RESULTS AND DISCUSSION

The numbers of breeding animals in Lithuanian native breeds are presented in Table 1. The numbers of animals from native breeds were stabilized and even have increased for some breeds. The greatest proportion of Lithuanian White pigs shown in this table were pigs improved by immigration of lean foreign breeds. Only minor residual part of Lithuanian White pigs without any immigration after the breed recognition is bred as closed population. It is impossibility to increase the numbers of purebred pigs due to insufficient subsidies for pigs. The effective population size (N_e) for the original old genotype of Lithuanian White pigs without introgression of other pigs is only 38. *Ne* is important for understanding the effects of varying numbers of males and females on genetic drift variance and inbreeding. The levels of risk status based on N_e are: endangered, where $N_e < 50$, vulnerable, where $N_e < 100$ and care, where $N_e < 200$ (Maijala, 1999). Effective population size for many Lithuanian breeds is below 50 till now, there is driftless reproduction and, therefore, the survival of the population is uncertain (Table 1). The effective population size of most other Lithuanian farm animal breeds is low too and could be characterized as the critical and endangered (Table 1). Some native breeds have low N_e because there are critically low numbers of purebred breeding males like Lithuanian White boars or native bull sperm. The effective population size from 2004 only for such breeds as Lithuanian Heavy-Draught, Lithuanian Blackface sheep that were developed in the 20th have increased.

After restoration and conservation of Lithuanian old native breeds' it seems that Lithuanian animal breeds could experience bottleneck effect. For example the population of Žemaitukai horses in 1994 contained 30 purebred horses with recorded pedigree. Therefore, their genealogical structure is drastically narrow. Semen doses were collected and stored from 9 ash-grey and 7 white-backed heterogeneous bulls. Further, the number of bulls is decreasing. The conserved herd of Lithuanian indigenous wattle pigs had 19 founders of which five were non-related boars and fourteen sows - from five non-related groups. Nowadays there is just one herd of these pigs. The herd of Lithuanian native coarse-woollen

Table 1: Numbers of Lithuanian rare breed animals and their changes in 2004-2012 and the effective population size of Lithuanian native domestic animal breeds by N_e FAO (Maijala, 1999)

Breeds	2004			2010			2012			Risk status
	No. animals	Breeding females	N_e	No. animals	Breeding females	N_e	No. animals	Breeding females	N_e	
Horses										
Žemaitukai horse	191	70	55	421	170	110	564	206	120	Critical/endangered
Large type Žemaitukai horse	125	80	42	280	165	95	544	237	70	Critical/endangered
Lithuanian Heavy Draught horse	498	190	153	990	420	270	1174	566	205	Vulnerable
Cattle										
Lithuanian White-Backed cattle	322	174***	20	820	420***	20	1011	502***	12	Critical
Lithuanian Ash-grey cattle	375	210***	40	961	482***	28	1291	634***	28	Critical
Lithuanian Black-and-White cattle (old genotype)	-	-	-	1108	520	33	1290	685	28	Critical
Lithuanian Red cattle (old genotype)	100	100	23	126	60	23	30	20	16	Critical
Pigs										
Lithuanian White pigs (old genotype)	1164**	160***	20	1160**	46***	27	121***	74***	38	Critical-maintained
Lithuanian indigenous (Wattle) pigs	62	40	33	122	42	32	90	45	30	Critical-maintained
Sheep										
Lithuanian Coarse-Woollen sheep	99	32	50	122	65	50	220	93	49	Critical-endangered
Lithuanian Blackface sheep	1587	666	225	2398	1615	225	4226	2109	157	Critical-maintained
Geese										
„Vištinės“ geese	360	180	-	104	72	-	489	310	-	Critical-maintained

* In 2010 the number of Vištinės geese decreased to 70 birds (42 females)
** - Lithuanian White pigs, including improved pigs of open population
*** - pure breed breeding females

sheep had 6 founders and the population of Lithuanian “Vištinės” geese was restored from 100 eggs in 1996. Their effective population size (N_e) is less than 50.

The inbreeding can be minimized by having a larger $N_e > 50$ and by using special mating schemes to maintain genealogical structure. The first decision in setting up conservation schemes was to carry forward the existing variability in the breeds. This is mainly concerned with the size of available resources, which could be adjusted by choosing individuals for conservation action from different lines and by carrying out planned mating between the chosen animals. The variability in the conserved populations should be maximized and the target should be to minimize the overall kinship (Šveistys, 1982; Oldenbroek, 1999). This could be most efficiently achieved if the pedigree information was available in the population. In the case of old Lithuanian animal breeds, except horses, there were no pedigree records available, and we had to use other possible information, such as geographical accounts, to avoid redundant use of individuals, unnecessarily increasing average kinship. In order to meet these requirements the founders were divided into at least four disconnected pedigree animal groups and developed mating plans on the basis of experience in the pig breeding system, prepared by Šveistys (1967; 1982). The progeny of the founder generation in one group are mated with the progeny of the founder generation in another non-related group. After the progeny of the generation is available, their mating with the progeny of the first generation is the third group, and is carried out in order to obtain the second generation, etc. (Table 2).

Table 2: Circular breeding scheme for small populations (Razmaitė and Šveistienė, 2003)

Generation	Disconnected pedigree animal groups							
	1		2		3		4	
	Female	Male	Female	Male	Female	Male	Female	Male
Founder generation, parents	A x B		C x D		E x F		G x H	
Daughters, sons	A1	B1	C1	D1	E1	F1	G1	H1
1st generation, parents	A1 x H1		C1 x B1		E1 x D1		G1 x F1	
Daughters, sons	A2	H2	C2	B2	E2	D2	G2	F2
2nd generation, parents	A2 x F2		C2 x H2		E2 x B2		G2 x D2	
Daughters, sons	A3	F3	C3	H3	E3	B3	G3	D3
3rd generation, parents	A3 x D3		C3 x F3		E3 x H3		G3 x B3	
Daughters, sons	A4	D4	C4	F4	E4	H4	G4	B4
4th generation, parents	A4 x B4		C4 x D4		E4 x F4		G4 x H4	
Daughters, sons	A5	B5	C5	D5	E5	F5	G5	H5

Application of such circular mating schemes with 4 disconnected pedigree animal groups allows minimizing inbreeding. The coefficient of inbreeding (by Wright) amounted to only 6.2 % after four generations. On application of similar mating schemes with 8 disconnected pedigree animal groups, the coefficient of inbreeding should amount to only 3.12 % after eight generations (Šveistys, 1982). It was a great achievement to form four non-related groups (genealogical lines and families) of Lithuanian old critical indigenous breeds.

The breeding of animal from native breeds and income from their production is not competitive compared with industrial breeds and does not correspond to farmers' and market´s demands.

The total numbers of some protected breeds are decreasing, and separate breeds have grown only due to the 5 year obligation to increase herds by participating in the Rural Development Programme. From 2005 animals of critical and endangered Lithuanian native breeds are receiving the subsidies from Rural Development Programme (Table 3). After the end of 2004-2009 programme for rare animal breeds, some farmers have stopped participating in the programme (for example "Vištinės" geese) due to insufficient support level. Consequently, the numbers of animals from Lithuanian native breeds are decreasing. Despite the numbers of animals from some rare Lithuanian breeds were stabilized, the numbers of sires in all breeds should be increased and higher requirements for pure breeding and participation in the programme must also be considered.

Moreover, some farmers already have started crossing native breeds with superior foreign breeds. Small payments for critical animal breeds did not promote keeping native animal breeds.

When animals are located only in one herd, there is a risk that accidents, disease outbreaks, disposal of the herd for economic, health, age or other unforeseen reasons and circumstances could increase the danger of breed disappearing. Currently, some breeds are conserved just at the Lithuanian Centre of Farm Animal Genetic Resources Coordination of the Institute of Animal Science (Table 4) where nucleus is maintaining. There are only one isolated herd of Lithuanian indigenous wattle pigs and Lithuanian White pigs of the old genotype without introgression of foreign breeds and also one breeding flock of Vištinės geese.

The Lithuanian Heavy-Draught horses and Lithuanian Blackface sheep are still popular among breeders and have the effective population size of vulnerable risk status (Table 4).

The breeding of animals from native breeds (genetic pool) and income from their production is not competitive compared with industrial breeds. The production of native breed animals is lower and in the majority of cases their market quality (especially meat animal) is lower and it is difficult to sell them in the market. It could be defined that nowadays native breed animals are kept not for commercial purposes but for breed restoration and herd stability maintenance by preserving biodiversity for future generations.

Therefore, compensatory payments have helped to conserve the genetic resources and stabilize the numbers of some Lithuanian farm animal breeds by

Table 3: The level of subsidies (€) for animals participating in Rural Development Programme from Lithuanian breeds

Breeds	Subsidies in € for animals, including males and females	Started in year	2005		2011	
			No. stakeholders	No. animals	No. stakeholders	No. animals
			Horses			
Žemaitukai horse	198		8	80	54	255
Large-type Žemaitukai horse	198	2005	7	103	28	420
Lithuanian Heavy Draught horse	191	2005	25	290	115	731
			Cattle			
Lithuanian Ash-grey cattle	180	2005	36	71	136	486
Lithuanian White-Backed cattle	180	2005	37	62	140	436
Lithuanian Black-and-White cattle (old genotype)	180	2005	35	165	122	878
Lithuanian Red cattle (old genotype)	180	2005	1	100	1	10
			Sheep			
Lithuanian Coarse-Woollen sheep	28	2005	2	27	6	166
Lithuanian Blackface sheep	28	2005	11	938	51	2440
			Pigs			
Lithuanian indigenou (Wattle) pigs	65	2005	0	0	3	49
Lithuanian White pigs (old genotype)	65	2005	3	184	4	72
			Geese			
„Vištinės“ geese	3	2005 2011	1	45	1	100

reproducing new herds or animals following special mating rules and schemes in order to minimize inbreeding increase and prevent single individuals from getting extreme levels of inbreeding. However, low effective population sizes of some breeds or their genotypes show that the amounts of the compensatory payments are not sufficient and do not promote the increase in numbers of purebred pigs and geese. The amounts of the compensatory payments should be increased in taking into account of very small animal populations in Lithuania.

Implications

Although the establishment of isolated herds with four non-related groups based on founder generation and implementation of special mating schemes had prevented the total disappearance of Lithuanian old animal breeds, the amounts of the compensatory payments are not sufficient for successful conservation of Lithuanian farm animal breeds.

Despite the numbers of animals from rare Lithuanian breeds were stabilized and even have increased for some breeds, the numbers of sires should be increased and higher requirements for pure breeding and participation in the programme must also be considered.

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Table 4: Distribution of animals from critical and endangered breeds at farms of different stakeholders (% of total population)

Breeds	Distribution of National nucleus		Private farmers
	National stud and sheep farm of special purpose	Lithuanian Centre of Farm Animal Genetic Resource Coordination	
Horses			
Žemaitukai horse	19.4	7.2	73.3
Large type Žemaitukai horse	34.8	0.0	65.2
Lithuanian Heavy Draught horse	1.6	0.0	98.4
Cattle			
Lithuanian White-Backed cattle	0.0	2.5	97.5
Lithuanian Ash-grey cattle	0.0	2.0	98.0
Lithuanian Black-and-White cattle (old genotype)	0.0	0.0	100.0
Lithuanian Red cattle (old genotype)	0.0	41.0	59.0
Pigs			
Lithuanian White pigs (old genotype)	0.0	69.9	30.1
Lithuanian indigenous (Wattle) pigs	0.0	88.4	11.6
Sheep			
Lithuanian Coarse-Woollen sheep	0.0	50.6	49.4
Lithuanian Blackface sheep	31.3	0.0	68.7
Geese			
„Vištinės“ geese	0.0	48.7	51.3

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ANIMAL GENETIC RESOURCES IN SERBIA

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ABSTRACT

In the last few decades, farm animal genetic diversity has rapidly declined. Therefore, it is in the interest of the international community to conserve the livestock genetics. *In situ* (live animal herds) model of genome conservation is expensive and limited for practical usage. Therefore, *ex situ* (*ex vivo*) conservation model is developed to cryopreserve animal genetic resources in genome (gene banks) to regenerate a particular population in future. In Serbia, only in situ (*in vivo*) model of animal genetic resources preservation is performed. Autochtone animal breeds preserved in Serbia: cattle (Busha and Podolian), Domestic buffalo, horses (Domestic mountain horse and Nonius), Balkan donkey, pigs (Mangalica, Moravka, Šiška, Resavka, Šumadinka), sheeps (Cigaja, Sjenička, Svrljiška, Pirotska, Karakačanska, Lipska, Krivovorska, Bardoka, Vitoroga pramenka), goats (Balkan goat), dogs (Šarplaninac, Serbian shepherd dog, Serbian hound, Serbian tricolor hound Serbian yellow hound), poultry (Sombor chicken, Banat chicken, Svrljig chicken, Kosovo chicken, Domestic turkey, Domestic goose, Domestic duck) and pigeons (Serbian flyer, Sombor flyer, Niš flyer, Backa tumbler and Vršac tumbler).

Key words: animal; genetic resources; preservation; *in situ*

INTRODUCTION

In the last few decades, almost all farm animal breeds are experiencing a significant decrease of genetic diversity (Prentice and Anzar, 2011). This is a result of intensive genetic selection for small number of productive and reproductive traits (Buerkle, 2007), application of modern biotechnologies in reproduction, that allowed the production a large number of progeny from a single individual, as well as use the effective methods of transport and long-term storage of sperm cells, oocytes and early embryos (Patterson and Silversides, 2003). Conservation of genetic biodiversity of domestic animals is a global imperative in the biological, economic and moral sense; biologically, because biodiversity is a key condition for survival of life on our planet, economically, because a human population uses a huge number of animal species for food, medicine, chemicals, technological materials and energy. Moral, because man, as dominant species, is responsible for the maintenance and protection of all other species of living organisms, with which it must live on this planet. Thus, biodiversity preservation in domestic animal breeds and gene banks formation is in the interest of the international community (Prentice and Anzar, 2011).

Gene banks are defined as systematic and organized collection, preservation and exploitation of genetic material, by *in situ* (*in vivo*) or *ex situ* (*ex vivo*) methods. The *in situ* (*in vivo*) method involves preservation and reproduction of the small herds of various animal species, breeds, and lines (Wildt, 1999; Stančić, 1999). The major advantages for *in situ* conservation relate to the availability of technologies and the utilization of the breeds. The *in situ* conservation of live populations requires no advanced technology. There are optimal sampling strategies and breeding strategies, but the basic needs of an *in situ* program are already available and affordable throughout the world. The farmers of every region and nation know how to manage and maintain their local strains. They already have the capability; all they require is a direction.

