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THE IMPACT OF ARTIFICIAL SELECTION ON RUNS OF HOMOZYGOSITY IN SLOVAK SPOTTED AND PINZGAU CATTLE

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ABSTRACT

The aim of this study was to analyse the distribution of runs of homozygosity (ROH) across the genomes of Slovak Spotted and Slovak Pinzgau cattle and to describe the autozygosity islands resulting from the selective breeding for traits of interest during the development of these breeds. The genome-wide data for a total of 236 animals were obtained by using two platforms: Illumina BovineSNP50v2 BeadChip and ICBF International Dairy and Beef v3. After quality control, the database of genotyping data consisted of 39,261 common SNPs across both breeds that covered overall length 2,497,077 kb of the genome with average distance between adjacent SNPs 63.67 kb. The ROH segments were defined as genomic regions with 15 or more consecutive homozygous calls with maximum gap between SNPs of 1 Mb and minimum density of one SNPs on every 100 kb. The distribution of ROH was analysed for five length categories (> 1 Mb, > 2 Mb, > 4 Mb, > 8 Mb, and > 16 Mb). The results showed that the ROH segments were present across the genome of all animals, with the average number of 54.59 ± 18.58 segments and the average length of 130.33 ± 58.40 Mb. The short segments (> 1 Mb) were the most frequent through the genomes and accounted for 70.26 % (Slovak Spotted) or 65.99 % (Slovak Pinzgau) of all segments detected. Thus, our results indicated that on average 6.11 % (Slovak Spotted) and 4.72 % (Slovak Pinzgau) of the genomes are autozygous. Moreover, the proportion of ROH > 16 Mb revealed that on average 0.45 % of the Slovak Spotted and 0.88 % of the Slovak Pinzgau genomes could be affected by recent inbreeding. Despite the fact that the distribution of ROH differentiated between breeds, the major fraction of chromosome residing in ROH was observed on BTA6 (13.49 % resp. 14.26 % of autosomal length in ROH). In this region we identified various QTLs and genes responsible for milk production (CSN1S1, CSN1S2, CSN2, CSN3), and coat colour patterns (KIT). Generally, our results confirmed that the regions displaying autozygosity in Slovak Spotted and Slovak Pinzgau cattle are linked mostly to milk production and muscle development thus ensuring selection for dual-purpose performance.

Key words: autozygosity islands; high-throughput SNP platforms; dual-purpose cattle; selection signatures

INTRODUCTION

Slovakia has a long tradition in breeding dual-purpose cattle, namely the Slovak Spotted and Pinzgau breeds. Both of those breeds, whose origin is composite of autochthonous Carpathian Red (extinct) and Carpathian Grey (extinct) from the 17th to the 18th century as well as Swiss Simmental and Austrian Pinzgau from the 19th century common in Austro-Hungarian Empire, belong to the main cattle breeds of national interest in Slovakia (Kasarda *et al.*, 2015). First imports of Pinzgau and Simmental purebred animals were organized long time ago before 1894 when system of cattle recording has started in the territory of Slovakia. Over the following decades, the population sizes of both breeds have been improved and in 1958 they were officially accepted as Slovak Spotted and Slovak Pinzgau cattle. The population sizes reached maximum in 1975 and 1978 for Slovak

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Spotted and Slovak Pinzgau, respectively. However, due to post-1990 changes of economic conditions the population size of both breeds has significantly decreased mainly due to the transformation processes in agriculture and utilization of Holstein cattle for crossbreeding (Kadlečík *et al.*, 2013). From long-term perspective, the population size is unfavourable mainly in case of Slovak Pinzgau cattle, which is registered by the UN FAO as threatened with extinction and it is classified as Animal Genetic Resource (AnGR) since 1994 (Kadlečík *et al.*, 2008).

