

GENOMIC AND PEDIGREE-BASED INBREEDING IN SLOVAK SPOTTED CATTLE

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ABSTRACT

The objective of this study was to evaluate the level of inbreeding in population of Slovak Spotted cattle and to compare its genomic and pedigree-based estimates. The genomic data have been obtained from in total of 37 AI sires and 50 sire dams genotyped by using Illumina BovineSNP50v2 BeadChip and ICBF International Dairy and Beef v3, respectively. The genealogical information have been obtained from the database of Breeding Services of the Slovak Republic, s. e. The pedigree file consisted of 109,686 individuals (105,229 dams and 4,457 sires), while the reference population included only living animals, AI sires (129) and dams (36,949). The genomic inbreeding (F_{ROH}) was computed as the length of the genome present in runs of homozygosity (ROH) divided by the total length of the autosomal genome covered by SNPs on the chip and the pedigree-based inbreeding (F_{PED}) was calculated based on assumption that inbreeding of an individual reflects the probability that both alleles in one locus are derived from the same ancestor or are identical by descent. The ROH segments greater than 4 Mb ($F_{ROH > 4Mb}$) covered in average 2.09 % of the genome, while the ROH segments greater than 16 Mb ($F_{ROH > 16Mb}$) achieved 0.43 % which indicated in analysed population recent inbreeding. Similarly, the increase of inbreeding across generation signalized the average F_{PED} computed from pedigree information (0.094%). However, the pedigree-based and genomic estimates of inbreeding differ from each other (in average $F_{ROH>4}=0.02$; $F_{PED}=0.004$). In recent generation, the obtained values of F_{ROH} indicated considerably higher degree of inbreeding.

Keywords: *Cattle, Genotyping data, Inbreeding, Pedigree analysis, Runs of Homozygosity.*

INTRODUCTION

The Slovakia has close to a century of tradition in breeding dual-purpose cattle breeds, namely the Slovak spotted and Slovak Pinzgau cattle. The beginnings of a production type transformation go back to 1972 when cross breeding (mainly combined crossing) with foreign breeds (Lowland Black-spotted and later on also

Holstein) has begun, with the purpose of acquiring a pronounced milk-type cattle population but retaining however a certain hereditary ratio (about 30 %) of Slovak spotted cattle in order to maintain its typically good meat utility value in the target population (Biros, 1997). Currently, the breeding of Slovak Spotted cattle continues in the dual-purpose direction focusing on high milk and beef production. Regular fertility, longevity, good adaptation potential and an ability to consume large amounts of bulk feed in connection with a high utility level are required from the Slovak Spotted cattle mainly with respect to the efficiency and economics on the farms. In 2017, totally 28,747 purebred cows have been registered in Slovak Spotted cattle herd book (ZCHSSD, 2018).

Generally, in livestock the inbreeding coefficient is recognized as one of the most important parameter reflecting the level of genetic diversity, relatedness of animals as well as individual fitness of in a population. The inbreeding coefficient of an individual can be described as the probability that two alleles at a locus in that individual are identical by descent and equals the coancestry between its parents (Howard *et al.*, 2015). Thus, the inbreeding coefficient can be considered as a key parameter mainly with respect to understanding of the amount of matings between related animals in a population (Gomez-Raya *et al.*, 2015). It has been shown that the high level of inbreeding leads to a reduction in fitness and overall productivity at the phenotypic level as well as to a higher risk of homozygosity for deleterious/lethal alleles at the genotypic level (Curik *et al.*, 2014; Howard *et al.*, 2015; Forutan *et al.*, 2018). Therefore, the periodic control of inbreeding in livestock populations is crucial for the preservation of animal genetic resources mainly in case of small populations and from a wider perspective for the population management and development of mating plans (Gomez-Raya *et al.*, 2015). Traditionally, inbreeding coefficient has been estimated based on pedigree-based relationships (Meuwissen and Luo, 1992). Pedigree-based inbreeding is based on Mendelian sampling probabilities, so that the inbreeding coefficients of full-sibs are always identical. Using pedigree information for calculating the level of inbreeding usually underestimates the true inbreeding coefficient, mainly due to incomplete pedigree information, especially for distant generations (Forutan *et al.*, 2018). This can be avoided for example by the use of genome-wide data that allow to estimate the level of inbreeding derived from the relative amounts of autozygosity due to recent as well as remote ancestors (Feren akovi *et al.*, 2013a). The best concept to quantify the level of genomic inbreeding based on true or realized autozygosity was suggested by McQuillan *et al.* (2008). The genomic inbreeding coefficient (F_{ROH}) is defined as an individual autozygosity reflecting the proportion of the autosomal genome, in which autozygosity is derived from the assumption that very long stretches of homozygosity (ROH) can only result from inbreeding (Curik *et al.*, 2017).