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The disadvantages of *in situ* conservation are brought about by a lack of complete control over the many factors which influence the survival of individuals and therefore the genetic makeup of the conserved population (Henson, 1992; Furukawa *et al*., 1998). The method *ex situ* (*ex vivo*) involves long-term storage of gametes (sperm cells and oocytes) (Johnston and Lacy, 1995; Stančić, 2000, Stančić *et al*., 2001; Stančić *et al*., 2002; Stančić *et al*., 2005; Stanković, 2012) or early embryos by cryopreservation technology (Stančić, 2004; Boettcher, *et al*., 2005; Pereira and Marques, 2008; Prentice and Anzar, 2011; Chrenek *et al*., 2013) as well as by cryopreservation of testicular or ovarian tissue somatic cells (Andrabi and Maxwell, 2007; Pereira and Marques, 2008). The major advantages for *ex situ* conservation is the relative cost of collecting, freezing and storage of frozen material, as compared to maintaining large scale live populations, has been estimated to be very low. In particular, once the material has been collected, the cost of maintaining a cryogenic store is minimal. Such banks require little space and few trained technicians. A very large number of frozen animals from a large number of populations can be stored in a single facility. Cryogenically preserved populations suffer from genetic loss due to selection or drift. The method places a sample in suspended animation and that sample remains genetically identical since the time of collection to the time of use. Frozen animal genetic resources can be made available to livestock breeding and research programs throughout the world. The principal disadvantages of *ex situ*, or cryogenic preservation lie in the availability of the necessary technology and access to the frozen populations (Henson, 1992; Furukawa *et al*., 1998).

The aim of this paper is to present the current situation of animal genetic resources preservation in Serbia.

ANIMAL GENETIC RESOURCE PRESERVATION IN SERBIA

Serbia accepted the FAO global rules for animal genetic resource conservation, which are defined in the Convention on Genetic Diversity (Rio de Janeiro, 1992). According to these rules, three state and several individual farms were formed in Serbia to preserve the herds of indigenous breeds of domestic mammals (cattle, horses, donkey, sheep, goats and pigs) and birds species (chickens, geese, ducks, turkeys and pigeons) (Stojanovic and Pavlovic, 2003).

According to the Serbian plan for animal biodiversity preservation for the period 2011-2018 („Official Gazette of RS“, no. 13/2011), the following breeds of domestic mammalian and avian indigenous species are *in situ* (*in vivo*) preserved: Cattle (Busha

Table 1: *In situ* preserved indigenous breeds in R. Serbia

Species	Breed	No. of animals in the preserved herds
Horse	Domestic mountain horse	80
	Nonius	90
Donkey	Balkan donkey	350
Cattle	Busha	750
	Podolian cattle	350
Buffalo	Domestic buffalo	1100
Pigs	Mangalica	2000
	Moravka	100
	Resavka	35
	Šiška	
	Šumadinka	
Sheep	Krivovirska	250
	Pirotska	60
	Lipska	250
	Bardoka	40
	Karakačanska	125
	Vlaška vintoroga	450
	Cigaja	400
	Svrljiška	200
	Sjenička	300
Goats	Balkanska	250
	Svrljiška	200
Dogs	Šarplaninac	200
	Serbian shepherd dog	200
	Serbian hound	150
	Serbian tricolour hound	150
	Serbian yellow hound	100
Chickens	Somborska kaporka	200
	Banatski gološijan	1000
	Svrljiška	200
	Kosovska	200
Turkeys	Domestic turkey	180
Geese	Domestic goose	300
Ducks	Domestic duck	100
Pigeons	Serbian flyer	200
	Sombor flyer	200
	Niš flyer	150
	Bačka tumbler	150
	Vršac tumbler	100

Source: „Official Gazette of RS“, No. 13/2011.

and Podolian), Domestic buffalo, Horses (Domestic mountain horse and Nonius), Donkey (Balkans donkey), Pigs (Mangalica, Moravka, Šiška, Resavka, Šumadinka) Sheep (Tsigai, Sjenička, Svrljiška, Pirotska, Karakatčanska, Lipska, Krivovirska, Bardoka, Vitoroga pramenka), Goats (Balkan goat), Dogs (Šarplaninac, Serbian shepherd dog, Serbian hound, Serbian tricolour hound Serbian yellow hound), Poultry (Sombor chicken, Banat chicken, Svrljig chicken, Kosovo chicken, Domestic turkey, Domestic goose, Domestic duck) and Pigeons (Serbian flyer, Sombor flyer, Niš flyer, Bačka tumbler, and Vršac tumbler). Number of populations of certain species of domestic mammals and birds, according to state records since 2011 is shown in Table 1.

CONCLUSION

According to FAO reports (2007), in all species of domestic animals an increasing decline in biodiversity is observed. Therefore, there is a global increasing demand for efficient biotechnological research methods of long-term conservation of genomes of existing species, breeds and lines of farm animals.

Preservation of genetic resources is carried out using method *in situ* (*in vivo*), forming small herds of certain species of animals, or *ex situ* (*in vitro*), using long-term cryopreservation of sperm, oocytes, embryos or reproductive tissue somatic cells (testis and ovaries). Thus, it is possible to perform multiplication of desirable genotypes, when the need arises, although the donor animals are dead for a long time. Although cryopreservation technology has progressed in recent decades, the success of survival of frozen oocytes and embryos is still not satisfactory. Previous studies have shown that early embryos are more tolerant to cryopreservation than oocytes. In addition, cryopreservation technology is complex and expensive and not available for widespread use. It is therefore necessary to combine the use of methods of *in situ* and *ex situ*, with the aim of successful conservation of biodiversity of domestic animal breeds.

In Serbia, only *in situ* method for indigenous breeds of mammalian and avian species is performed.

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FARM ANIMAL GENETIC RESOURCES IN SLOVAKIA

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ABSTRACT

Despite lower production and abilities to compete with high-productive breeds, local breeds are still important for countries as their heritage. The cultural and environmental roles of these breeds have to be taken into account and proper efforts have to be put on their conservation and sustainable utilization. The legislative basis, activities, support and state of endangered of animal genetic resources in Slovak Republic will be presented in the paper.

Key words: animal genetic resources; monitoring; conservation; local breed

INTRODUCTION

In the past decades there was a decreasing trend in the population size of indigenous breeds in Slovak republic. This was mainly due to lower productivity of local breeds and lower abilities to compete with high-productive imported breeds. However local breeds keep their historical value, they are important in production of local animal products and help in supporting the employment in the marginal regions. In the present the cultural and environmental values of local breeds are recognized.

Legislation

The state administration promotes the conservation of the biological diversity of farm animals by passing and sticking to legislative measures the most important of which is Act No. 194 of 1998 on Farm animal breeding. This act specifies the rights and duties of authorized organizations and certified breeders` associations to carry out their professional activities connected with animal breeding. The breeding programs are developed for all major livestock species and breeds and are regularly updated. The breeding is performed with respect to minimize inbreeding in the populations.

The breeding Act No. 194/1998 includes the paragraph treating the issue of „protected farms“. Under §2 section 7 of act 194/1998 protected farms are defined as farms keeping animals of endangered breeds that are used for conservation of breed gene pool and maintenance of its historical value. Under §13 section 2 of act 194/1998 the transfer of breeding stock and genetic reserves originating from such farms is controlled and permitted only after approval of the state. In the present such legislation is not sufficient and legislation ensuring capacities (personal and financial) for management of animal genetic resources and legal basis for running the national gene bank is needed. The long-term national program for animal genetic resources protection should be adopted in order to ensure minimization of extinction risk and support for sustainable utilization of local breeds. As the European Union signed Nagoya protocol in 2011 and is preparing the regulation establishing rules governing access and benefit sharing for genetic resources and traditional knowledge associated with genetic resources Slovak Republic will also have to adopt this legislation in the near future.

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Monitoring

The important part of animal genetic resources issue is their identifying and monitoring. Slovakia was the member of EFABIS (European Farm Animal Biodiversity Information System) project financed by EU (2002-2005) with aim to create a net of databases enabling regular and automated synchronization of data among the single national nodes. In the present the Slovak EFABIS node is updated on yearly base. National EFABIS node is hosted and managed by Animal Production Research Centre Nitra. The monitoring of animal genetic resources is performed via cooperation with Breeding Services of the Slovak Republic, s.e. and authorized breeders` organizations of respective breeds. There were 48 breeds population data updated for year 2011. Detailed numbers are presented in table 1. There are several other breeds monitored in Slovakia. However, these are not registered in the database as these are mainly recently imported exotic breeds reared mostly in one farm.

Table 1: List of updated population data by species

	Breeds together	Updated for year 2011	Extinct	Not updated
Cattle	14	11	3	0
Goose	4	2	0	2
Duck	3	1	0	2
Goat	3	2	1	0
Rabbit	43	0	0	0
Chicken	30	15	0	15
Horse	11	0	0	0
Turkey	1	0	0	0
Sheep	11	11	0	0
Pig	10	6	2	2
Japanese quail	4	0	0	0

Conservation

The most of animal genetic resources conservation is realized via rearing of animals on the farms. Beside *in situ* conservation which is supported via Rural Development Programme (RDP) there are *ex situ in vivo* and *ex situ in vitro* conservation efforts aimed on maintaining breeding animals and sperm of endangered local breeds.

The local breeds including original type of Valachian sheep, Oravka chicken, Zobor and Nitra rabbit, Mangalica pig and Japanese quail are maintained in *ex situ in vivo* conditions at Animal Production Research Centre Nitra. Slovak University of Agriculture in Nitra is active in *ex situ* conservation of Oravka chicken.

The efforts for *ex situ in vitro* conservation exist in Slovakia though not organized. Insemination centers and National Stud keep the samples, mainly the sperm for the commercial use. Since 2009 Animal Production Research Centre Nitra hosts and manages the database CRYOWeb with aim to register the samples of endangered breeds of farm animals available for long-term conservation. In the present there are samples kept and registered at APRC Nitra and partner insemination center.

Support

State incentives have been provided for live animals (purebred females) reared in their natural environment since the mid-1990s. However, this support varied in both the amount per head and the breeds to cover, as public funds are limited (Oravcová *et al.*, 2004).

In 2003 the autochthonous and possibly endangered breeds and species under state incentives were as follows: the Slovak Spotted and Slovak Pinzgau cattle, the Valachian sheep, the Hutsul, Lipitsa, Furioso, Nonius, Shagya-Arab, Noric of Murany and Slovak Sport Pony horse breeds. Since January 2003 state subsidies had covered other sheep, hen and quail breeds like the Improved Valachian and Tsigai sheep, Oravka hens, Japanese quails as well as breeding animals of geese.

After Slovakia entered the European Union the funding scheme was changed. During period 2004-2006 the support was provided via Rural Development Plan

(2004-2006). List of supported species and breeds are listed in table 2. Since 2007 Slovakia has provided the support for the conservation of genetic resources in agriculture via Rural Development Programme (2007-2013) in accordance with Council Regulation (EC) No. 1698/2005 and Commission Regulation (EC) No. 1974/2006. The support is provided as the compensation of income loss due to lower production (RDP 2007-2013). Species and breeds eligible for this support are listed in table 3.

Table 2: Species and breeds supported via RDP 2004-2006

Species	Breed
Sheep	Valachian (original type)
Goat	White Shorthaired
Horse	Slovak Warmblood, Hutsul, Furioso, Nonius, Slovak Sport Pony, Lipitsa, Shagya Arab, Noric of Murany
Chicken	Oravka, Plymouth Rock, Rhode Island Red, New Hampshire, Vlaška, Sussex
Goose	Slovak White, Suchovy
Turkey	Bronze

Table 3: Species and breeds supported via RDP 2007-2013

Species	Breed
Cattle	Slovak Pinzgau
Sheep	Valachian (original type)
Goat	White Shorthaired
Horse	Slovak Warmblood, Hutsul, Furioso, Nonius, Slovak Sport Pony, Lipitsa, Shagya Arab, Noric of Murany
Chicken	Oravka, Plymouth Rock, Rhode Island Red, New Hampshire, Vlaška, Sussex
Goose	Slovak White, Suchovy

Table 4: Number of registered purebred females of supported breeds

Breed	2004	2009	2011
Pinzgau	2258*	1600	1763
Valachian	40	30	102
White Shorhaired	769	744	934
Slovak Warmblood	280	1857	1650
Hutsul	60	119	129
Furioso	67	267	156
Nonius	19	58	22
Slovak Sport Pony	35	177	92
Lipitsa	70	140	152
Shagya Arab	80	156	171
Noric of Murany	54	239	100

* year 2008

State of supported breeds

In tables 4 and 5 numbers of registered purebred females reported by Oravcová *et al*. (2010) are presented and updated with numbers for the year 2011.

Oravcová *et al.* (2010) attributed the decrease of Pinzgau registered cows in the first period (2004-2009) to crossbreeding. In next two years the number of Pinzgau registered cows stabilized with slight increase.

The numbers of registered purebred sheep and goats were stabilized in the first period and increased in the second period (2009-2011).

In horses number of registered purebred mares increased in the first period and Oravcová *et al.* (2010) stated support fostered this increase. However in the second period numbers were decreasing in some breeds while stabilized in Hutsul, Lipitsa and Shagya-Arab.

Situation in poultry is the most alarming. Despite the support no farmer submitted request for subsidies and the numbers of purebred registered females have been falling during both periods except Slovak White goose in the first period.

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This paper and the monitoring of animal genetic resources is performed in cooperation with authorized breeders` associations, Breeding services of Slovak Republic, s.e. and we would like to express our thanks to all who are participating in this work.

Table 5: Number of poultry purebred females in registered farms of Slovak association of breeders

Breed / Species	2007	2009	2011
Oravka / chicken	623	485	295
Slovak White / goose	46	56	29
Suchovy / goose	96	85	25

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DIVERSITY OF CATTLE BREEDS IN SLOVAKIA

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ABSTRACT

The aim of the study was to assess the diversity based on the pedigree information in three cattle breeds. The pedigree information was available from The Breeding Services of the Slovak Republic, s. e. The pedigree analyzed populations consisted of (reference populations in brackets) 274,756 (94,357) Holstein, 109,686 (36,949) Slovak Spotted and 9,756 (2,501) Slovak Pinzgau cattle. Indices of pedigrees completeness differed by breeds but in the 5th generations their values were 18.4 % in Slovak Pinzgau, 56 % in Slovak Spotted breed and 63.8 % in Holstein. Generation interval of Slovak Pinzgau was 7.2 years, tending to be shorter as the size of breed population was bigger. There were 30 % inbred Slovak Pinzgau, 42.8 % in Slovak Spotted and 83 % in Holstein cows and sires in the reference populations. The average inbreeding coefficient ranged from 0.36 % for the Slovak Spotted to 1.32 % for the Holstein. The highest average individual increase of inbreeding ΔF_i = 0.29 % was found for the Holstein and the average individual relatedness coefficient *AR* = 0.8 % in Slovak Spotted cattle. Inbreeding trends in the reference populations as well as purebred animals were positive with increasing average values by animal birth years since 1990.