Globally, local breeds are rarely subject to modern selection techniques. However, selection programs will be required if local breeds are to remain a viable livelihood option for farmers. Selection in such small populations needs to take into account accurate inbreeding control that is one of the most important criteria in the evaluation of the degree of endangerment of the given breed (Gandini *et al.*, 2014). It is generally accepted that the population is endangered if the increase in inbreeding in the population per generation is higher than 1 % (Kasarda and Kadlečík, 2007). It should be noted also that the rate of inbreeding in cattle still has increasing tendency and there is a strong correlation between level of inbreeding and reduced fitness. Moreover, the high level of inbreeding and reduced variability in populations will result in inbreeding depression and reduced selection response in breeding programs. Thus, maintaining genetic diversity is crucial in cattle breeding populations (Zhang *et al.*, 2015).

Traditionally, the inbreeding coefficient is estimated by the degree of parental relatedness based on the pedigree data, while the genomic inbreeding is based on the proportion of the genome that is autozygous. Moreover, pedigree-based inbreeding is based on Mendelian sampling probabilities, so that the inbreeding coefficients of full-sibs are always identical. Using pedigree information for calculation of the level of inbreeding usually underestimates the true inbreeding coefficient mainly due to incomplete pedigree information, especially for distant generations (Zhang *et al.*, 2015; Forutan *et al.*, 2018). Calculating the inbreeding coefficient based on genomic data is more accurate for estimation of genome autozygosity and for detection of both past and more recent inbreeding effects than are estimated from pedigree data. The better results of genomic inbreeding coefficient suggest that it can be used to infer information about the history and inbreeding levels of a population in the absence of genealogical information (Peripolli *et al.*, 2017).

The application of high-throughput single nucleotide polymorphism (SNP) platforms allows for determination of autozygous segments based on the identification of runs of homozygosity (ROH) genotypes (Kim *et al.*, 2013), whose frequency, size and distributions in the genome are affected by many factors such as artificial selection, recombination rate, linkage disequilibrium and population structure or mutation rate (Peripolli *et al.*, 2017). Generally, the ROH are defined as continuous homozygous segments that are common in individuals and populations. ROH segments would be expected within an individual when both identical haplotypes share a common ancestor, and this should be correlated to the inbreeding coefficient as defined by the probability that two genes at a locus are identical by descent (Kim *et al.*, 2013; Mastrangelo *et al.*, 2018). One of the most important factors that affect the ROH patterns in various genomic regions is artificial selection of superior animals. Such selection pressure increases mainly the proportion of homozygous genotypes around the target locus involved in the genetic control of phenotypic traits of interest. Thus, ROH segments can be also defined as genomic regions with reduced diversity and, consequently, high homozygosity around the selected locus that might harbour targets of positive selection and are under strong selective pressure (Pemberton *et al.*, 2012). Because of this, the analysis of ROH segment distribution in the genome provides an information about how the architecture of genome can disclose a population's genetic background. By revealing the molecular changes in populations over time, genome-wide information is crucial to understanding antecedent genome architecture and, therefore, to maintaining diversity and fitness in endangered livestock breeds (Peripolli *et al.*, 2017).

The aim of this study was to analyse the ROH segment distribution in the genomes of Slovak Spotted and Slovak Pinzgau cattle, to determine the impact of artificial selection on the architecture of their genomes and to describe the genomic regions mostly affected by strong selection pressure during the development of those breeds.

MATERIAL AND METHODS

Database of genotyping data

In order to analyse the impact of artificial selection on genome architecture the genotyping data for a total of 236 animals, representing the nucleus of both Slovak Spotted and Slovak Pinzgau cattle, were obtained. The sample from Slovak Spotted cattle consisted of 37 AI sires and 48 dams that were genotyped by using two platforms, Illumina BovineSNP50v2 BeadChip (AI sires) and ICBF International Dairy and Beef v3 (dams). The samples of Slovak Pinzgau cattle covered living animals (19 active breeding bulls, 35 dams of sires, and 79 dams of dams) as well as AI doses deposited in reproduction centres (18 animals). In case of Slovak Pinzgau cattle, all of animals were genotyped by using Illumina BovineSNP50v2 BeadChip in a commercial lab.

Quality control of data

The data cleaning was performed by using PLINK 1.9 (Purcell *et al.*, 2007; Chang *et al.*, 2015). The quality control of genotyping data was carried out to remove markers assigned to unmapped regions or with unknown chromosomal position according to the latest bovine genome assembly (Btau 4.0) and SNPs positioned to sex chromosomes. In the following step, the consensus map had to be constructed, because of the two different genotyping platforms used for animals' genotyping. The final consensus map file consisted of 40,033 markers. 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.

