

Genetic diversity and production potential of animal food resources

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The submission aims to present results of the five-year research project, oriented on the evaluation of genetic diversity of selected populations of economically important animal species in Slovakia, their sustainable adaptation and production potential in the context of preservation of genetic resources and food safety. Under the supervision of Department of Animal Genetics and Breeding Biology, Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra run between 2015-2019 project called Molecular-genetic diversity and production potential of animal genetic resources in Slovakia (APVV-14-0054). Considering the difficulty and complexity of studied issues was research realized in close collaboration with the University of Natural Resources and Life Sciences Vienna (BOKU) and Zagreb University. Erosion of genetic diversity represents the main threat for food safety of mankind. Individuals of economically important animal species groups accumulate risks and threats of loss of sustainable adaptation as a reaction to the environment due to intense selective breeding. It is therefore important and needed to focus on permanent monitoring and evaluation of diversity of economically important breeds based on the diverse parameter and suitable methods.

Keywords: Genetic diversity, economically important breeds, Animal genetic resources, Slovakia

1 Introduction

In the context of transformation and globalisation processes are ever often discussed questions of food safety and tools for its maintenance. Already in 2007, Commission for genetic resources for nutrition and agriculture FAO, resp. International technical conference in Swiss city Interlaken approved Global Plan for Animal Genetic Resources and Interlaken Declaration. It contains 23 strategical priorities, which shall beside others help erase extreme poverty and ensure sustainable development. No poverty is the No. 1 within the Sustainable Development Goals to transform our World under gesture of UN and protection of biodiversity is part of that Global Plan fo Action represented under Goal No. 15: Life on Land. Needed is, therefore, global collaboration, as well as intense development of programmes and politics of sustainable utilisation and development, protection and description of Animal genetic resources at the national level.

This submission represents the results of the research project aimed at the evaluation of animal genetic diversity of economically important species in Slovakia, which was realised at the Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra, under gesture of Department of Animal *Genetics* and Breeding Biology: Molecular-genetic diversity and production potential of animal genetic resources in Slovakia (APVV-14-0054).

2 Research of genetic diversity as a base for the protection of animal food resources

With the effort to stop the erosion of animal genetic diversity it is important to give attention to the genetic diversity of economically important animal species. Intense genetic interventions and systematic breeding are sources of the high risk of inbreeding and production of inbred progeny, which has a negative impact especially on the genetic diversity of small populations.

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Individuals within such groups accumulate risks and threats of loss of adaptation mechanisms as a reaction to the environment. It is therefore important and needed to focus on permanent monitoring and evaluation of diversity of economically important breeds based on the diverse parameter and suitable evaluation methods. Estimation of genetic diversity loss and monitoring of changes of each population from the point of view of all elements entering the breeding process is therefore essential. It is a simple action, but even complex of the network of supporting each other.

A project called Molecular-genetic diversity and production potential of animal genetic resources in Slovakia (APVV-14-0054) was carried under the supervision of Department of Animal Genetics and Breeding Biology, Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra between 2015–2019. The project team consisted of 9 researchers, from which two new workplaces have been generated by the project and early career scientists employed.

Core themes of the project were:

- a) Knowledge and preservation of the diversity of breeds and the preservation of genetically important animals with high production potential.
- b) Identification of population-specific variants of SNP markers as a condition of long-time survival.
- c) Establishment of SNP panels for milk and meat production in cattle as well as performance in horses.

Populations for research included dairy (Jersey), beef (Limousine, Charolais, Hereford, Angus, Piedmontese and Romagnola) and dual-purpose (Slovak Pinzgau, Slovak Spotted, Carpathian Brown) in case of cattle and warm-blood (Furioso, Nonius, Lipican, Slovak warm-blood) as well as cold-blood (Norik) horses. During the whole project period were established complex methodologies for determination of identifiers of “higher-order” not only in cattle and horses but even in other livestock and wild species (Kasarda et al., 2016a; Kadlečík et al., 2017a; Moravčíková et al., 2018a). An integral component of complex methodology was the determination of genetic diversity or uniqueness of genetically close populations and sub-populations (Kasarda et al., 2016b; Moravčíková et al., 2017a; Kukučková et al., 2018a; Moravčíková et al., 2018b).