Key words: cattle; diversity; pedigree analysis; relatedness; inbreeding

INTRODUCTION

Breeding of Slovak Pinzgau and Slovak Spotted dual purpose breeds has a long tradition in Slovakia. The first imports of Pinzgau and Simmental purebred animals were organized long time ago before 1894 when system of cattle recording has started on territory of Slovakia. The size of both breeds was improving and in 1958 they were officially accepted as Slovak Pinzgau (P) and Slovak Spotted (S) breeds. Further breed development was positive from the point of population size and production. Purebred Slovak Spotted breed reach a maximum population size in 1975, Slovak Pinzgau in 1978. After 1990 size of both breeds has decreased significantly due to transformation processes in agriculture and exploitation of Holstein sires for crossbreeding. Today, Slovak Pinzgau is endangered breed. Imports of Holstein to Slovakia started about 1970 and since 1992 breed is managed under Slovak Holstein Association. All three breeds passed through many changes that modified sizes of their populations, structure and breeding strategies over the last 25 years. Therefore, these populations are not under steady-state conditions and the possible consequences were not yet observed. One possibility to describe diversity and its evolution across generations is analysis of pedigree information. Genetic variability of populations and similarity of subpopulations in Austrian cattle breeds determined by analysis of pedigrees was described by Soelkner *et al.* (1998). Roughsedge *et al.* (1999) quantified genetic contributions to a dairy cattle population using pedigree analysis. Gutiérrez *et al.* (2003) analyzed herdbooks in order to know gene flow, population structure and potential danger for genetic diversity loss of eight Spanish local beef cattle breeds. Pedigree information for analysis of diversity in cattle populations was used also by Bartolomé *et al.* (2010) and Kadlečík and Pavlík (2012).

The objective of this study was to assess diversity of three cattle breeds basing on pedigree information.

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Diversity was analyzed in terms of the parameters of the probability of identity by descent.

MATERIAL AND METHODS

Three breeds were involved in the analysis: Holstein (H), Slovak Spotted (S) and Slovak Pinzgau (P). Both breeds, Slovak Spotted and Pinzgau are dual purpose types. Pedigree data registered by The Breeding Services of the Slovak Republic, s. e. were assumed to be representative of the whole (pedigree) population under milk recording system. The results of the reference population (RP) were independently analyzed and compared with purebred animals (H0, S0, P0). The pedigree populations consisted of 274756 Holstein, 109686 Slovak Spotted and 9756 Slovak Pinzgau cattle. Reference population created cows living in 2009 and sires in insemination with frozen genetic material deposited in AI centres in Slovakia. The number of animals by breeds in reference and purebred groups is presented in Table 1. Parameters of diversity in three breeds were estimated using Endog v. 4.8 (Gutiérrez and Goyache, 2009).

Quality of pedigree information was estimated according to the index of pedigree completeness (PEC) (MacCluer *et al.*, 1983), calculated as follows: $2\ C_{sire}C_{dam}\ /\ C_{sire} + C_{dam}$, where C_{sire} and C_{dam} are contributions from the paternal and maternal lines. $C = \frac{1}{d}\sum_{i=1}^{d} g_i$, where g_i is the proportion of known ancestors in generation i; and d is the number of generations that are taken into account (Gutiérrez *et al.*, 2009). Generation intervals were defined as average age of parents at the birth of their offspring (either used for reproduction or not).

Diversity of breeds was evaluated by the parameters based on probability of identity by descent. The individual coefficient of inbreeding (F_i) reflects the probability that two individuals received two identical alleles by descent (Gutiérrez *et al.*, 2009). Inbreeding coefficient value was computed according to algorithm of Meuwissen and Luo (1992). The average relatedness (*AR*) reflects the probability that an allele randomly chosen from the whole population in pedigree belongs to a given animal (Gutiérrez *et al.*, 2009). The individual increase in inbreeding (ΔF_i) was calculated by means of the classical formula $\Delta F_i = 1 - \sqrt[t-1]{1 - F_i}$ where F_i is individual coefficient of inbreeding and t is the equivalent complete generation (Gutiérrez *et al.*, 2009). Effective population size (N_e) was defined as the number of breeding animals that would lead to actual increase in inbreeding if they contributed equally to the next generation (Gutiérrez *et al.*, 2009).

RESULTS AND DISCUSSION

Unbalanced development of three analyzed cattle breeds led to changes in population size and structure. Massive exploitation of Holstein sires for crossbreeding with Slovak Spotted but mainly Slovak Pinzgau cows led to decreasing of both breed population sizes. It was the reason that sizes of analyzed reference populations were different as well as number of purebred animals within each breed. Number of animals in reference and purebred populations within the breeds is presented in Table 1.

An independent analysis of pedigree completeness level of the breeds is important since all results in terms of parameters based on identity by descent and probability of gene origin are dependent on it. The percentages of parents and the other generations of ancestors including founders known are shown in Figure 1. The breed with the highest pedigree completeness was Holstein followed by Slovak Spotted and Slovak Pinzgau. Quality of pedigrees was higher in breed with large population size. This evaluation was based on index of pedigree completeness, namely the

Table 1: Description of analyzed breeds

Breed		n	% of inbred animals
Holstein	RP	94357	82.58
	H_0	37657	95.52
Slovak Spotted	RP	36949	42.77
	S_0	17355	66.76
Slovak Pinzgau	RP	2501	29.99
	P_0	1184	48.40

description of the completeness of each ancestor in the pedigree to the 5th parental generation (MacCluer *et al.*, 2012). Completeness of pedigrees was higher mainly in Holstein and Slovak Spotted compared with results of Baumung and Sölkner (2002) in Pinzgau, Tux-Zillertal and Carinthial Blond breeds and Maignel *et al.* (1996) in French dairy breeds. Nevertheless, pedigrees of breeds under study should be considered as incomplete because in the 5th generations indices values were only 18.4 % for Slovak Pinzgau, 56 % for Slovak spotted breed and 63.8 % for Holstein.

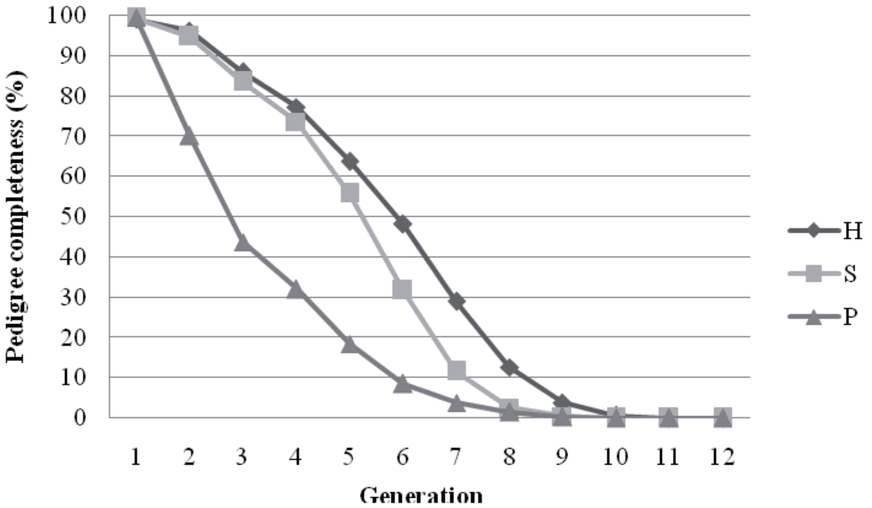

Fig. 1: Pedigree completeness in reference populations

Table 2 reports the average length of generation intervals for the three breeds and purebred animals calculated for all offspring. Generation intervals ranged from 5.7 to 7.2 years in reference populations, tending to be shorter as the population size was larger. Generation intervals of purebred animals were shorter in all analyzed breeds than in reference populations. Generation intervals were longer, as reported by Bozzi *et al*. (2006) in three Italian beef cattle breeds as well as Maignel *et al.* (1996) in French dairy breeds.

The long-term survival of population depends on the maintenance of sufficient genetic variation for individual fitness and population adaptability. Characteristics of diversity derived on probability of identity by descent are presented in Table 3.

The average inbreeding coefficient of a population is often used as a parameter of its homozygosity level. The average coefficient of inbreeding was found to be variable among the evaluated breeds. In Slovak Spotted and Slovak Pinzgau breeds level of inbreeding

Table 2: Generation intervals by breeds

Parameter		H	H_0	S	S_0	P	P_0
	n	19356	9051	8401	4811	419	260
Generation interval	$\overline{x}$	5.70	5.59	6.90	6.76	7.22	7.01
	s	3.02	2.86	3.26	3.19	3.77	4.08

Table 3: Parameters based on probability of identity-by-descent

Parameter	Holstein		Slovak Spotted		Slovak Pinzgau	
	RP	H_0	RP	S_0	RP	P_0
F in %	1.32	1.79	0.36	0.57	0.38	0.54
AR in %	1.41	1.59	0.80	0.90	0.61	0.74
ΔF_i in %	0.30	0.39	0.09	0.14	0.21	0.29
Realised N_e via ΔF_i	166.00	124.90	526.90	341.70	220.60	168.60

in reference populations was low with inbreeding coefficients under 0.4 % and 3.7-fold lower than level of inbreeding in reference population of Holstein. Inbreeding in purebred animals of all three breeds had higher values of coefficients than in the reference populations of H, S and P breeds. In S and P breeds the other parameters like increase in inbreeding and average kinship were more promising than in Holstein population. Better results in Slovak Spotted and Slovak Pinzgau reference populations were obtained in spite that these breeds have smaller populations, even the percentage of inbred animals in the reference population was smaller (S = 42.77 %, P = 29.99 %). If these results are related to lower quality of pedigrees, system of genetic management of populations should be studied in the future. Results of Slovak Spotted and Slovak Pinzgau breeds are better than obtained by Baumung and Sölkner (2002) in Pinzgau, Tux-Zillertal and Carinthian Blond breeds and are comparable with some of Spanish beef cattle breeds in values of intensity of inbreeding and Italian beef cattle breeds (Bozzi *et al*.,2006). In Holstein, Slovak Spotted and Slovak Pinzgau breeds the results of effective population size were better than in mentioned publications. Results of inbreeding in inbred reference and purebred populations are presented in Table 4.

Table 4: Parameters based on probability of identity-by-descent in inbred animals

Parameter	Holstein		Slovak Spotted		Slovak Pinzgau	
	RP	H_0	RP	S_0	RP	P_0
F in %	1.60	1.87	0.85	0.86	1.26	1.12
AR in %	1.53	1.65	0.95	0.97	0.76	0.79
ΔF_i in %	0.36	0.41	0.21	0.21	0.69	0.60

Evolution of inbreeding intensity over year of birth in Holstein, Slovak Spotted, and Slovak Pinzgau breeds in reference and purebred populations is presented in Figures 2 and 3. All three breeds showed an increase in inbreeding by years of animal birth, but only in Holstein reference population (also in purebred animals) increasing of inbreeding over 1 % was recorded (in 2003).

The results of Holstein are the most problematic in comparison with the other two breeds due to very high ratio of inbred animals in reference population (82.58 %, but 95.52 % purebred animals), gain and trend in inbreeding as well as high level of kinship in the both reference and purebred populations. This breed has large population size but results did not reflect it. Obtained results indicate that Holstein could be considered as genetically small breed. Very similar results were obtained by Maignel *et al.*(1996), who argued results of analyzed French dairy breeds by selection of a very few bull sires and wide spreading of elite germplasm concentrate gene origin on a few families.

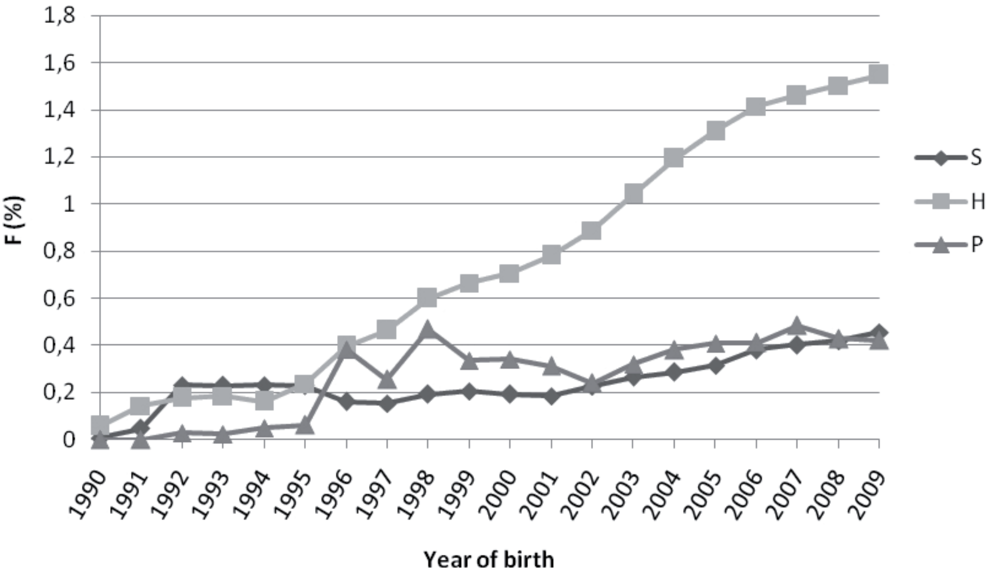

Fig. 2: Trends in inbreeding by reference populations of breeds

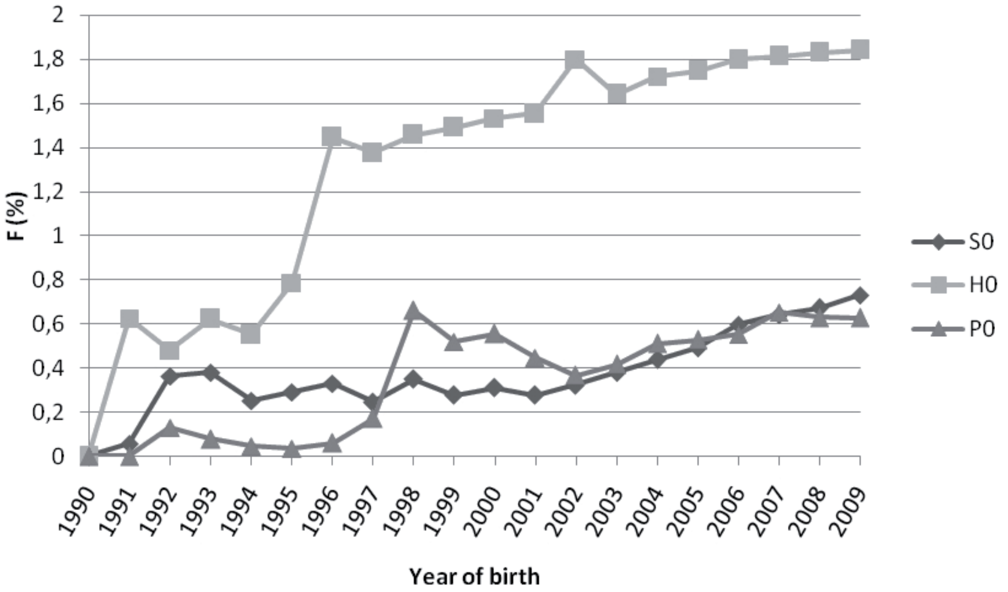

Fig. 3: Trends in inbreeding by purebred populations of breeds

CONCLUSION

Pedigree analysis of Holstein, Slovak Spotted and Slovak Pinzgau breeds based on parameters of identity by descent demonstrates that the genetic status regarding genetic variability differs among breeds. The results of Holstein are the most unexpected in comparison with the other two evaluated breeds due to very high ratio of inbred animals in reference population (82.58 %, 95.52 % purebred animals), gain and trend in inbreeding as well as high level of kinship in the both reference and purebred populations. This breed has large population size but results did not reflect it. Obtained results indicate that Holstein could be considered as genetically small breed. Pinzgau breed is endangered due to small size and negative development in population during the last two decades, mainly. The most promising results are noted in Slovak Spotted breed. The causes of these differences can be related to breeding policy of the breeds.