Distribution of ROH segments in the genome

The ROH segments were defined according to Ferenčaković *et al.* (2013) as genomic regions with 15 or more consecutive homozygous calls with maximum gap between consecutive SNPs of 1 Mb and minimum density of one SNPs per every 100 kb. Because of the theoretical relationship between the distribution of identity by descent (IBD) fragments and the number of generation since common ancestor the minimum length of ROH segments was set to 1 Mb. The distributions of ROH segments in the genome were analysed separately for five length categories (> 1 Mb, > 2 Mb, > 4 Mb, > 8 Mb, and > 16 Mb). Heterozygous calls were not allowed across ROH categories, except length > 16 Mb with one permissible call. Missing calls per windows were not allowed for lengths > 1 Mb and > 2 Mb, while one missing call was accepted for length > 4 Mb, two for > 8 Mb and four for > 16 Mb. The total number of ROH detected, the average length of ROH (in Mb) and the sum of all ROH segments per animal were calculated for each ROH length category within and across analysed breeds. The proportion of autosomes covered by ROH was then expressed for length > 1 Mb as the pools of overlapping segments within animals per each breed.

The subsequent analysis of genome-wide selection signatures was based on the assumption that the identified autozygosity islands across the genome of Slovak Spotted and Slovak Pinzgau cattle are results of selective breeding for traits of interest defined in their breeding objectives. The autozygosity islands, characterized by SNPs with extreme frequency in ROH segments > 4 Mb across specific genomic regions, were determined based on the calculation of runs incidence per each SNP by using Plink 1.9 (Purcell *et al.*, 2007; Chang *et al.*, 2015). The genome-wide occurrence of SNPs in ROH was then expressed as the frequency (%) of overlapping ROH shared among samples and visualised by R package qqman (Turner, 2014). The genome-wide significance threshold for SNPs under selection (with extreme ROH frequency) was determined based on the corresponding boxplot distribution. All of SNPs with appropriate level of significance were assigned to the genomic QTL (quantitative trait loci) location according to the Bovine Genome Database (http://bovinegenome.org). To identify genes located in ROH regions under the most intense selection pressure, the Genome data viewer of the bovine genome UMD3.1.1 was used (https://www.ncbi.nlm.nih.gov/genome/gdv/).

RESULTS AND DISCUSSION

After quality control of genotyping data, the database consisted of 39,261 common SNPs for both Slovak Spotted and Slovak Pinzgau cattle that

covered overall length 2,497,077 kb of the genome. The average distance between adjacent SNPs was 63.67 kb; minimum distance was 0.02 kb and maximum distance between markers was 4428.95 kb.

The ROH segments were identified across the genome of all analysed individuals, with average number of 54.59 ± 18.58 segments and average length of 130.33 ± 58.40 Mb. The detailed descriptive statistics of the ROH number and length by categories for each breed is given in Table 1. The average sums of the ROH length calculated per animal and averaged per breed were higher in Slovak Spotted (152.48 Mb) compared to Slovak Pinzgau cattle (117.86 Mb) and represent in average 6.11 % or 4.72 % of their autosomal genome covered by SNP markers. As expected from these results, the average number of ROH per animals was similarly higher in Slovak Spotted (71.86) compared to Slovak Pinzgau cattle (44.88). The obtained proportion of the genome autozygosity per animals was comparable to results reported for dual-purpose breeds (Ferenčaković *et al.*, 2011; Marras *et al.*, 2015; Szmatoła *et al.*, 2016). On the other hand, both of the analysed breeds showed lower genome autozygosity compared to those reported for dairy cattle. For example Purfield *et al.* (2012), Kim *et al.* (2013) and Mastrangelo *et al.* (2016) identified dairy cattle as one of the most homozygous animals among various cattle breeds. Such high genome autozygosity in dairy cattle could be a consequence of intensive artificial selection as well as repeated use of superior and proven sires in breeding practices (Peripolli *et al.*, 2018). Figure 1 shows the chromosome-wise distribution of ROH segments with minimum length 1 MB across breeds. As can be evident from other results as well (Table 2, Figure 3), the major fraction of autosome residing in ROH was observed on BTA6 for both analysed breeds (13.49 % resp. 14.26 % of autosomal length in ROH), while the lowest coverage by ROH was found on autosome BTA19 for Slovak Spotted and on BTA24 for Slovak Pinzgau cattle.