Different methods for determination of admixture level of populations were used based on Bayesian approach and discriminant analysis of principal components (DAPC) (Moravčíková et al., 2017b, c; Lehocká et al., 2020) evaluation of genetic differentiation of populations

based on linkage disequilibrium (LD) with simultaneous identification of genomic regions showing selection signatures linked on their genetic differentiation (Kukučková et al., 2016a,b), determination of the level of genetic differentiation of populations based on Wright's F_{ST} index and determination of genomic regions characteristic for particular breeds and local populations, respectively (Lehocká et al., 2019a). As part of the methodology for evaluation of the effect of selection on genome structure and identification of genomic regions significantly affected by the specific selection type, were evaluated parameters as extended haplotype homozygosity (EHH) integrated haplotype score (iHS) (Kasarda et al., 2015), Wright's F_{ST} on the whole genome level (Moravčíková et al., 2018c), level of autozygosity based on the distribution of homozygous segments (ROH) (Kasarda et al., 2018a; Moravčíková et al., 2018d; Moravčíková et al., 2019a) and the variability of linkage disequilibrium (Kasarda et al., 2016c; Moravčíková et al., 2019b). An integral part of the genetic diversity monitoring of cattle and horse breeds was the evaluation of observed heterozygosity (H_o) and gene diversity (H_e), the average number of alleles (MNA), effective number of Alleles (A_{ne}), Shannon information index (I) in case of STR markers solely (Kasarda et al., 2016d; Vostrá Vydrová et al., 2018). Wright's FIS index as the molecular equivalent of inbreeding coefficient calculated based on pedigree information, genomic inbreeding coefficient based on the distribution of homozygous segments (ROH) in the genome, inbreeding coefficient (F) and its increase (ΔF) based on pedigree analysis (Kadlečík et al., 2016, Kadlečík et al., 2017b; Kasarda et al., 2017a; Kasarda et al., 2019), level of population fragmentation based on F -statistics and analysis of molecular variance (AMOVA), Nei genetic distances at intra- and inter-population level (D_a), migration level and genetic drift intensity (Kukučková et al., 2016c; Kukučková et al., 2018b; Miluchová et al., 2018; Moravčíková et al., 2020), present and historical effective population size (N_e) based on molecular and pedigree data (Kadlečík et al., 2016; Kukučková et al., 2016b; Moravčíková et al., 2017d). In cattle, the genotype-phenotype associations were tested concerning milk production (Trakovická et al., 2017) and beef quality (Miluchová et al., 2018; Trakovická et al., 2018a, b, c; Trakovická et al., 2019). In pigs, the effect of candidate genes responsible for carcass traits and meat quality were analysed (Trakovická et al., 2016a, b). In terms of the well-being and functional traits of dairy cattle, the optimal methodology for reliable claw traits measurement were suggested (Vlček et al., 2016a, b; Vlček et al., 2017a, b, c). The genetic parameters for the claw traits and metabolic diseases were estimated based on the Bayesian and REML approaches (Kasarda et al., 2018c, Kasarda et al., 2019a).

Considering the difficulty and complexity of studied issues was research realized in close collaboration with the University of Natural Resources and Life Sciences Vienna (BOKU) and Zagreb University for comparison analyses, in particular. The national platform constituted of breeding associations and organizations in Slovakia. Results of each research tasks were presented on national, as well as on international level in form of in total 73 publications (Where 9 in CC, 21 indexed in WOS and SCOPUS, 1 scientific monograph with the renowned foreign publisher, 3 scientific monographs in Slovakia).