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PINZGAUER CATTLE IN SLOVAKIA

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ABSTRACT

The decrease in the population of Pinzgau cattle leads to changes in the production type from dairy to beef in system of suckler cows. Limousine breed was used as referential population for better description of results of Pinzgau cattle. The aim of the investigation was to compare growth intensity and muscle thickness measured by ultrasound of Pinzgau (39 heads) and Limousine (37 heads) heifers kept under low input condition in mountain region of Upper Orava in Slovakia. Live weights were obtained at birth (W0) and before pasture seasons at average age of 21 months (W1) and 33 months (W3) and after pasture season at average age of 26 months (W2). Ultrasound measurements of *musculus longissimus thoracis et lumborum* at loins part and *musculus gluteus* above rump were done at 21 months. Growth intensity was calculated for periods within WB, W1, W2 and W3 respectively. Average muscle thickness measured by ultrasound in Pinzgau heifer group was 49.03 mm at loins and 91.74 at rump. Both ultrasound measured values were significantly higher in Limousine heifers (12 mm in loin and 13 mm at rump). Because of the fact that fat layers were higher in Pinzgau cattle, the portions of muscle layer from whole tissue layer were lower. W1, W2 and W3 were higher in Limousine group; the differences were 50.66, 55.33 and 37.37 kg, respectively. Average daily gain from birth to W1, W2 and W3 was significantly higher in Limousine heifers. Due to lower weight gain increase in Limousine heifers for last two controlled periods, average daily gain between WB and W2 or W1 and W2 was higher in Pinzgau than in Limousine heifers. This pilot study proved hypothesis that Pinzgau cattle has less musculature in the hind part of the body. More research is needed for better characteristics of beef production traits important for suckler cow´s low input production system to create enhanced breeding protocol for Pinzgau breed.

Key words: Pinzgau cattle; ultrasound; muscle thickness

INTRODUCTION

As a consequence of the farm animal breeding and intensive use of a relatively small breed number there is a progressive decrease in the genetic variability. The loss of genetic variability can lead to decrease in breed adaptation abilities, to worsening of the health state and, as a final consequence, to the reduction in its farm use. Many of the original (autochthonous) farm animal breeds, which were substituted by more efficient breeds in the past, are now endangered and preserved "*in situ*" as small populations in some regions. Possible extinction of these breeds would also mean irrecoverable loss of the genetic variability and so the loss of unique gene and allele combinations that would be very useful in future e.g. for the generation of new farm animal genotypes. (Table 1). The Slovak Pinzgauer cattle belong to these endangered breeds in the Slovak Republic.

Since early 90`s here is visible decreasing trend of Pinzgau cattle population in Slovakia. Milk production has been the most important trait in the majority of farms. Pinzgau cows were considered as non-sufficient in milk yield, what led to crossing with dairy breeds, most likely Red Holstein. Pinzgau is, however, suitable to very extensive production systems (low input) with using seasonal grazing of permanent pastures in mountain regions of Slovakia. Its walking ability, rusticality and overall robustness with milk

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production above 3500 kg from grazing let it to be appropriate to produce heavy weaners for later fattening in suckler cow´s production systems. Kica *et al*. (2001) proved that young bulls of the breed are good for fattening. In our previous work (Polák *et al*., 2005) we proved that muscle thickness of Pinzgau bulls is slightly smaller than in Simmental but significantly higher than in Holstein. Farmers in mountain regions have not been competitive in comparison to lowland dairy farms, and have been changing production from dairy to beef in system of suckler cows. This trend is visible in Table 2 after year 2008. In 2008, significant decrease in number of Pinzgau cows was recorded in milk performance, but in 3 consequent years the trend was increasing.

The experiment was set in order to help breeders not only by subsidies for keeping animal genetic resources. The aim of our investigation was to characterise growth intensity and muscle development in purebred Pinzgau cattle under suckler cow´s (cow-calf unit) production system in comparison to specialised beef breed, Limousine, kept under the same condition of mountain region of Upper Orava.

Table 1: Breed standard of Pinzgauer cattle in different countries

Country	Performance Type	Live weight bull/cow (kg)	Height of withers bull/cow (cm)	No. of cows
Austria	meat-milk	1100/650	147/138	2500
Slovakia	meat-milk	1150/590	150/138	2000
Germany	meat-milk	1000/700	144/135	1000
Italy	meat-milk	1150/675	140/132	2300
Romania	meat-milk	900/500	134/127	36000
South Africa	meat	1050/600	147/137	-
USA	meat	1100/650	-/-	26000

Table 2: Number of Pinzgau cows in milk and beef performance

Breeding year	Number of first lactations	Milk yield in first lactation	Total number of lactations	Average milk yield all lactations	Average number of finished lactations per cow	Percentage of cows in performance control	Number of cows in beef performance control	Number of purebred calves in beef performance control
2011/2012	525	4044	2299	4567	3,40	82.00	1071	623
2010/2011	607	3756	2564	4420	3,57	82.20	1943	608
2009/2010	584	3755	2865	4506	3,64	85.12	1589	569
2008/2009	807	4155	3978	4777	3,65	80.82	1490	492
2007/2008	762	4015	3798	4653	3,75	70.17	1628	673
2006/2007	926	3943	4553	4543	3,35	85.63	1853	883

(Breeding Services of Slovak Republic, state enterprise, Results of milking, beef and suckler cows performance control in Slovakia, 2008, 2009, 2010, 2011, 2012 and 2013)

MATERIAL AND METHODS

Limousine breed was used as referential population for better description of results of Pinzgau cattle. Growth intensity and muscle thickness measured by ultrasound were determined on 39 Pinzgau and 37 Limousine heifers. All heifers were contemporaries came from the same herd and were kept in low input system in mountain region of Upper Orava in Slovakia.

Live weights were measured before second pasture seasons at average age of 21 months (W1), after second pasture season at average age of 26 months (W2) and before third pasture period at average age of 33 months (W3). Ultrasound measurements of *musculus longissimus thoracis et lumborum* at loins and *musculus gluteus* above rump (Polák *et al.,* 2001) were done on 21 months before first pasture season and before this date. Echocamera Aloka PS2 with UST 4403 probe, 3.5 MHz; 1720 mm was used for measuring muscle thickness. SAS 9.2. was used.

The age was selected due to the fact that growth ability and muscle thickness are minimally affected by gravidity in this age because heifers are just before first conception or only at very early stage of gravidity. Growth intensity was calculated for periods among W1, W2 and W3, respectively.

RESULTS AND DISCUSSION

It is difficult to discuss our results with other authors, because Pinzgau cattle and its beef production has not been analysed, or existed results were obtained on bulls or steers growing under different conditions. Average muscle thickness measured by ultrasound in Pinzgau heifers group was 49.03 mm on loins and 91.74 at rump (Table 3). Both ultrasound measured values were significantly higher in Limousine heifers (12 mm in loin and 13 mm at rump). In our previous works (Polák *et al*., 2004; Polák *et al*., 2007) ultrasound measurements were obtained smaller. It can be due to the fact that in those studies 9 months young bulls or steers kept in extensive pastures in high latitude were used. Because of the fact that fat layers were higher in Pinzgau cattle, the portions of muscle layer from whole tissue layer were lower. W1, W2 and W3 were higher in Limousine group; the differences were 50.66, 55.33 and 37.37 kg, respectively. Average daily gain from birth to W1, W2 and W3 was significantly higher in Limousine heifers (Table 4). Due to lower weight gain increase in Limousine heifers for last two controlled periods average daily gain between WB and W2; W1 and W2 was higher in Pinzgau than in Limousine heifers.

Table 3: Ultrasound measurements of Pinzgau and Limousine heifers

Variable	Limousine	Pinzgau
Muscle thickness on rump	105.05	91.74
Whole tissue layer on rump	113.11	100.64
Muscle percentage from tissue layer on rump	92.85	91.15
Muscle thickness on loin	61.03	49.03
Whole tissue layer on loin	69.49	58.15
Muscle percentage from tissue layer on loins	87.74	84.28

Table 4: Weights and average daily gains at different ages of Pinzgau and Limousine heifers

	Limousine	Pinzgau
W1	442.51	391.85
W2	514.89	459.56
W3	588.22	550.85
ADG 1 – 2	458.38	452.56
ADG 1 – 3	402.95	424.36
ADG 2 – 3	360.03	406.23

CONCLUSION

Since early 90`s here is visible decreasing trend of Pinzgau cattle population in Slovakia. Milk production has been the most important trait in the majority of farms. Pinzgau cows were considered as non-sufficient in milk yield, what led to crossing with dairy breeds, most likely Red Holstein. Pinzgau is, however, suitable to very extensive production systems (low input) with using seasonal grazing of permanent pastures in mountain regions of Slovakia. Its walking ability, rusticality and overall robustness with milk production above 3500 kg from grazing let it to be appropriate to produce heavy weaners for later fattening in suckler cows production systems. This pilot study proved hypothesis that Pinzgau cattle has less musculature and high fat layer in hind part of the body. However, heifers of Pinzgau breed are able to growth rapidly in a certain period of their life, than their contemporaries from Limousine breed. Additional research is needed for better characteristics of beef production traits important for suckler cow´s low input production system to create enhanced breeding protocol for Pinzgau breed.

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GENETICALLY MODIFIED ANIMALS AS POTENTIAL GENETIC RESOURCES

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ABSTRACT

Recent genome programs in livestock animals revolutionized genetic selection. Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used to estimate genomic breeding values.

Recombinant proteins produced by GMO livestock animals with high pharmaceutical value are already reached the market and these products expected to spread in the forthcoming years. Unfortunately even this approach, namely the production of recombinant proteins by livestock animals is developing more slowly than it potentially could. Pharmaceutical companies may be reluctant to adopt these techniques just because they are new and because they suffer from the GMO negative image but perhaps also because they presently make substantial profit with proteins prepared from cultured cells.

With the advent of novel transgenic technologies the number of valuable GMO large animals as models of human diseases for translational research has been growing exponentially. The emerging novel method, genome editing, which enables the targeted transfer of a favourable allele, leaving unaltered otherwise the genome of the animal and therefore rising less bioethical and regulatory issues might contribute to increase productivity and in parallel sustainability of livestock production in the near future.

Key words: transgenic animal; application; genetic resources

INTRODUCTION

Although population growth in developed nations has reached a plateau, no slowdown is predicted in the developing world until about 2050, when the population of the world is expected to reach 9 billions (United Nations, 2008). To meet the global food demand will require nearly double the current agricultural output, and 70 % of that increased output must come from existing or new technologies (United Nations, 2002).

Recent genome programs in livestock animals revolutionized genetic selection. Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used to estimate genomic breeding values. Although predicting genetic merit using DNA diagnostics may be less precise than directly testing the performance of every animal, the reduction in generation interval by far offsets it. However even this approach has limitations, since relies on existing genetic variation. If a trait such as disease resistance does not exist in the population it is not possible to select for it. The characterization of novel, economically important allelic forms of genes, or newly described genes in disease resistance could be the outcome of research with genetically modified model organisms where their effect on fitness can be evaluated.

Phenotypic effects of different genes are variable, therefore genetic modification is appropriate to add major effect genes, whereas genetic selection is applied to all genes including the lesser-effect genes.

Transgenic technology has been originally developed in basic research to examine gene functions in model animals, but scientists proposed that this

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technology might have immediate applications for both biomedical and agricultural purposes, when extended to livestock animals.

The applications of transgenic (GMO) livestock technology in biomedicine are in more advanced stage due to greater economical incentive and public acceptance. The main biomedical applications of GMO livestock are (1) biopharming (live bioreactors) for large scale production of pharmaceutically important proteins, (2) xenotransplantation: animal to human transplantation of "humanised" organs and (3) livestock animal models for human diseases.

The main areas of agricultural applications of transgenic technology are (1) increased production efficiency, (2) improved animal welfare and health, (3) improved food safety and quality and (4) reduced environmental footprint.

BIOMEDICAL APPLICATIONS

(1) Biopharming

Recombinant protein expression in milk of GMO animals has been extensively studied in the last twenty years and underwent improvement recently both from methodological point of view and in terms of reaching market. The U.S. FDA issued its first approval for a biological product produced by genetically engineered animals in 2009, for ATryn (human antithrombin III) to treat a rare clotting disorder www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm109074.htm.

Patients suffering from hereditary antithrombin deficiency are at high risk of blood clots during invasive medical interventions, such as surgery, and during and after childbirth. ATryn is a therapeutic protein derived from the milk of goats; the current manufacturer is rEVO Biologics - formerly GTC Biotherapeutics, Inc. - (www.gtc-bio.com). The amount of ATryn purified per year from transgenic goats is equivalent to those obtained from 90 000 human blood samplings. Other recombinant proteins produced in bovine, goat or rabbit milk are in the pipeline, at different phases of clinical trials. Ruconest™ (Rhucin® in non-European territories) is a recombinant human C1 inhibitor approved for the treatment of angioedema attacks in patients with hereditary angioedema in all EU countries and produced in rabbit milk by Pharming Group (http://www.pharming.com/).