Figure 2 shows the differences between the number of ROH segments (> 4 Mb, > 8 MB, and > 16 Mb) and the length of the genome covered by them. The results indicated that the number of ROH segments as well as the length of genome covered by them were considerably different

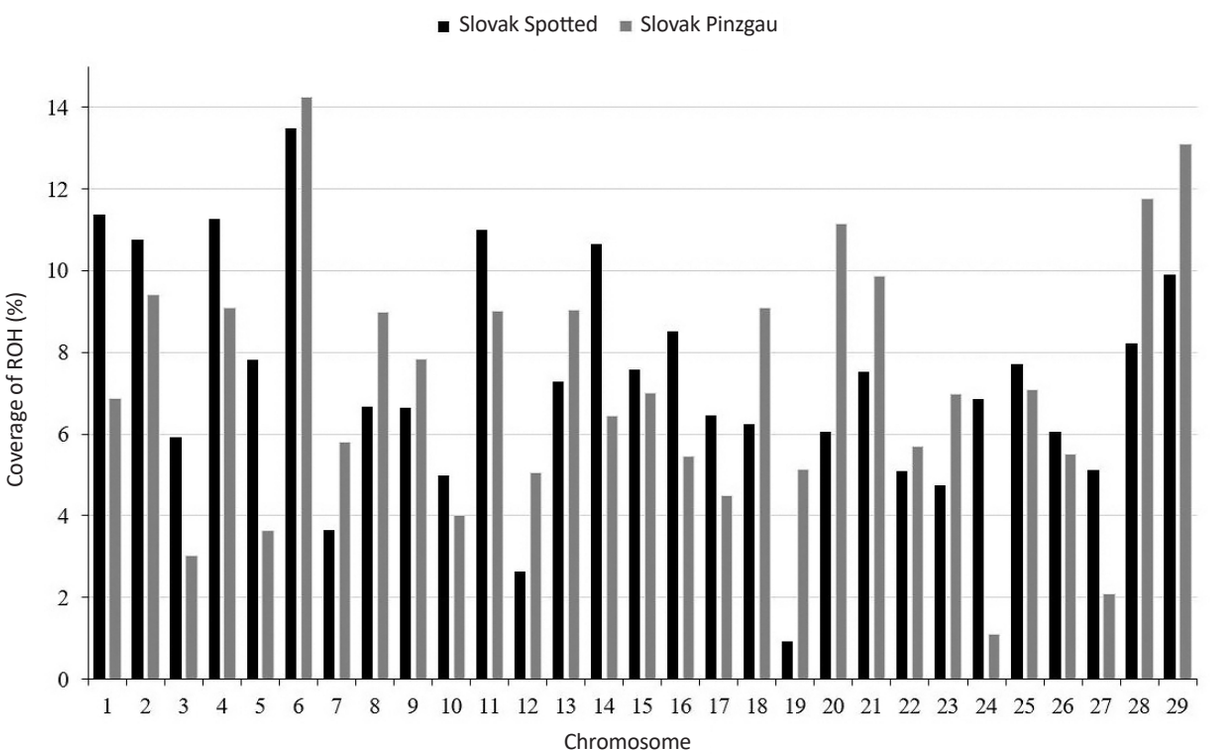

Figure 1. Percentage of autosome coverage by ROH (minimum ROH length set to 1Mb)

depending on the studied animal, which is likely a result of the distinct distances from the common ancestors (Mészáros *et al.*, 2015).