The objective of this study was an analysis of the trend of inbreeding in Slovak Spotted cattle by using pedigree-based (F_{PED}) and genomic (F_{ROH}) estimates with respect to compare both approaches and to obtain more realistic view on situation in current population.

MATERIAL AND METHODS

The pedigree database of Slovak Spotted cattle included in total of 109,686 individuals (105,229 dams and 4,457 sires). The reference population (RP) covered only living animals (129 AI sires and 36,949 dams) that were included in performance testing in 2013. The genealogical information were obtained in cooperation with the Breeding Services of the Slovak Republic, s. e. The genotyping database consisted of totally 87 animals (37 AI sires and 50 sire dams) that were genotyped in commercial lab by using two platforms, Illumina BovineSNP50v2 BeadChip (AI sires) and ICBF International Dairy and Beef v3 (sire dams). Animals for genotyping were selected based on the previous pedigree analyses that were performed to select only most representative animals in terms of the Slovak Spotted population gene pool. The quality of genealogical information expressed as the pedigree completeness was evaluated based on the equivalent complete generations of ancestors and pedigree completeness index described by MacCluer *et al.* (1983) using Endog v4.8 software (Gutiérrez and Goyache, 2005). The quality of genotyping data was tested by using PLINK 1.9 (Chang *et al.*, 2015). Because of the two different genotyping platforms used for animals' genotyping, the consensus map had to be firstly constructed. The consensus map file included overall 40,033 markers. Subsequent quality control of genotyping data were performed to remove all of SNPs with unknown chromosomal position or localized on unmapped genomic regions based on the bovine genome assembly Btau 5.0.1 and markers located on sex chromosomes. In the subsequent SNP pruning only samples with lower than 10 % of missing genotypes, autosomal SNPs with call rate higher than 90 % and minor allele frequency higher than 1 % that adhered to mendelian inheritance patterns were retained.

The level of pedigree-based inbreeding (F_{PED}) was characterized by using two measures: increase in inbreeding (ΔF_{PED}) and individual increase in inbreeding (F_{PEDi}). The increase in inbreeding was calculated using Endog v4.8 (Gutiérrez and Goyache, 2005) according to Gutiérrez *et al.* (2009) as follows:

$$\Delta F_{PED} = \frac{(F_t - F_{t-1})}{(1 - F_{t-1})},$$

where F_t and F_{t-1} are the average inbreeding at the t^{th} generation and $1 - F_{t-1}$ is increase of inbreeding in last generation. The individual increase in inbreeding was computed as:

$$\Delta F_{PEDi} = 1 - \sqrt[t]{1 - F_i},$$

where F_i is the individual coefficient of inbreeding and t is the equivalent complete generations (Maignel *et al.*, 1996).

The genomic inbreeding (F_{ROH}) was calculated for each individual as the length of the genome present in runs of homozygosity (ROH) divided by specified length of the autosomal genome covered by all SNPs that retained in the database after quality control of genotyping data (2,496,829 kb):

$$F_{ROH} = \frac{\sum k \text{ length}(ROH_k)}{L},$$

where k is the number of ROH identified for each individual in kilobases and L is the total length of the genome covered by SNPs in chip (McQuillan *et al.*, 2008). The genome-wide distribution of ROH segments were scanned using PLINK 1.9 (Chang *et al.*, 2015). The ROH were determined based on following criteria: the minimum number of SNPs included in the ROH segments was fixed to 15; the minimum length of ROH was set to 1 Mb; minimum density of one SNPs on every 100 kb; maximum gap between consecutive SNPs of 1 Mb; one heterozygous call was allowed only for length >16 Mb. In addition, one missing call was allowed for length >4 Mb, 2 for >8 Mb and 4 for >16 Mb. Feren akovi *et al.* (2013b) showed that the 50K panel is not enough sensitive for the precise determination of segments that are 1 to 4 Mb long, so that only for three ROH length categories were taken into account (ROH > 4 Mb, ROH > 8 Mb and ROH >16 Mb) to differentiate between ancient and recent inbreeding.