Transgenic rabbits carrying a 110 kb rabbit genomic fragment encoding the IgG binding Fc receptor (FcRn) were created (Catunda *et al.*, 2012). The FcRn transgenic rabbits showed improved IgG protection and augmented humoral immune response indicating that FcRn overexpression on a large genomic fragment brings significant advantages for the production of polyclonal antibodies (http://www.immunogenes.com).

(2) Xenotransplantation

The gap between the number of patients suffering from complete organ failure and the number of donors is increasing in Western populations and became a life threatening problem for them. The chronic shortage of human organs for transplantation initiated research alternatives to human organs, namely the use of organs from animals as xenografts and stem cell therapy. Pigs are currently thought to be the best candidates for xenotransplantation. The risk of cross-species disease transmission is decreased because of their phylogenetic distance from humans. They are readily available; pig organs are similar in size and physiology to human organs. New infectious agents are less likely since they have been in contact with humans through domestication for many generations. The importance of this research field is underlined by the 4936 publications in PubMed (http://www.ncbi.nlm.nih.gov/pubmed).

Current experiments in xenotransplantation most often use GMO pigs as the donor, and baboons as human models. The most profound barrier to pig-to-primate xenotransplantation is the rejection of the grafted organ by a cascade of immune mechanisms commonly referred to as hyperacute rejection, acute humoral xenograft rejection, immune cell-mediated rejection, and chronic rejection. The protective efficacy of all strategies is strictly dependent on a sufficiently high expression level of the respective factors with the required spatial distribution. Multi-transgenic pigs for clinical xenotransplantation which combine the most important genetic modifications with three, four, or five different genetic modifications were produced recently (Ayares *et al.*, 2013). The islets of the multi transgenic pigs were tested in diabetic monkeys and demonstrated prolonged survival, function and complete normalization of blood glucose levels for up to 1 year.

(3) Livestock animal models for human diseases

The domesticated pig has turned into an important disease model, and preclinical testing of novel therapies in human disorders. The cystic fibrosis model pig, created with recombinant adeno-associated virus vector and somatic nuclear transfer to delete the porcine gene developed a disease remarkable similar to human (Rogers *et al.*, 2008; Stoltz *et al.*, 2013). Using Sleeping Beauty DNA transposition and cloning by somatic cell nuclear transfer, minipigs expressing a human gain-of-function mutation in the proprotein convertase subtilisin/kexin type 9 gene were created. Initial characterization revealed severe hypercholesterolemia, and spontaneous development of progressive atherosclerotic lesions (Al-Mashhadi *et al.*, 2013). Duchenne muscular dystrophy and diabetes porcine models were created in E. Wolf's laboratory with targeted gene mutation and somatic nuclear transfer (Klymiuk *et al.*, 2013; Renner *et al*., 2013). An autosomal dominant mutation resulting retinitis pigmentosa in humans was modelled in minipigs with somatic nuclear transfer (Ross *et al.*, 2012).

Like pig, the rabbit is more prosperous as an experimental animal model than the rodents in different biomedical aspects. Long-lasting effects of early prenatal development on health and complex diseases (*hypertension, atherosclerosis, diabetes mellitus, hyperlidemia, and obesity*) cannot be adequately mimicked in mice (Duranthon *et al*., 2012). Transgenic rabbits expressing GFP (green fluorescent protein) provide a way to visualisation of fine anatomical structures and cell morphology through in vivo imaging (Katter *et al*., 2012). Beyond that GM rabbit models of human cardiac disorders-cardiac electrophysiology and cardiac hypertrophy- turned out to be extremely useful in pharmacological studies and disease prevention (Senthil *et al*., 2005; Bentzen *et al*., 2011). The first transgenic rabbit model of retinitis pigmentosa (Kondo *et al*., 2009), carries a 100 kb long rabbit rhodopsin BAC clone which was modified to model a dominant negative human mutation. Contrary to rodent models rabbit has large eye and the scientific community has substantial knowledge on its anatomy and ophthalmology (Jones *et al*., 2011), therefore more useful in translational experiments into clinical practice.

AGRICULTURAL APPLICATIONS

(1) Increased production efficiency

There are theoretical limitations to the production capacity of livestock because of the limits of genetic diversity that is available for selection and propagation. It is unlikely that existing genetic variation will continue to generate the rate of gain obtained in the past. It is very likely that genomic selection and genetically-modified animals will be required and that they will may be accepted. After more than 15 years of research and development the transgenic atlantic salmon expressing the chinook salmon growth hormone gene (AquAdvantage salmon developed by AquaBounty Technologies), are already on the way to the table, albeit with considerable opposition from environmental groups (Ledford, 2013). By the end of May 2013, the public comment period officially ended. The FDA is now scheduled to finalize its assessment.

(2) Improved animal welfare and health

A recent publication with the title: New phenotypes for new breeding goals in dairy cattle stated that „New breeding goals should be defined in dairy cattle to face new challenges for sustainable production, to restore functional traits and to address societal demands" (Boichard *et al*., 2012). Among others this publication proposes that global climate change will increase pathogen pressure, raising new questions to be answered.

Transgenic cattle produced in the USA express antibacterial protein lysostaphin in their milk, which dramatically enhanced the resistance of these cows to infection by Stapylococcus aureus, the most common cause of mastitis. This genetic improvement could improve the well-being of millions of dairy cattle and decrease the economical costs of mastitis ($2 billion per year in the USA).

The two main limitations of the agricultural approaches are:

i.; the dissemination of the transgene in herds through artificial insemination is slower than in plants;
ii.; the lack of public acceptance, which may shy away potential producers.

Beyond that, any GMO strategy would need to be more cost effective than vaccination and other disease prevention strategies to compete and to become the preferred disease prevention policy. Currently, there are no treatments for more than 50 % of all livestock diseases. Even for those diseases for which treatment is available some issues limit their effectiveness e.g. virus serotypes against which the vaccine is not protective or the global concerns about the expansively use of antibiotics (Wall *et al*., 2009). Transgenic technology offers novel disease prevention strategies using RNA interference. This strategy is very promising to attack virus-caused diseases (foot and mouth disease, avian influenza, porcine reproductive and respiratory syndrome).

(3) Improved food safety and quality

Current production systems provide safe animal food products with good nutritional qualities but there is room for improvement. Functional foods are increasingly fashionable in the industrialized world. Milk is an important food and therefore introduction of antimicrobial properties into milk could be beneficial for the consumer. GMO goats that are producing the human antibacterial protein lysozyme in their milk were created in the USA (Maga *et al*., 2006). Their milk was consumed by pigs as a human model of the gastrointestinal tract and showed beneficial effect on their intestinal microflora. This GMO goat milk could be left at room temperature for at least two days, which is especially important in developing countries (Wall *et al*., 2009).

Transgenic cattle were created in China, which express human lactoferrin and alfa-lactoglobulin to make it more similar for human milk. Genetically modified goat expressing a converting enzyme in its milk, to decrease long-chain saturated fatty acids content and increase to their monounsaturated forms, was created in California. Consumption of this milk with higher proportions of monounsaturated fatty acids might have beneficial effect on human cardiovascular health (for review: Fahrenkrug *et al*., 2010). A transgenic rabbit model was created to illustrate the unique ability of transgenic technology to provide novel foods tailored for specific dietary requirements of patients suffering from the genetic disorder phenylketonurea. In this study, a low phenylalanine mutant rabbit κ-casein was expressed at high level in milk and this modified κ-casein could be purified with a simple one step purification step in the Agricultural Biotechnology Centre, Gödöllő, Hungary (Baranyi *et al*., 2007).

(4) Reduced environmental footprint

Livestock transgenesis could contribute to keep agriculture sustainable. The main objects are the following: more effectively utilize both feed and animal resources and reduce pollution. In the intensive pork industry the manure-based environmental pollution is a critical problem. The transgenic pig producing phytase enzyme called Enviropig was developed at the University of Guelph by Canadian scientists (http://www.uoguelph.ca/enviropig/). The pigs are expressing phytase in a saliva specific way, which allows the pigs to digest phytate, the most abundant plant derived source of phosphorus in the pig diet. Phytate passes undigested into manure without this enzyme, increasing the environmental load of pork production. This genetic modification reduces the excretion of undigested phosphor in faces by 30-60 %, which could ameliorate surface water eutrophisation and the environmental footprint of phytase production as food supplement (Golovan *et al.*, 2001). In 2010, Canada has approved limited production of the Enviropigs, in strictly controlled environments for further research. It seemed to be one of the first approval for a meat originated from genetically engineered livestock for human consumption.

The University of Guelph stopped the program and euthanized the pigs, in 2012 after it couldn't find any partner to fund the project. However, the genetic material will be stored at the Canadian Agricultural Genetics Repository Program.

Ecotoxicological studies resulted transgenic fishes which produce special reporter proteins upon environmental stress (Seok *et al*., 2008).

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THE EFFECT OF HERBAL PLANTS *YUCCA SHIDIGERA* AND *CURCUMA LONGA* ON RABBIT FEMALE REPRODUCTION

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The aim of the study was to evaluate the effect of *Yucca shidigera* and *Curcuma longa* herbal additive on rabbit female reproduction. White New Zealand line rabbit females (two months old) were used in the experiment. The animals were divided into the control (C: without *Yucca shidigera* and *Curcuma longa* addition, n = 29) and experimental groups (E1: *Yucca shidigera*, n = 17; E2: *Yucca shidigera*, n = 12; E1: *Curcuma longa*, n = 15; E2: *Curcuma longa*, n = 12). All the studied animals were housed in the wired-floor cages at the air temperature of 22 ± 3ºC, humidity 75 ± 5 %, *ad libitum* access to water and feed. The rabbits in the control group (C) were fed with a commercially available feed; in the first experimental groups (E1) 5 g of *Yucca shidigera* and *Curcuma longa* plant powder was added to 100 kg normal feed. In the second experimental groups (E2) 20 g of *Yucca shidigera* and *Curcuma longa* plant powder was added to 100 kg normal feed. The animals were fed for 50 days and weighed weekly. The rabbit females were hormonally treated and inseminated using heterospermic insemination dose (0.5 ml I.D. per female) after reaching the sufficient weight (at least 3.5 kg). Conception rate, kindling rate, the number of liveborn and number of stillborn young rabbits were evaluated in this experiment.

The highest conception and kindling rate ($p < 0.05$) were found out in the *Yucca shidigera* E2 group (100 %-conception rate; 100 %-kindling rate) and *Curcuma longa* E2 group (83.3 %-conception rate; 80.0 %-kindling rate), when compared to control group (65.5 % -conception rate; 58.6 % -kindling rate). Both herbal additives improved reproduction parameters in rabbit females; furthermore, *Yucca shidigera* addition was more efficient than *Curcuma longa* addition in our experiment.

Key words: rabbit does; *Yucca shidigera*; *Curcuma longa*; conceptional rate; kindling rate

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COMPARISON OF GENETIC VARIABILITY BETWEEN SLOVAK AND CZECH VALACHIAN SHEEP POPULATIONS

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The aim of this study was to compare the genetic variability in small populations of Valachian sheep maintained as genetic resources in Slovakia and the Czech Republic. Ninety eight animals from different regions of Slovakia (SR) and forty eight animals from the Czech Republic (CZ) were individually genotyped using 18 microsatellite markers in two multiplex PCR. The number of alleles, frequency of alleles, heterozygosity, polymorphism information content, Hardy-Weinberg equilibrium and null allele frequency estimate were calculated for all microsatellites using Cervus 3.0.3. All loci were polymorphic in both populations showing between 4 alleles (D5S2, McM527, SPS113, TCRVB6 in CZ) and 22 alleles (OarFCB304 in SK), PIC between 0.377 (TCRVB6 in CZ) and 0.887 (HSC in SK). The Slovak population showed apparently higher average number of alleles per locus (15.05 in SR vs 7.27 in CZ), higher mean expected heterozygosity (0.83 ± 0.06 in SR vs 0.67 ± 0.11 in CZ) and mean observed heterozygosity (0.75 ± 0.08 in SK vs 0.61 ± 0.13 in CZ) than the Czech population. The deviation from Hardy-Weinberg equilibrium ($P \leq 0.05$) was detected only in two loci common for both populations, D5S2 and MAF 214, probably due to the presence of null-alleles. Despite the dramatic reduction in the population size of Valachian sheep breed in both countries in the past these data suggest higher genetic variability in the Slovak population.

Key words: Valachian sheep; genetic resources; genetic variability; microsatellites

ANTIOXIDANT STATUS OF PORCINE OVARIAN GRANULOSA CELLS EXPOSED TO IRON *IN VITRO*

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The objective of present study was to determine the dose-dependent effect of iron (Fe) *in vitro* on total antioxidant status (TAS) of porcine ovarian granulosa cells. The cells were cultured with iron sulphate ($FeSO_4.7H_2O$) at various doses of Fe in the experimental groups 0.17, 0.33, 0.5 and 1.0 mg.ml^{-1} (E1, E2, E3, and E4) for 18 hrs. The cells without Fe exposure served as the control. TAS was analysed by spectrophotometer Genesys 10. Antioxidant status was dependent on Fe doses and decreased in all experimental groups, when compared to the control. Statistical analysis showed significantly lower value ($P < 0.05$) in the E4 group with the highest dose of Fe in comparison with the control group. Trace elements may adversely affect reproductive system of animals through oxidative stress induction. The research in the area of antioxidant system and iron effects will be worthy of further investigation.

Key words: porcine ovarian granulosa cells; iron; antioxidant status

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CRYOPROTECTANTS USED FOR SEMEN FREEZING AFFECT RABBIT REPRODUCTIVE PERFORMANCES

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The aim of this study was to compare the effect of two different cryoprotectants (CPAs), dimethylacetamide (DMA) and dimethylsulfoxide (DMSO) on the fertility and prolificacy of rabbit does artificially inseminated with cryopreserved semen. Semen was collected using an artificial vagina from 32 mature hybrid rabbit bucks. Ejaculates were pooled (four ejaculates/pool) in order to avoid individual differences and transported to the laboratory in a water bath at 30°C and cooled at 5°C for 90 minutes. Each pool was divided into 2 semen samples, that were diluted 1:1 (v:v) with the freezing extender composed of Tris-citric acid-glucose, 2 % sucrose (as a non-permeable CPA) and 12 % DMA or 16 % DMSO (as permeable CPAs) to give a final concentration of 1 % sucrose and 6 % DMA or 8 % DMSO. After dilution, the semen was packed into 0.25 ml plastic straws, equilibrated at 5°C for 45 min and frozen in liquid nitrogen vapour for 10 min before plunging into liquid nitrogen for storage (-196°C). Three groups of rabbit does (n = 114) were inseminated with a fresh semen and with semen frozen using the DMA and DMSO protocols. All the does were i.m. treated with cyclogonine. (20 IU/doe) for estrous synchronization 48 h before insemination. At the time of insemination, each female was treated by intramuscular injection of buserelin acetate to induce ovulation (1 μg/doe). Fertility for each doe was checked by abdominal palpation 12 days after the insemination. Kindling rate, total number of kids born and the number of young born alive were determined at parturition. Similar fertility rates and litter size (total number of born) were recorded in the DMSO group (79.8 % and 7.7 ± 0.3 young per kindling) and fresh semen group (81.6 % and 8.6 ± 0.3), what was higher ($P \leq 0.05$) in comparison with the DMA (47.4 % and 6.7 ± 0.4) group. Moreover, the number of kids born alive in DMSO group (7.2 ± 0.3) was lower ($P \leq 0.05$) in comparison with fresh semen (8.3 ± 0.3) but higher ($P \leq 0.05$) compared to the DMA (5.9 ± 0.4) group.
In conclusion, our results show that DMSO is more effective cryoprotectant than DMA for rabbit sperm freezing, because the semen frozen with DMSO showed a fertility potential similar to that of fresh semen.