Based on the applied criteria in ROH analysis across all length categories, overall 8,694 ROH segments for Slovak Spotted and 10,270 for Slovak Pinzgau cattle were identified. As described in many studies, the length and frequency of ROH can give insight into the history of the population in which an individual occurs and the history of that individual's ancestors (Howrigan *et al.*, 2011; Curik *et al.*, 2014; Szmatoła *et al.*, 2016). Thus, the length of ROH can be used to determine the age of inbreeding (Curik *et al.* 2014). It has been shown that very long autozygous ROH are expected to originate from recent common ancestors, while most of short ROH are likely derived from more remote ancestors. But sometimes, short ROH might persist in a population for a very long time, much above defined base population, as a consequence of the lack of recombination or just by chance (Curik *et al.*, 2017). Howrigan *et al.* (2011) revealed based on simulated sequence data that the expected length of autozygous segments follow an exponential distribution with average value equal to ½ g Morgan (g is the number of generations since common ancestor). In cattle, Ferenčaković *et al.* (2013) showed that the ROH > 1 Mb date back ~50 generations, > 2 Mb ~25 generations, > 4 Mb ~12.5 generations, > 8 Mb ~6 generations, and > 16 Mb ~3 generations. In our study, the total length of runs of homozygosity for both breeds was composed mostly from short segments (> 1 Mb) that accounted for 70.26 % (Slovak Spotted) and 65.99 % (Slovak Pinzgau) of all segments detected. The ROH segments of 2 – 4 Mb long, representing the 25 – 12 .5 generations from common ancestor, accounted for 27.4 % (Slovak Spotted) and 28.98 % (Slovak Pinzgau). The lowest proportion within the total length of ROH was found for the longest segments (> 16 Mb) that accounted for 0.45 % and 1.29 % of all segments detected. In terms of the genetic diversity those results indicated that on average 0.45 % of the Slovak Spotted genome and 0.88 % of the Slovak Pinzgau genome could be affected by recent inbreeding.

In subsequent analysis the impact of artificial selection on genome architecture was tested based

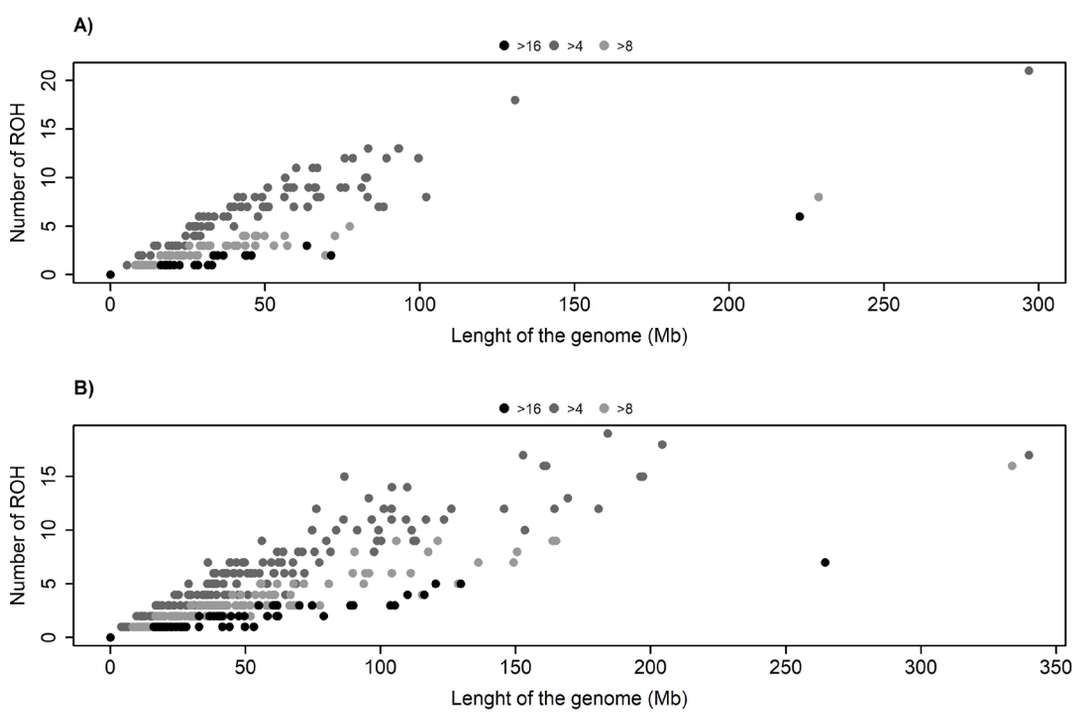

Figure 2. The relationship between the number of ROH segments and the length of genome covered by them (minimum ROH length set to 4 Mb, 8 Mb, and 16 Mb) for Slovak Spotted (A) and Slovak Pinzgau (B) cattle