3 Obtained results

The results of the project show, that Slovak Pinzgau and Slovak Spotted are significantly endangered. In case of Slovak Pinzgau it is based on analyses of homozygous runs longer than 16 Mb, reflecting present inbreeding, which present 0.81% of the genome (Kukučková et al., 2017a, b; Kasarda et al., 2019b). Similar results were obtained in case of Slovak Spotted, whereas 0.43% of the genome was covered by homozygous segments

(ROH) (Kasarda et al., 2018a, Kasarda et al., 2019c). Those observations clearly point a relatively large proportion of inbred animals in the present generation in case of both breeds. Level of endangerment was further evaluated by effective population size. In the case of Slovak Pinzgau was estimated effective population size cca. 30 individuals with loss of 7.01 animal per generation (Kukučková et al., 2017b; Moravčíková et al., 2017d). Effective population size in Slovak Spotted was 38.69 with loss of 7.64 animal per generation (Lehocká et al., 2019b). Both values are below the recommended minimum effective population size limit (50 animals) for the preservation of genetic diversity and could be considered as alarming. Even in the case of beef breeds was observed also the linear decrease of effective population size, however, the values were between (20–120 animals). The decrease was more rapid in the case of Limousin cattle. In the case of observed horse populations, results show a relatively stable level of genetic diversity (Kasarda et al., 2020).

Relatively high heterozygosity was observed in the case of Slovak Spotted ($H_o = 0.69$; $H_e = 0.70$) based on STR

Table 1 SNP marker panel significantly associated with milk performance in Pinzgau cattle (Kasarda et al., 2017a)

CHR	Illumina ID	Position (bp)	-log (p-value)	QTL trait
1	ARS-BFGL-NGS-18066	111357945	4.77e-06	Milk Yield, Dressing percentage
7	BTB-00955523	105621232	4.74e-06	Milk Yield, Protein Yield, SCS score
8	Hapmap48090-BTA-81304	60269047	4.47e-06	
9	Hapmap60949-rs29020404	52283151	7.68e-06	Marbling Score, Milk, Protein and Fat yield
15	ARS-BFGL-NGS-12339	20018872	1.05e-06	
	ARS-BFGL-NGS-118767	24021537	2.93e-06	
16	BTA-38204-no-rs	3075859	2.52e-06	
18	ARS-BFGL-NGS-15438	53224638	5.18e-06	

Table 2 SNP marker panel significantly associated with milk performance in Slovak Spotted (Moravčíková et al., 2018e)

CHR	Illumina ID	Position (bp)	-log (p-value)	QTL trait
2	ARS-BFGL-NGS-102253	62853776	2.66E-05	Fat content
4	ARS-BFGL-NGS-76618	104909380	2.85E-05	Fat content
6	Hapmap58359-rs29011329	7514029	2.37E-05	Fat content
8	BTB-01415809	47479350	1.50E-06	Protein content
11	ARS-BFGL-NGS-39507	74139241	7.63E-06	Milk production, Protein content
12	BTB-00500829	68846890	3.72E-06	Protein content
14	Hapmap50356-BTA-42148	17401030	1.91E-05	Fat content
15	ARS-BFGL-NGS-44706	76438547	2.50E-05	Protein content
15	ARS-BFGL-NGS-25994	76466667	2.50E-05	Protein content
17	ARS-BFGL-NGS-96040	12766838	2.89E-05	Fat content
21	ARS-BFGL-NGS-103866	5690539	3.03E-05	Fat content

markers, whereas FIS index didn't show any significant effect of inbreeding on genetic diversity. Further analysis showed a higher level of heterozygosity and gene diversity of Slovak Pinzgau cattle ($H_o = 0.75$; $H_e = 0.73$) in comparison to Slovak Spotted, confirmed by negative FIS (-0.02) index. In total, a higher level of genetic diversity in comparison to other local cattle (Holstein, Simmental, Montbeliard, Austrian Pinzgau) was also indicated by other parameters, including (MNA = 7.82) and Shannon information index ($I = 1.55$) (Kasarda et al., 2019b; Kasarda et al., 2019d). In case of horses were analysed 3 warmblood breeds (Lipizzan, Furioso, Nonius). For the analyses were used animals representing gene-pool of those breeds in Slovakia. The observed level of genetic diversity and heterozygosity (0.89) within populations show the dominance of heterozygous animals and therefore a good level of genetic (Moravčíková et al., 2016; Kasarda et al., 2016d; Kasarda et al., 2018d; Kasarda et al., 2019e).