Key words: rabbit; spermatozoa; DMA; DMSO; cryopreservation; fertility

PATULIN – INDUCED CHANGES IN HAEMATOLOGICAL PARAMETERS OF RABBITS FED BY STRAWBERRY LEAVES

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The goals of the presented study was to determine effect of 2 week application of strawberry leaves at various doses and acute dose of patulin on selected haematological parameters of rabbit‘s blood: total white blood cell count (WBC), lymphocyte count (LYM), granulocyte count (GRA), lymphocyte percentage (LY %), medium size cell count (MID), medium size cell percentage (MI %), granulocyte percentage (GR %), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDWc). Fifteen rabbits of Californian broiler line were used in this experiment. The animals were divided into four groups (control group C and experimental groups E1, E2 and E3). Animals were fed *ad libitum* using KKV1 feeding mixture (FM) with strawberry leave inclusion as follows: control group without strawberry leaves; group E1 received feed mixture with 0.5 % of strawberry leaves, group E2 -1.0 % and group E3 -1.5 %. All groups received patulin in injectable form at 10 μg.kg^{-1} for 14 days twice a week. Blood was collected into the tubes and the parameters were analysed using Abacus Junior Vet. We observed significant higher level of MID ($P < 0.05$) in experimental group E2 than in the control and E1. Also, increase in this parameter was detected in the E3 in comparison with the E2. Significant change ($P < 0.05$) in MI % was detected in the experimental group E3 in comparison with the E2 as well as between control group and experimental group E2. Other haematological parameters were without significant differences ($P > 0.05$). This decrease is probably mediated through enhancement and activation through macrophage or some cytokine production. In this study the values of other parameters (WBC, LYM, LY %, GRA, GR %, RBC, HGB, HCT, MCV, MCH, MCHC, RDWc, PLT, MPV and PDWc) in all groups corresponded with normal hematological values in rabbit´s blood. The values of these haematological parameters were not influenced ($P > 0.05$) by patulin application combined with strawberry leaves.

Key words: haematological parameters; strawberry leaves; patulin; rabbit

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CARNIOLAN BEE IN SLOVAKIA

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In Slovakia, the original local bee belongs to the Carniolan bee (*Apis mellifera carnica*). Kepena (1975), an employee of the Institute of Apiculture in Liptovský Hrádok, confirmed this fact by detailed morphometric measurements. The origin of Carniolan bees is located in the area of the Carpathians. Carpathians formed a natural boundary of its original distribution to the north and east. Carniolan race is the best race for the climatic conditions of the Central Europe, but it can also survive outside this zone. These bees are peaceful and quiet. In the spring they have a rapid development. They are adapted to mild and severe

winter very well. To overcome the winter, they are grouped in medium weight clusters, 1 to 1.5 kg of winter bees. They have very economical consumption of stocks during the winter as well as during a whole year. They collect nectar and honeydew very well from all insectophile / insect-pollinated plants and they can quickly shift to new food sources. They lid stocks airy. Carniolan honeybee race derives several ecotypes. We classify all accepted Slovak lines (Tatranka, Vojničanka, Sitňanka, etc.) to the Carpathian ecotype.

Bees, kept in our area since ancient times, have been attacked by bees imported from other countries. We can say that besides Carniolan bee, Italian bee also participated in the development of Slovak bees, but not in such a large extent. Slovak Carniolan bee may also be negatively affected by uncontrolled individual import of mothers from other breeds. Bastardization of Slovak Carniolan bee population may lead to reduction of bee activity and raise of their aggression. Affiliation to Carniolan race is assessed numerically by evaluating of the typical morphological features. For the conservation of the gene pool of the original Carniolan bees it is necessary to involve also modern molecular methods for the verification of genetic markers, besides the traditional ones.

Due to these characteristics Carniolan bees are the most widespread in central Europe because they have adapted to the climate and environmental conditions. The fact that the current Slovak Carniolan bees are racially profiled and best adapted to the conditions of Slovakia is proved by the fact that Slovakia has not been affected by large colony losses (so-called CCD) to so high extent as it is happening abroad.

In Slovakia, the breeding of Carniolan bees is covered by queen breeders organized into the Association of Slovak Carniolan bee breeders. Its members have to prove that they kept Carniolan bee, what is currently determined by morphometric measurement of wings and according to colour of tergits. The DNA mapping of bees reared in Slovakia is performed using molecular biology.

After Slovenia, we are probably the second country in Europe, which aims using pure-bred breeding to keep only one breed, autochthonic one, which lives and has been developed here for long period. It is unethical and unnecessary to import other breeds and try to introduce them here. In Slovakia, from the breeders‘ point of view, the only Carniolan race could be kept. For recovery of the gene pool, pure-bred Carniolan lines may be imported from abroad with permission from authorized breeders‘ organization - Institute of Apiculture Liptovský Hrádok, Animal Production Research Centre Nitra.

Key words: Carniolan bee; bee breeding; morphometric measurement of wings; importation of queen bees

THE RELEASE OF ESTRADIOL BY PORCINE OVARIAN GRANULOSA CELLS AFTER AMYGDALIN ADDITION *IN VITRO*

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Amygdalin, sometimes termed as vitamin B_{17}, is a cyanogenic glycoside abundant in the seeds of bitter almond, apricots of the *Prunus* genus and other rosaceous plants. It has been used as a traditional drug because of its wide range of medicinal benefits, including curing or preventing cancer, relieving fever, suppressing cough and quenching thirst. The aim of this *in vitro* study was to examine the secretion activity (steroid hormone estradiol) of porcine ovarian granulosa cells after amygdalin addition. Granulosa cells from non-cyclic and cyclic porcine ovaries were incubated with amygdalin at the doses of 1, 10, 100, 1000 and 10000 µg/mL for 24 hours and compared to the control without amygdalin addition. The release of estradiol by granulosa cells from non-cycling and cycling porcine ovaries was assessed by ELISA. Our results showed that amygdalin at the highest dose (10 000 µg/mL) significantly ($P \leq 0.05$) affected releasing of estradiol by granulosa cells from non-cyclic and cyclic porcine ovaries. In the experimental groups with lower doses of amygdalin no statistically significant differences in the estradiol release were found. In conclusion, our results indicate possible dose-dependent effect of amygdalin on secretion activity of porcine ovarian granulosa cells.

Key words: amygdalin; secretion activity; estradiol; ovarian granulosa cells

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CHARACTERIZATION OF ORAVKA BREED: CHICKEN GENETIC RESOURCE

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Growth and some reproduction traits (fertilization, hatchability and egg weight) of Oravka breed were measured in two chicken lines (OR2 and OR3). The in situ conservation flock kept in Animal Production Research Centre Nitra (APRC Nitra) during the period between 2009 and 2012 was included in the experiment. During each season the breeding males outside the APRC Nitra flock were used. The breeding females were originated from the mating between females raised in the APRC Nitra flock and those new males. The weight at age of 5, 12 and 20 weeks was monitored. The average weights of OR2 were ranged from 371.3 ± 68.9 g to 538.1 ± 79.4 g at 5 weeks, from 1246.1 ± 254.0 g to 1464.5 ± 242.2 g at 12 weeks and from 2076.3 ± 381.8 g to 2286.1 ± 535.4 g at 20 weeks of age. The fertilization rate for each line and season was higher than 84 % except for OR2 line in the season 2011/2012. The hatchability from fertilized eggs was higher than 80 % except for OR2 line in the season 2011/2012. The average weights of eggs in the middle of laying period (from March to May) were ranged from 53.9 ± 3.5 g to 56.6 ± 4.3 g for OR2 and from 52.9 ±4.5 g to 56.4 ±3.6 g for OR3 during the whole experiment.

Key words: Oravka; growth; fertilization; hatchability; egg weight; genetic resources

EFFECT OF GENOTYPE ON SOME EXTERNAL AND INTERNAL EGG QUALITY PARAMETERS OF JAPANESE QUAIL (*COTURNIX JAPONICA*) IN GENETIC RESOURCE

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A research was carried out to determine effect of different genotypes of Japanese quail (*Coturnix japonica*) in genetic resource on some external and internal egg quality parameters. The birds were housed as 1 male and 3 females per cage of 0.12 m^2 area at Animal Production Research Centre Nitra and fed with a mixture of 9.0 MJ ME and 145.0 g of crude protein during the experiment. Feed and water were given *ad libitum*. Analysis of external and internal parameters of Japanese quail eggs was performed in the laboratory of the Department of Poultry Science and Small Animal Husbandry at the Faculty of Agrobiology and Food Resources of the Slovak University of Agriculture in Nitra. This research was conducted to investigate the effects of genotype on egg weight, egg length, egg width, egg shape index, shell weight, percentage of shell, shell thickness, shell strength, albumen weight, percentage of albumen height, albumen width, albumen length, albumen index, Haugh unit, yolk weight, percentage of yolk, yolk height, yolk width, yolk index and yolk colour. We have found significantly higher values for meat type in terms of all egg parameters ($P \leq 0.05$). In case of shell parameters, we observed significant ($P \leq 0.05$) difference between genotypes only in shell weight in benefit of the meat type. There were significant ($P \leq 0.05$) differences found between the genotypes in points of albumen height and albumen index for laying Japanese quail. The significant ($P < 0.05$) difference in benefit of the meat type was found in yolk weight, yolk percentage, yolk height and yolk index. For all other characteristics no significant differences in egg quality between the laying and the meat type of Japanese quail were observed.

Key words: Japanese quail; egg; external quality; internal quality

VITRIFIED RABBIT EMBRYOS AND SPERM AS POTENTIAL GENETIC RESOURCES

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The aim of our study was to assess the effect of Ficoll 70 on the post-thaw quality of New Zealand White rabbit embryos and spermatozoa. Rabbit embryos at morula stage were cryopreserved using OPS (open pulled straw) vitrification method (CIM - CO_2 independent medium + 40 % EG + 18 % ficoll + 0.3 M sucrose). Rabbit ejaculates of good initial quality (> 75 % motility) were diluted in a freezing medium composed of commercial diluent, 8 % dimethylsulphoxide and 1 % sucrose enriched with 0.5, 1, and 2 % Ficoll 70, respectively. The semen samples were packed in straws and frozen in liquid nitrogen vapour according to the protocol.

The cleavage rate of vitrified/warmed early morula stage embryos up to the hatched blastocyst stage *in vitro* was 64.70 % after 2 hours of storage in liquid nitrogen. Computer Assisted Sperm Analyser showed that 2 % Ficoll 70 had a positive effect ($p < 0.05$) on the total motility and progressive motility (E4; 42.235 ± 5.695 and 30.385 ± 5.488) of rabbit spermatozoa when compared to the medium without Ficoll 70 (E1; 35.684 ± 7.468 and 24.968 ± 6.030), respectively.

Our preliminary results indicate that vitrification medium enriched with Ficoll 70 had a positive effect on the quality of cryopreserved rabbit embryos and spermatozoa compared to medium without Ficoll 70. However, other experiments are required to confirm our suggestion.

Key words: rabbit; embryo; spermatozoa; cryopreservation

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THE EFFECT OF RAPAMYCIN ON PORCINE OVARIAN GRANULOSA CELLS PROLIFERATION, APOPTOSIS AND STEROIDOGENESIS

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The target of rapamycin (mTOR)-dependent intracellular mechanisms can play an important role in regulation of ovarian cell functions, but its effects and mechanisms of action are studied insufficiently. The aim of this *in vitro* study was to examine the effect of rapamycin, a plant mTOR inhibitor, (at the additions of 0; 1; 10; 100 μg/ml) on proliferation, apoptosis and hormone release of porcine granulosa cells. The presence of the proliferation-associated peptide (PCNA) and apoptotic peptide (BAX) in the ovarian granulosa cells was determined by immunocytochemistry. The secretion of hormones (progesterone, testosterone) was analysed by RIA. It was observed that the addition of rapamycin decreased the PCNA and increased the BAX accumulation in the ovarian cells at all doses added. The rapamycin treatment also suppressed progesterone release (at dose of 1 μg/ml), whereas the release of testosterone was unaltered. These observations demonstrate the involvement of the mTOR signalling system in control of female reproductive functions via changes in ovarian cell proliferation, apoptosis and steroidogenesis. Rapamycin, an inhibitor of mTOR system, can directly affect these ovarian cell functions and, therefore, it could be potentially useful for the suppression of ovarian functions.

Key words: Rapamycin; mTOR; proliferation; apoptosis; steroidogenesis; granulosa cells

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THE EFFECT OF CHOSEN BEE PRODUCTS ON BIOCHEMICAL CONSTITUENTS OF CHICKEN BLOOD

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The aim of this work was to investigate the effect of chosen bee product (propolis and bee pollen) inclusion to the diet on some serum biochemical parameters (glucose, total cholesterol, triglycerides) of chickens. Broiler chickens (n = 99) were divided into 3 groups. First group without any addition served as a control (C, n = 33). Experimental groups received propolis extract (E1, n = 33) in feed mixture at the dose of 200 mg.kg^{-1}, bee pollen extract (E2, n = 33) at the dose of 800 mg.kg^{-1}. After 42 days of feeding chickens were slaughtered and blood samples (n = 10 in each group) were collected. The blood serum was separated from whole blood by centrifugation at 3000 g for 30 min. The concentrations of serum components in blood serum of broiler chickens were analysed using automatic analyser Microlab 300 (Merck, Germany) using Ecoline kits according to the manufacturer guidelines. Significant decrease in serum glucose in the experimental group E2 (11.76 mmol.l^{-1}), when compared to the control (14.70 mmol.l^{-1}) and the E1 (14.73 mmol.l^{-1}) group, was measured. The addition of propolis extract and bee pollen extract to the diet of broiler chickens had slight or no effect on values of triglycerides, cholesterol, as differences among the groups were insignificant ($P > 0.05$).