Table 1. The length (Mb) and number (in brackets) of ROH by categories within analysed breeds

Category	Mean ± SD	Lower 95 % CI	Upper 95 % CI	Range	Genome coverage %
Slovak Spotted					
> 1 Mb	152.48 ± 53.18 (71.86 ± 16.42)	141.01 (68.32)	163.95 (75.40)	21.38 - 396.13 (14.00 - 101.00)	6.11
> 2 Mb	83.83 ± 46.66 (21.28 ± 8.64)	73.76 (19.42)	93.89 (23.15)	3.11 - 317.54 (1.00 - 36.00)	3.36
> 4 Mb	50.73 ± 37.90 (6.99 ± 3.65)	42.56 (6.20)	58.91 (7.78)	0.00 - 296.79 (0.00 - 21.00)	2.03
> 8 Mb	23.10 ± 29.57 (1.69 ± 1.49)	16.73 (1.37)	29.48 (2.02)	0.00 - 228.91 (0.00 - 8.00)	0.93
> 16 Mb	11.22 ± 28.04 (0.46 ± 0.92)	5.17 (0.26)	17.27 (0.66)	0.00 - 222.70 (0.00 - 6.00)	0.45
Slovak Pinzgau					
> 1 Mb	117.86 ± 57.67 (44.88 ± 11.23)	108.58 (43.07)	127.13 (46.69)	18.83 - 398.71 (13.00 - 70.00)	4.72
> 2 Mb	76.58 ± 53.98 (13.44 ± 6.52)	67.89 (12.39)	85.26 (14.49)	2.16 - 350.75 (1.00 - 34.00)	3.07
> 4 Mb	58.96 ± 51.28 (6.26 ± 4.21)	50.71 (5.59)	67.20 (6.94)	0.00 - 339.89 (0.00 - 19.00)	2.36
> 8 Mb	38.50 ± 43.76 (2.54 ± 2.39)	31.47 (2.16)	45.54 (2.93)	0.00 - 333.68 (0.00 - 16.00)	1.54
> 16 Mb	21.95 ± 35.06 (0.88 ± 1.22)	16.31 (0.69)	27.58 (1.08)	0.00 - 264.49 (0.00 - 7.00)	0.88

on the assumption that the regions with increased ROH frequencies are most likely consequences of selective breeding for traits of interest during the development of analysed breeds (Kim *et al*., 2013; Curik *et al*. 2014). The consecutive ROH segments identified in the genome close together reflected mainly the existence of a founder alleles broken down by recombination over generation (Biscarini *et al*., 2014). In our study, the autozygosity islands were defined as specific genomic regions where SNPs had extreme ROH frequency (minimum ROH length set to 4 Mb). According to the boxplot distribution the genome-wide significance threshold for those SNPs was set to 6.5 % for Slovak Spotted, 5.9 % for Slovak Pinzgau and 5.4 % for a whole population (Figure 3). As Figure 3A and 3B show, the overlapping ROH were evident across the genomes of both breeds, but the distribution of identified autozygosity islands was not uniform and differentiated between breeds. The detailed description of each region with extreme ROH frequencies, corresponding number of SNPs, number of genes and quantitative trait loci (QTL) is listed in Tables 3 and 4.