RESULTS AND DISCUSSION

As expected, the higher level of pedigree completeness across first five generations was found in reference population (81.37 %) than in pedigree file (44.94 %). The maximum number of generation detected was 12. Even if the dams showed generally lower level of pedigree completeness, taking into account only first 5 generations in both groups more than 80 % of the ancestors were known. In agreement with previous studies the completeness of pedigrees had decreasing tendency with increasing number of known generations (Bernardes *et al.*, 2016; Sarmiento *et al.*, 2016; Utrera *et al.*, 2018).

The average value of pedigree-based inbreeding was in range from 0.14 % (pedigree file) to 0.76 % (group of sires). In group of sires the highest individual increase in inbreeding between generations was found (0.19 %), while the average F_{PED} was almost the same regardless of gender (0.25 %) (Figure 1A). Because of this we can expect in the next generations the increase of inbreeding level with the same intensity in both dams and sires groups. The obtained proportion of inbred animals in reference population of Slovak Spotted cattle (42.77 %) is comparable with Irish Simmental population (< 50 %), but the average F_{PED} in group of inbred animals (0.85 %) was much lower than in Irish Simmental breed ($F_{PEDi} = 2.21$ %) (McParland *et al.*, 2007). Similarly, the proportion of inbred animals found in Slovak Spotted cattle is about half lower than in Slovak Holstein cattle (83 %; Pavlík *et al.*, 2012). In reference population, the higher proportion of inbred animals showed the group of sires (Figure 1B) that confirmed study of Pavlík *et al.* (2012) as well. Pavlík *et al.* (2012) reported for Holstein cattle significantly lower proportion of inbred animals in group of dams (82.97 %) compared to sires (98.82 %). This results are logical and clearly reflect the utilization of reduced number of sire lines in breeding practices.

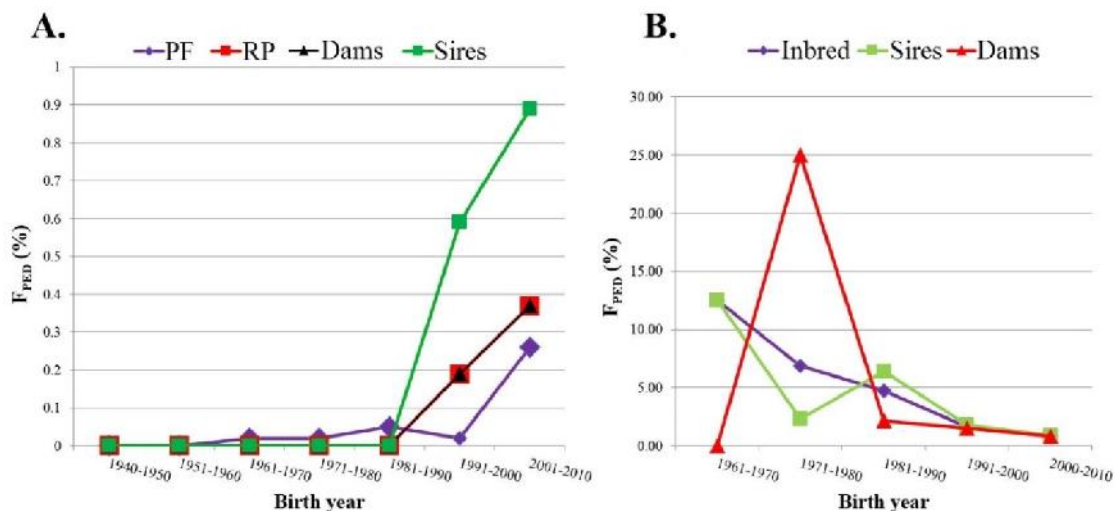


Figure 1. Trend of pedigree-based inbreeding by birth year in Slovak Spotted cattle (A – whole population and B – inbred animals; PF – pedigree file, RP – reference population).

It has been shown that inbreeding coefficients derived from the distribution of ROHs with different length in the genome reflect differently remote common ancestors from the past generations. Various studies reported that the $F_{ROH>4Mb}$ is related to the proportion of autozygosity originating from chromosomal segments present in ancestors that were born 12-13 generations ago. The inbreeding coefficient derived from the distribution of ROH segments greater than 8 Mb ($F_{ROH>8Mb}$) is related to the proportion of autozygosity originating from ancestors that were born 6-7 generations ago and $F_{ROH16>Mb}$ presents in ancestors that were born 3-6 generations ago (Howrigan *et al.*, 2011; Feren akovi *et al.* 2013a; Curik *et al.*, 2014). In Slovak Spotted cattle, the ROH segments greater than 4 Mb cover in average 2.09 % of the genome, while the ROH>16 Mb achieved 0.43 %. This signaled that around 0.5 % of its genome is affected by mating of relatives in recent population. Figure 2 shows boxplot distribution of F_{ROH} by each length category.