Genomic information was used to characterize the structure of observed populations and evaluation of admixture level in Slovak Pinzgau and Slovak Spotted in

connection to other European cattle and subsequently identified genomic regions, which could be specific especially for local populations of Pinzgau or other endangered breeds. The analyses identified clear differentiation among 15 populations, as well as an expected higher level of genomic similarity between Slovak Pinzgau and Austrian Pinzgau cattle (Kukučková et al., 2017b; Kasarda et al., 2019b). According to a more general view, Cika and Pinzgau were closest breeds. Regarding the relatively low value of F_{ST} index, resulting from a high level of genetic similarity between individuals, identification of regions representing differences between Slovak and Austrian population was possible. Strongest signals were observed on Chr. 6. Regions with high homozygosity were detected on Chr 2, 4 and 11 (Kasarda et al., 2015; Kukučková et al., 2017b; Kasarda et al., 2018a; Moravčíková et al., 2018c).

Subsequent GWAS analysis was realized in Slovak Pinzgau and Slovak Spotted. In both cases was the aim to identify genomic positions associated with milk performance and proportion of fat and protein in milk. In the case of Pinzgau cattle data consisted of information about 7729

Table 3 SNP marker panel for milk performance and reproduction (Moravčíková et al., 2017a)

CHR	SNP ID	Position (bp)	Gene	Trait
1	rs109007595	35014129	POU1F1	Production and composition of milk, urea, SCS
1	rs41608610	81589478	DGKG	Milk fatty acids, UFA, Production of milk, SCS
2	rs43706906	79923716	STAT1	Production and composition of milk, urea, SCS
4	rs110559656	93257549	LEP	Milk fatty acids, SFA, Production of milk, SCS
5	rs41604573	70471512	RIC8B	α -LA
6	rs29024684	87396306	Casein family	α S1-CN, α -LA, milk protein content
6	rs41577868	37983812	ABCG2	Milk fatty acids, UFA, Production of milk, SCS
6	rs41653166	95988438	CSN2	β -CN
6	rs41654958	90730485	Casein family	Protein content
6	rs42225005	67643584	Casein family	β -LG
9	rs29018912	17726910	MEI4	α -LA
13	rs110270855	1278678	PLCB1	Production and composition of milk, urea, SCS
13	rs41624761	1655502	PLCB1	Production and composition of milk, urea, SCS
13	rs41630716	60242262	MC3R	κ -CN
18	rs41572288	54450227	GRLF1	Production of milk, SCS, urea, acidosis
19	rs109686238	14673538	CCL3	Milk fatty acids, SFA
19	rs110562092	13887927	ACACA	Milk fatty acids, SFA, Production of milk, SCS
20	rs41640170	36097136	EGFLAM	Milk protein content
22	rs29020976	39491373	PTPRG	α S1-CN
24	rs29016076	34928812	ABHD3	β -LG
26	rs41606739	33003665	GPAM	α -LA
26	rs41624917	15383866	PLCE1	Production and composition of milk, urea, SCS

daughters (130087 milk records) 35 sires, with genomic information available. Identification of genomic regions associated with milk performance and its content was made using a linear mixed model. Results of analyses confirmed signals especially in regions of QTLs associated with milk performance, protein and fat content as well as somatic cell count or marbling (Table 1), confirming dual-purpose character of Pinzgau cattle (Kasarda et al., 2017a). Similarly as in the case of Pinzgau population were observed associations between SNP markers and breeding values for milk, fat and protein (in kg and %) in Slovak Spotted. A linear model with random regression was used to analyse data. SNP markers with significant effect were located predominantly in genomic regions of Chr 8, 11 a 12. In table 2 is the list of SNP markers with the most important effect on the variability of observed traits (Moravčíková et al., 2018e).

4 Conclusions

The results of the five-year research project, oriented on the evaluation of genetic diversity of selected populations of economically important animal species in Slovakia, their sustainable adaptation and production potential in the context of preservation of genetic resources and food safety served as the background for this review. Erosion of genetic diversity represents the main threat for food safety of mankind. It is therefore important and needed to focus on permanent monitoring and evaluation of diversity of economically important breeds based on the diverse parameter and suitable methods. Expected results of such research will be the identification of unique genomic regions for the particular populations with national importance and identification of regions affected by selection on the genome-wide level and application of comprehensive methodologies of the genomic data utilisation in animal production and protection of animal genetic resources.

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