Key words: propolis; bee pollen; broiler chickens; blood biochemistry

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SPERMIOPHAGES IN RABBIT EJACULATES AFFECT FUNCTIONAL PARAMETERS OF SPERMATOZOA

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The objective of our study was to access the influence of spermiophages on the membrane stability and motility of the rabbit spermatozoa. Semen samples from rabbit males were collected using an artificial vagina and spermiophages were analysed using an Alexa-AcLDL fluorescent dye (Acetylated Low Density Lipoprotein; Molecular Probes, USA). The sperm samples were divided into two groups basing on the number of spermiophages. Spermatozoa were stained for plasma membrane integrity (*peanut agglutinin*, PNA-FITC) and occurrence of dead/necrotic sperm (propidium iodide, PI). The quality of semen samples was evaluated by CASA system (Sperm Vision™, Minitube, Germany). Our results show that the percentage of the spermatozoa with disturbed plasma membrane was lower in the experimental group S1 with less than 20 % of spermiophages in sperm samples, than in the experimental group S2 with 40 % of spermiophages (S1 vs. S2; 16.83 ± 2.33 vs. 20.03 ± 2.21). The negative impact of spermiophages was expressed also by the higher number of dead/necrotic spermatozoa (S1 vs. S2; 7.57 ± 1.66 vs. 10.89 ± 1.46) and the lower motility (M) and progressive movement (PM) of the spermatozoa (S1 vs. S2; M: 84.05 ± 7.22 vs. 79.33 ± 6.89; PM: 70.35 ± 7.04 vs. 68.29 ± 6.84). These preliminary results indicate that the higher occurrence of spermiophages may have negative influence on the quality of rabbit spermatozoa.

Key words: rabbit; spermatozoa; spermiophages; plasma membrane; motility

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PRODUCTION AND EXTERIOR TRAITS OF ORIGINAL VALACHIAN BREED

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The objective of this study was to analyse the population of original Valachian breed in Slovakia in concern to the animal genetic resource. Breed promotion and breeding operations are managed under the guidance of the Original Valachian Breeders' Club, which works under the umbrella of the Sheep and Goat Breeders' Association of the Slovak Republic. The breed standard and breeding programme are in place. Breeding is aimed at maintaining of genetic variability and of typical wool and exterior characteristics (colour variability and both horned and polled individuals). At the end of 2012, there were 31 living adult males and 225 living adult females registered in the flock book. The animals are kept in two nucleus and five multiplier flocks. The number of ewes per flock ranges

from 6 to 93. In the period between 2010 and 2012 the average fertility was 90.3 ± 12.0 %, the average fecundity was 123.4 ± 23.1 % and the average prolificacy was 136.4 ± 17.1 %. In milk recorded ewes, the average milk yield per 150-day lactation length was 96.3 ± 6.1 l, the average fat percentage was 7.660 ± 0.462 %, the average protein percentage was 5.920 ± 0.221 % and the average lactose percentage was 4.580 ± 0.066 %. The average daily gain in male and female lambs till weaning at age of 45 to 55 days was 246.70 ± 81.51 g and 242.90 ± 48.80 g, respectively. According to the analysis of breed phenotype, which was done in four original Valachian flocks in 2010 (in total 102 heads), 48.0 % were polled animals and 36.3 % were animals with lyre horns (so called "širaňa" animals). The majority (30.4%) were the animals of "bekaša", "murga" or "mucha" colour pattern (white coat with black spots on head and legs), 28.4 % were the animals of "lajka" colour pattern (wool with black and white hairs, head and legs are mostly black) and 18.6 % were the animals of "belica" colour pattern (white coat and wool without any markings). About 52 % were the animals with white wool and 15.7 % were the animals with black wool. About 42 % were the animals with hair diameter between 37.1 and 40.0 µm (grade D) and 39 % were the animals with hair diameter between 40.1 and 43 µm (grade DE). None animals with reduced auricle (so called "čulka" animals) were found. According to PrP genotyping (there were 55 tested individuals in the period between 2010 and 2012), 36.4 % were the animals of the ARR/ARR genotype and 38.2 % were the animals of the ARR/ARQ genotype. The ARR allele frequency was 0.582 and the ARQ allele frequency was 0.327. The monitoring based on genetic and phenotypic evaluation of the original Valachian sheep population, aimed at the maintaining typical adaptability, production and exterior traits, is recommended to be done regularly.

Key words: sheep; original Valachian breed; animal genetic resource; production traits; exterior; wool

EFFECT OF GREEN TEA POLYPHENOLS (GTPP) ALONE OR IN COMBINATION WITH T-2 TOXIN ON STEROID HORMONE PRODUCTION BY THE OVARIAN GRANULOSA CELLS

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Green tea extracts have beneficial effects on live organisms. It is well known that green tea has strong antioxidant, anticarcinogenic, anti-inflammatory and anti-radiation effect. T-2 toxin is a mycotoxin, which occurs predominantly in cereals and cereal-based products. T-2 toxin is considered to be a strong cytotoxic mycotoxin. The aim of present study was to examine the ability of ovarian granulosa cells (GCs) to produce progesterone and estradiol after addition of green tea polyphenols (GTpp) either alone or in combination with T-2 toxin. Ovarian granulosa cells were incubated without (control) or with green tea polyphenols (1, 10, 100 µg.ml^{-1}) and in combination of green tea polyphenols (50 µg.ml^{-1}) with T-2 toxin (1, 5, 50 ng.ml^{-1}) (experimental groups) for 48h. After the addition of GTpp (1, 10, 100 µg.ml^{-1}) no significant changes in secretion of progesterone were observed. Secretion of estradiol by ovarian GCs was significantly stimulated after the addition of GTpp (1 µg.ml^{-1}). No significant changes in estradiol secretion were observed after the addition of higher doses (10, 100 µg. ml^{-1}). After addition of green tea polyphenols with T-2 toxin no significant changes in progesterone secretion were observed. Estradiol secretion was significantly stimulated after the addition of GTpp (50 µg.ml^{-1}) with T-2 toxin (5 ng.ml^{-1}). No significant changes in estradiol secretion by ovarian GCs were observed after the addition of GTpp (50 µg.ml^{-1}) with T-2 toxin (1, 50 ng.ml^{-1}). In conclusion, because of the lack of information about the effect of GTpp and its combination with T-2 toxin on ovarian cells, it is necessary to continue in this investigation. Green tea extracts with their high antioxidant status could have protective effect against cytotoxicity of mycotoxins.

Key words: Green tea polyphenols (GTpp); T-2 toxin; progesterone; estradiol; granulosa cells

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LH-INDUCED PROGESTERONE SECRETION BY PORCINE OVARIAN GRANULOSA CELLS AFTER FUSARIOTOXINS EXPOSURE

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Normal ovarian steroid production in most mammals occurs via the "two cell/two gonadotropin" model. Granulosa cells (GCs) become "luteinized" after aspiration from follicles because they have limited oxygen and cholesterol supply, thereby are able of progesterone synthesis in large amounts. Luteinizing hormone (LH)-induced cultivation of GCs reveals a large and rapid rise in progesterone production in porcine ovarian granulosa cells from non-cyclic gilts. The aim of the study was to examine the LH-induced progesterone secretion by porcine ovarian GCs after addition of *Fusarium* toxin deoxynivalenol (DON), zearalenon (ZEA) and T-2 toxin each at dose of 100 ng/ml for 24h. Direct measurements of ovarian progesterone content in culture medium were accomplished by ELISA. The doses of 0.1, 1 and 10 µg/ml of LH in culture medium significantly ($P \leq 0.05$) increased progesterone secretion by GCs. The combination of the lowest LH dose (0.1 µg/ml) and 100 ng/ml of T-2 toxin (but not ZEA and DON) significantly ($P \leq 0.05$) decreased GCs progesterone release. Progesterone secretion was significantly ($P \leq 0.05$) stimulated by 1 µg/ml of LH in combination with DON, ZEA and T-2 toxin (each at dose of 100 ng/ml). DON and ZEA (but not T-2 toxin) each at dose of 100ng/ml in combination with 10 µg/ml of LH significantly ($P \leq 0.05$) increased progesterone secretion. Our findings reveal that *Fusarium* toxins can act as a possible endocrine disruptor in the steroidogenesis.

Key words: DON; ZEA; T-2 toxin; LH; progesterone; granulosa cells

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SCREENING FOR TOLL-LIKE RECEPTOR GENE POLYMORPHISM IN THE TRADITIONAL CATTLE BREEDS

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The traditional breeds are supposed to be a valuable source of genes in the breeding programmes for sustainable agriculture. Notably, the genes responsible for the health traits might be of interest due to the presumed adaptation of the traditional breeds to the local environmental and breeding conditions and thanks to a higher intravarietal diversity. Additional variants of the microbe resistance genes are demanded by the changing spectrum of pathogens, legal and practical limits on the antibiotic prevention and therapy, and by the eroding genetic diversity in modern breeds. Therefore, a screening has been carried out for the structural polymorphism in the innate immunity receptors of the TLR family in two historical Czech cattle breeds that are conserved in frame of the national program of Conservation and Use of Farm Animal Genetic Resources. The survey in the Czech Red and Czech Pied cattle will include 50 individuals of each breed and is aimed at the genes coding for receptors participating in the interactions with the bacterial pathogens in frame of the innate immunity. The sequencing of the PCR-fragments of the TLR4 gene discovered four haplotypes. To date, no new gene variants specific for the local breeds have been revealed. The comparatively low diversity in the completed locus TLR4 can be ascribed to the population bottleneck in the history of both breeds and to the outcrossing with modern breeds. Nevertheless, the polymorphism in the highly conserved TIR domain of the TLR4 receptor resulting in the exchange Ile674Thr has been shown to occur in the nucleus herds of both breeds. The difference in the amino acid character has been predicted to associate with a functional change of the molecule. Accordingly, the variant of the receptor with threonine has been reported to lead to an increased infection resistance in the Canadian and Chinese Holstein populations. The validation of its effect in both historical breeds and in the production herds of the Czech Pied cattle is a logical next step.

Key words: cattle; disease resistance; diversity; innate immunity; Toll-like receptor

THE EFFECTS OF BENZOIC ACID AND PROTEIN LEVEL ON URINE PH AND AMMONIA EMISSION IN PIGS

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Twelve hybrid gilts (initial body weight 29.9 ± 1.7 kg) were used for the evaluation to identify the effect of benzoic acid and dietary protein in the diet on urine pH and ammonia of the slurry in growing piglets. We used two protein concentrations (high protein 18.8 %, HP and low protein 14.0 %, LP) and two benzoic acid levels (0.0 % and 1.0 %). The same energy level (13.3 MJ. kg) in the diets was achieved by means of the supplementation with rapeseed oil. The piglets were housed in metabolic cages and fed with two equal doses at 7 a.m. and 5 p.m. at a daily rate of 90 $g.kg^{0.75}$. Water was supplied *ad libitum*. Each experimental period consisted of a 6-day adaptation, which was followed by a 4-day collection phase, when faeces and urine were collected using bladder catheters. Experimental data were subjected to ANOVA and when significant value was observed for treatment effect, the differences between means were assessed using Fisher‘s LSD procedure.

Nitrogen (N) and dry matter intake were not significantly affected in any diets. The amount of retained nitrogen from intake was significantly increased only in pigs fed the diet with benzoic acid and HP. We found a significant decrease in urine pH (-0.7 and -0.9) in both experimental groups fed with benzoic acid diets, regardless of the nitrogen content in the diet. The coefficients of excretion determination between hippuric acid and urine pH were R2 = 0.57 both for the HP and LP diets. The higher but not significant decrease in ammonia nitrogen was observed in the experimental LP groups.

Key words: ammonium excretion; benzoic acid; urine pH; piglets

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EFFECT OF QUERCETIN AND T-2 TOXIN ON SERUM ALBUMIN AND BILIRUBIN CONTENT IN RABBITS

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The aim of the present paper was to determine the effect of long-term application of quercetin at various doses and T-2 toxin at acute dose on the content of bilirubin (BR) and albumins in rabbit‘s blood.

The animals were divided into two control groups (C1 and C2) and six experimental groups (E1 – E6). Experimental groups received quercetin (Sigma Aldrich, Saint Louis, USA)

in injectable form (intramuscularly) at 10 μg.kg^{-1} in E1 and E2 group, 100 μg.kg^{-1} in E3 and E4 group and 1000 μg.kg^{-1} in E5 and E6 group without T-2 toxin for 90 days. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) was applied to C2, E2, E4 and E6 groups at dose 0.08 mg per kg of body weight 72 hours before slaughter. Control group (C1) was injected with water (Imuna Pharm a.s. Šarišské Michaľany, Slovak Republic).

After T-2 toxin treatment statistical analysis showed insignificantly (P > 0.05) lower levels of bilirubin in the experimental groups (E4, E6) in comparison with the control group with T-2 toxin (C2). When differences between groups with/without T-2 toxin were compared, insignificant increase in the groups treated with T-2 toxin (C2, E2 and E6) and decrease in the E4 group was found. Our results are consisted with results of other authors.In case of albumins slight, but not significant, decrease in the content of albumins in all experimental groups (with or without T-2 toxin) vs. the control group was observed. Based on the literature natural substances could slightly modify the serum content of bilirubin and albumins and consequently influence antioxidant balance in the organism. To prove this assumption further research using quercetin and T-2 toxin is required.

Key words: quercetin; superoxide dismutase; rabbits

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EXPERIMENTAL TESTING OF LAPAROSCOPIC INTRAUTERINE INSEMINATION OF MOUFFLON FEMALES WITH FROZEN SEMEN

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The aim of the research was to evaluate the use of laparoscopic intrauterine insemination (LII) of moufflon females using deep-frozen moufflon semen. Fourteen moufflon females were synchronized during the anestric period using CIDR and i.m. administration of FSH. The LII was performed by deposition of 0.03 ml of thawed moufflon semen (20x10^6 motile spermatozoa) into the middle of each uterine horn using laparoscope and inseminating pipette, fitted with a 25-gauge needle either 40 or 48 h after the CIDR removal. Ovulation was monitored by ultrasonography with an abdominal linear probe as well as by laparoscopic examination. Embryos at blastocyst stage were recovered retrogradually using a Folley catheter. The highest ovulation rates were detected between 56 and 60 h after the CIDR removal. Recovery rate of embryos was 30 % and 38 %, fertilization rate 43 % and 58 %, percentage of unfertilized oocytes - 56 % and 41 %, of intact-transferable embryos - 28.2 % and 33.8 % and percentage of damaged embryos - 64.6 % and 66.7 % after insemination following 42 and 50 h after the CIDR removal, respectively. The results showed that the middle of the uterine horn is an acceptable deposition site for the insemination dose (ID). Insemination at 48 h after the CIDR removal seems to be better than at 40 h when using LII with frozen-thawed semen. The volume of ID and number of progressively motile sperm cells per ID were sufficient. Therefore, LII can be used successfully in moufflon and may be implemented to use in controlled reproduction.