A total of 18 overlapping autozygosity islands located on seven autosomes (BTA4, BTA6, BTA11, BTA16, BTA18, BTA28, and BTA29) were detected across both breeds (Table 2). As shown in figure 3C, a strong selection signal was found mainly on BTA6. Several genes responsible for various traits were identified directly in the regions of these homozygous segments. Within the second autozygosity island on BTA2, the genes encoding insulin-like growth factor binding protein (IGFBP1 and IGFBP3) were found. IGFBP as a structural gene responsible for the multiple influences of insulin-like growth factors (IGFs) system is considered as a candidate gene for growth and production traits (Othman *et al*., 2014). The KIT gene was identified on the BTA6 in the fifth region. In cattle,

the KIT gene is responsible for coat colour pattern and is recognized as a candidate gene for the spotting locus (Fontanesi *et al*., 2016). In addition, the MC1R gene (melanocortin receptor 1) that determines the basic coat color in cattle (Dorshorst *et al*., 2015) was found in the homozygous region on BTA18. In the sixth region on BTA6 the genes for casein family were detected (CSN1S1, CSN1S2, CSN2, CSN3). For example the CSN2 A1 genetic variant is considered as a riskfactor in milk intolerance and in other important human diseases (Massella *et al.*, 2017). On BTA11 the POMC gene (proopiomelanocortin), which is a candidate for carcass traits in beef cattle, was identified. POMC is the precursor for several peptide hormones produced by post-translational processing, some of which are involved in energy homeostasis, including α-melanocyte stimulating hormone (MSH), corticotropic hormone (ACTH) and β-endorphin. In cattle, this gene plays an important role in ingestive behaviour, energy homeostasis and hot carcass and shipping weights (Garza-Brenner *et al.*, 2017).