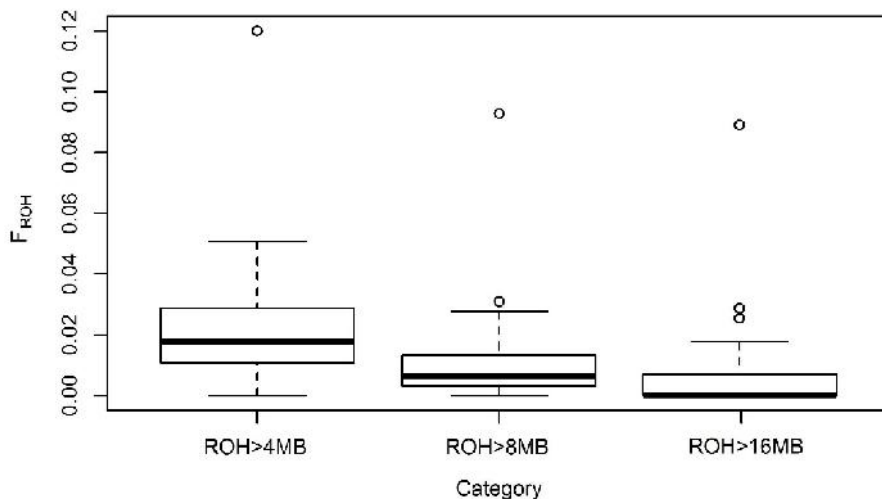


Figure 2. Boxplot distribution of genomic inbreeding derived from different ROH length categories.

As you can see on the figure 2 as well as in table 2 the value of $F_{ROH>4MB}$ indicated considerably higher degree of genomic inbreeding compared to the pedigree-based estimate regardless of gender ($F_{ROH} = 2\%$ vs. $F_{PED} = 0.36\%$). This also points out that the level of true inbreeding in current population of Slovak Spotted cattle can be significantly underestimated. As previous studies have shown an increase in inbreeding over 1% can lead to a reduction in fitness as well as overall performance at the animal's phenotypic level. For example, Bjelland *et al.* (2013) reported for dairy cattle significant association between increase of 1% of F_{ROH} and decrease of milk yield, some liner-type traits, increase in days open and maternal calving difficulty. Similarly, Pryce *et al.* (2014) and Ferencik *et al.* (2017) revealed inbreeding depression for milk yield and reproduction performance with stronger unfavourable effects for F_{ROH} related to closer ancestors (longer ROH segments).

Table 1. Genomic inbreeding by F_{ROH} category in group of sires and dams

Group	Category	Mean \pm SD	Range	Lower 95% CI	Upper 95% CI
Sires	$F_{ROH>4Mb}$	0.020 ± 0.011	0.004 - 0.051	0.016	0.024
	$F_{ROH>8Mb}$	0.007 ± 0.007	0.000 - 0.023	0.005	0.010
	$F_{ROH>16Mb}$	0.002 ± 0.005	0.000 - 0.018	0.001	0.004
Dams	$F_{ROH>4Mb}$	0.022 ± 0.018	0.000 - 0.120	0.016	0.027
	$F_{ROH>8Mb}$	0.011 ± 0.015	0.000 - 0.093	0.007	0.015
	$F_{ROH>16Mb}$	0.006 ± 0.014	0.000 - 0.089	0.002	0.010

SD – standard deviation, CI – confidence interval

CONCLUSION

Both pedigree-based and genomic estimates of inbreeding coefficient per generations indicated risk of increase of inbreeding in current population of Slovak Spotted cattle. The obtained level of inbreeding didn't show significant differences between sires and dams. Thus, in next generations we can expect the increase of inbreeding with the same intensity in both dams and sires group. However, the obtained level of recent inbreeding depended on the applied approach. The comparison between F_{PED} and F_{ROH} values clearly points out that the level of true inbreeding in current population is considerably underestimated most likely due to the incomplete pedigrees in distant generation. The F_{ROH} value around 2 % represents a risk for population especially in view of its production and reproduction performance. Therefore, it would be desirable to take into account not only pedigree data but also genomic information mainly in management of Slovak Spotted population nucleus.

ACKNOWLEDGEMENT

This study was supported by the Slovak Research and Development Agency (APVV-14-0054 and APVV-17-0060).

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