Key words: moufflon; frozen semen; laparoscopic insemination; embryo recovery rate

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IMPACT OF SELECTED METABOLIC PARAMETERS IN PROFITS AND QUALITY OF EMBRYOS IN FALLOW DEER DONORS

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The aim of this work was to study the observation of prevalence serum cholesterol value, urea and total protein before and after superovulation on the yield and quality of embryos in fallow deer donors. Positive correlation was found between the level of cholesterol and superovulatory response (r = 0.64), production of embryos (r = 0.55) and transferable embryos (r = 0.51). Levels of urea were in negative correlation in the superovulatory response (r = 0.34), to flushed embryos (r = 0.58) and transferable embryos (r = 0.61). The influence of total proteins in blood serum of donor fallow deer on effectiveness of embryo transfer was not proved in our experiments.

Key words: biochemical parameters; blood serum; fallow deer; heat

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TYPICAL ALLELES IN THE GENOTYPES OF NATIONAL RABBIT BREEDS

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The present Pattern Book of Rabbit Breeds by the Slovak Association of Rabbit Breeders contains more than 50 registered breeds and dozens of varieties. Their classification is based on live weight (giants, medium, small and tiny – dwarf) and fur structure (fur-bearing, Angoras, short-haired coat – rex). A group of national rabbit breeds is part of the

national heritage; they are the result of breeding activities of breeders associated in the national breeders' organization. These breeds represent in some cases unique genotypes with typical configuration of alleles, which do not occur in other zootechnical units. These breeds are a source of genetic variability, which is used to improve them further or in new breeding. In spite of the fact that the frequency of populations of national breeds is limited by the breeders' basis, these animals are regularly exhibited during exhibitions at home and abroad and they often award prizes. At present there are following national breeds in Slovak herds (with the year of origin):
Slovak grey-blue rex (1965)
Blue of Holic (1975)
Rabbit of Nitra (1977)
Zemplin pastel rabbit (1987)
Zobor rabbit (2005)
Liptov bold-spotted rabbit (2005)
Dwarf Slovak pastel rex (2005)
Slovak pastel rex (2007)
At the same time, these zootechnical units are bearers of unique nucleotide sequences, which can be used for a number of practical and experimental applications in the future.

Key words: rabbit; national breeds; alleles; coat colour

EXPRESSION OF THE CELL MEMBRANE ANTIGENS CD9 AND CD41/61 IN TRANSGENIC RABBITS WITH THE RECOMBINANT HUMAN FACTOR VIII (HFVIII)

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The blood coagulation is a complex cascade process in which a set of blood factors is comprised. Some cell surface molecules (CD antigens) mediate the interaction of thrombocytes and coagulation factors.

In current study, we investigated whether the insertion of the hFVIII gene construct into the rabbit genome could modify the expression of cell surface molecules CD9 and CD41/61 taking part in the process of blood coagulation. The presence of both molecules in blood and milk have been analysed by indirect immunofluorescence and ELISA during two lactations of transgenic rabbits and in the udder tissue after the killing of animals, in comparison with the expression of the same molecules in non-transgenic rabbits. The study showed that the insertion of the WAP-hFVIII does not influence significantly the expression of CD9 and CD41/61 on the blood cells, milk somatic cells and mammary gland tissues.

Key words: transgenic animals; CD molecules; factor VIII; rabbits

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EXPLORING GENE DIVERSITY IN CZECH TRADITIONAL BREEDS INCLUDED INTO THE CONSERVATION PROGRAMME

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Biotechnology based on the genetic markers provides a potential for effective management of genetic resources of animals, including the control of their health and development of new products with specific (desirable) properties.Genetic markers are used alongside to improve the efficiency of selection programs. For the assessment and prevention of inbreeding the polymorphic variant testing of selected candidate genes is used. In practice, the genes with high association to one of the indicators of these traits have been used.

The most common techniques to determine the genetic diversity of livestock breeds are the screening for microsatellite profiles, mtDNA variability, AFLP technique and variation present in the coding region and noncoding parts of particular genes. To assess inter- and intra-varietal diversity in the conserved traditional breeds, we have used various methods of molecular genetics.

The aim of this study was to evaluate the occurrence of gene diversity in goat, sheep, hen and horse breeds included into the National Programme of Conservation and Exploitation of Genetic resource. For analysis in different species, we have used different number of polymorphic microsatellite markers recommended by ISAG or FAO.

Goat: 7 recommended microsatellite markers were used. Results indicate that high level of genetic variations has been maintained in two Czech dairy goat breeds (White Short-Haired (WSH) and Brown Short-Haired (BSH). The low value of calculated genetic distance between them (D = 0.0517; SD = 0.0075) was expected since both breeds are related and have been bred for the same natural conditions. On the other hand, both breeds exhibited relatively high level of heterozygosity.

Sheep: 6 microsatellite loci divided into 2 multiplex were used to detect genetic variation in Sumava sheep, Valachian sheep and Improved Valachian sheep breeds native to the territory of the Czech Republic, engaged into the Animal Genetic Resource. The results showed high level of polymorphism in the second multiplex. Heterozygosity of loci in the first multiplex was lower than in the second in the followed sheep breeds.

Horse: 16 recommended microsatellite markers in Old Kladruber horse were used. The results showed that the average number of alleles per microsatellite locus was 8.25 with a range of 4 to 14. The estimated average value for the observed heterozygosity across microsatellite loci was 0.637, while the estimated mean value of genetic diversity was 0.678. Based on the result, the genetic diversity (heterozygosity) in monitored Old Kladruber horse is consistent with other populations of horses.

Rabbit: For molecular analysis we used 7 breeds of rabbit and 17 microsatellite markers, divided into 5 multiplex. The analysis revealed better than expected large differences

between breeds (due to their relatively recent common origin). This is underlined by the finding of unique alleles that are unique to each breed. With their help they can be reliably identified. From this we can also conclude that breeds carry the unique genes within a population of rabbits. Further results show that despite the limited number of individuals thrive maintain desirable genetic variability. The Fis coefficient (can be seen as a measure of inbreeding) acquires medium to high values at least in numerous breeds.

Chicken: To date, we have used 16 microsatellite loci to characterize Czech Gold Speckled Hen. Average values of heterozygosity (0.37), polymorphic information content (0.38) and total number of alleles (4.19), as well as a high frequency of the most common allele (0.7) show a high level of inbreeding, high intensity of selection and other factors negatively affecting genetic variability. The above-mentioned parameters obtained in the control group correspond to approximately half of the normal value for this type of markers in other livestock populations or to one third of the value of wild populations.

In conclusion, microsatellite analysis is one of the powerful tools for evaluating gene diversity, genetic variation within species and their populations. The obtained data are (or will be) a guide for breeding measures for the management of conserved breeds.

Key words: gene diversity; microsatellite DNA; polymorphism

QUALITY OF BOVINE EMBRYOS FOLLOWING CRYOPRESERVATION AND STORAGE

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Cryopreservation procedure still causes significant morphological and biochemical alterations, which may lead to cell death and loss of embryo viability. The loss of viability can be attributed among other factors also to the length of storage in liquid nitrogen. One potential way to improve post-thaw survival and post-transfer efficiency is to modify post-thaw culture conditions to more closely simulate embryo physiology *in vivo*. One of macromolecules, which can regulate embryonic development, may be insulin-like growth factor I (IGF-I), which promotes development to the blastocyst stage, increases blastocyst cell number and decreases proportion of apoptotic blastomeres in *in vitro*-produced embryos. The goal of this study was to examine the effect of IGF-I added during the post-thaw culture (48h) of bovine embryos stored for twenty years in liquid nitrogen on their post-thaw survival and quality.

The embryos recovered from the Czech Fleckvieh dairy cows within the years 1989-1990 were frozen using slow-freezing procedure. Following thawing the embryos were either processed immediately (the control group) or cultured alone (Exp 0) or in the presence of rhIGF-I (10 or 100 ng/ml; Exp10 and Exp100, resp.). Following 48h post-thaw culture 41 % of the embryos developed up to advanced blastocyst stage. Thawed embryos were represented mostly by the grade III actin quality and less of them (12 %) were of the grade II actin, whilst none grade I actin embryos were noted. In the cultured embryos IGF-I at both doses significantly elevated the cell number compared to non-cultured embryos. However, in comparison to embryos cultured without IGF-I, only higher IGF-I dose (100 ng/ml) elevated the total cell number. Apoptotic (TUNEL) index was significantly lowered by both IGF-I doses. These observations indicate that addition of IGF-I during post-thaw culture, can improve the quality of bovine embryos following long-term cryostorage.

Key words: embryo; post-thaw viability; apoptosis; actin; IGF-I

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KNOCK-OUT STEM CELLS IN PHYSIOLOGICAL RESEARCH

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Knockout mice have been widely used in human and veterinary medicine, biology, pharmacology and other related fields to the study of gene function, drug and therapeutic development and disease research. Mice remain the most efficient and easily genetically manipulated animal research model. Their genetic similarity to humans allows valuable advancements in human health research. Generation of knockout mice is based on introduction of specific mutation(s) into the genome of embryonic stem cell, subsequent generation of chimeric animals and transmission of the mutant allele to the offspring. In conventional knockout, the targeted gene is functionally eliminated. Conditional knockout enables a post-natal or tissue-specific gene inactivation. Knockout mice with a reporter gene are used to monitor target gene expression. In my presentation I will focus on several models of knockout mice previously or currently used in our laboratory. I will discuss our results in studies related to male infertility, developmental separation of blood and lymphatic vessels, restenosis and introduce our current projects in the area of cancer research. Finally, I will give a brief overview of current requirements of the Ethic commission of Medical Faculty in Vienna and Austrian Federal Ministry for Science and Research to breed knockout mice and to accomplish the experiments.

Key words: knockout mice; stem cells; gene function; gene expression; disease research

DIRECT IMMUNOMAGNETIC SEPARATION OF MESENCHYMAL STEM CELLS FROM THE RABBIT BONE MARROW

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The objective of this study was to assess the effectiveness of MACS technique used for the isolation of mesenchymal stem cells (MSCs) from the rabbit bone marrow. Rabbit bone marrow was harvested from humanely sacrificed New Zealand White rabbits (n = 4) under sterile conditions and mononuclear cells

(BMMCs) were isolated using ficoll centrifugation. BMMCs were divided and placed into prepared tubes, incubated with Anti-SSEA-4 or Anti-MSCA-1 microbeads (Miltenyi Biotec, Germany) and sorted using AutoMACS Pro Separator (Miltenyi Biotec, Germany) according to the producer's manual. Inactivated rabbit serum was also used in order to exclude non-specific labelling to Fc receptors. The control samples were not sorted. After MACS sorting APC-conjugated Labelling Check Reagent (LCR; Miltenyi Biotec, Germany) was used to control the sorting efficiency. Labelled cells before and after sorting were evaluated using the flow cytometer FACSCalibur (BD Biosciences, USA). At least 100,000 events (cells) were analysed in the control samples and negative fractions. In the positive fractions at least 10,000 cells were analysed. Propidium iodide was used to exclude the dead cells from the evaluation. The sorting efficiency, proved by the LCR positivity in both positive fractions (SSEA-4$^+$ and MSCA-1$^+$), was significantly higher ($P < 0.05$) in comparison to the sample before sorting or negative fractions. We observed higher percentage of MSCs using SSEA-4 Microbeads (34.70 ± 2.29) in comparison to the MSCA-1 Microbeads (7.00 ± 0.87). Our observations indicate that MACS technique could be potentially used for the positive selection of MSCs from rabbit bone marrow. However, further experiments are required in order to substantiate this assumption.

Key words: rabbit; MACS; MSCs; SSEA-4; MSCA-1

Acknowledgement: This work was supported from the grants of Slovak Research and Development Agency: APVV LPP-0119-09 and APVV-556-011.

THE HEALTH-PROMOTING FATTY ACIDS OF MILK FAT AND MILK QUALITY IN RELATION TO THE BREED OF DAIRY COWS REARED IN SLOVAKIA

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The aim of this study was to investigate the effect of gene pool on milk production and fat nutritive value of raw cow's milk from different upland farming areas in Slovakia. We examined Braunvieh (B, n = 8), Pinzgau (P, n = 74), Holstein (H, n = 105), Slovak Spotted (S, n = 61) and Red Holstein (R, n = 120) cows. We have found that P had significantly lower values of the total amount of produced milk, fat and protein compared to the other breeds. The B and H breeds had the lowest content of the fat-free dry matter (FFDM) and dry matter (DM) in the milk. The R and S breeds reached the highest content of fat and protein, and also the highest FFDM and DM. The S and P breeds showed significantly lower content of saturated fatty acids (SFAs 69.34 % and 70.89 %, respectively). This parameter was also affected at the value of the atherogenic index (AI), which is the lowest in both breeds. The highest values of unsaturated fatty acids (UFAs 30.66 % and 29.11 %, respectively) and essential fatty acids (EFAs 3.33 % and 2.98 %, respectively) were observed. From those the most distinguished FA, the conjugated linoleic acid (CLA) had significant proportion in milk of the P (0.67 %) and S (0.56 %) breeds. We conclude that among the observed breeds of dairy cows the lowest values of the health-undesirable components of milk fat in relation to cardiovascular diseases of people were observed in the P and S breeds.

Key words: cow's milk; milk fat; fatty acids; breed; gene pool

EFFECT OF EPICATECHIN ON HAEMATOLOGICAL PARAMETERS OF RABBITS: TWO-WEEK EXPOSURE

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In this study haematological parameters (total white blood cell count, lymphocyte count, medium size cell count, granulocyte count, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, platelet percentage, mean platelet volume and platelet distribution width) in blood of rabbits after epicatechin administration during two weeks were analysed. The animals were divided into four groups: control group (C) and experimental groups (E1 – E3). Experimental groups received epicatechin in the injectable form at 10 µg.kg^{-1} in E1, 100 µg.kg^{-1} in E2 and 1000 µg.kg^{-1} in E3 for 14 days three times a week. Significant decrease ($P < 0.05$) of mean corpuscular haemoglobin concentration in E2 and E3 groups in comparison with the C group was observed. Higher platelet and medium size cell count in the experimental groups and slight decrease in haemoglobin in the experimental groups was found in comparison with the control group but without significant differences ($P > 0.05$). Other haematological parameters were not affected by this natural antioxidant. Epicatechin has a number of positive effects on organism including the improvement of vasodilation, reduction of blood pressure, insulin resistance and glucose tolerance, the attenuation of platelet reactivity and the improvement of immune responses and antioxidant defence systems.

Key words: epicatechin; haematology; rabbit

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