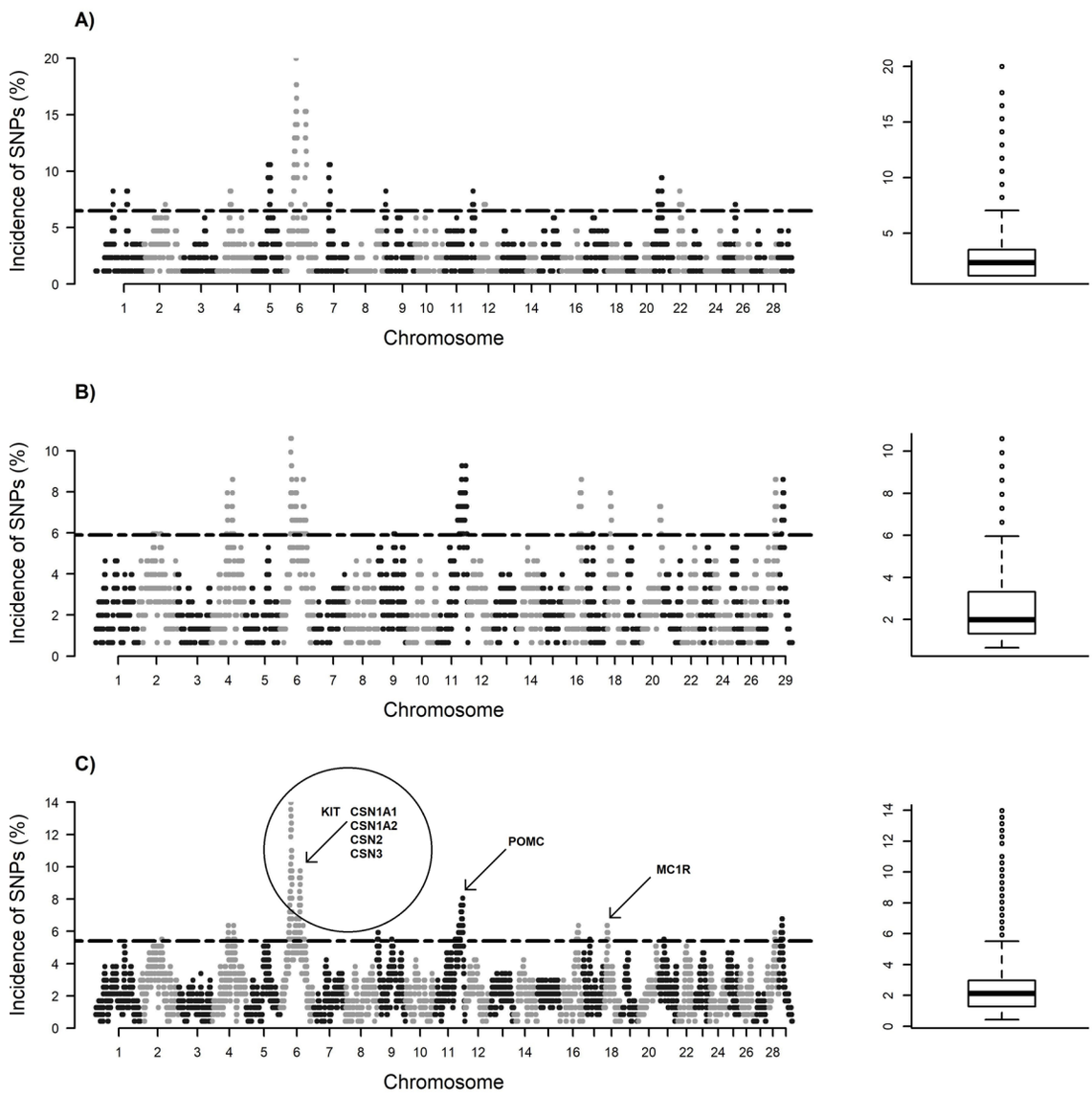

Figure 3. Genome-wide occurrence of SNPs in ROH for Slovak Spotted (A) and Slovak Pinzgau (B) cattle and incidence of runs for each SNP across both populations (minimum ROH length set to 4 Mb)

Table 2. Overlapping autozygosity islands across the genome of analysed breeds

BTA	Start position (Mb)	End position (Mb)	Length (Mb)	No. of SNPs	No. of genes	QTL traits
4	54.55	58.31	3.76	56.00	22	Somatic cell score
	76.07	76.90	0.82	17.00	5	
	78.39	80.71	2.31	35.00	12	
6	51.59	51.99	0.40	12.00	1	Birth weight, Yearling weight, Stature, Strength, Marbling score, Milk yield, Protein and Fat yield, Protein and Fat percentage, Backfat EBV
	54.14	59.31	5.17	87.00	23	
	63.28	63.89	0.62	6.00	2	Stature, Strength, Body, Rump width, Suspensory, ligament, Teat placement, Foot angle, Quality of udder, Quality of feet and legs, Udder depth, Protein and Fat percentage
	67.32	73.31	5.99	88.00	67	
	82.05	87.40	5.35	39.00	61	
11	72.56	73.98	1.42	22.00	38	Yield grade
	75.50	76.35	0.86	14.00	6	
	79.66	81.28	1.63	24.00	9	
	85.74	89.02	3.28	52.00	37	Fat yield
	90.39	91.88	1.49	20.00	5	
	92.79	101.22	8.43	124.00	217	
16	57.89	66.11	8.22	134.00	79	Fat depth, Yield grade, Hot carcass weight
18	11.84	15.10	3.26	44.00	72	Hot carcass weight, Dystocia (maternal effect)
28	30.84	33.10	2.26	22.00	18	Protein and Fat percentage
29	7.48	14.12	6.64	108.00	47	Milk Speed, Temperament, Marbling score, Milk yield, Birth weight

In the genome of Slovak Spotted cattle, overall 19 genomic regions distributed across 12 autosomes (BTA1, BTA2, BTA4, BTA5, BTA6, BTA7, BTA9, BTA11, BTA22, and BTA25) were found to be under intense selection pressure. The longest ROH was found on BTA6 (30,812,012:42,866,973 bp), while the shortest on BTA2 (71,209,253:71,657, 308 bp). Inside the ROH island on BTA6 various QTLs were reported, including those that affect milk yield (Viitala *et al.*, 2003), protein and fat yield (Kühn *et al.*, 1999), birth weight (Casas *et al.*, 2000), stature and strength (Hiendleder *et al.*, 2003), longissimus muscle area (Casas *et al.*, 2003), and daily gain (Lee *et al.*, 2011). For the Slovak Pinzgau cattle a total of 21 genomic regions with extreme ROH frequencies on BTA2, BTA4, BTA6, BTA9, BTA11, BTA16, BTA18, BTA20, BTA28, and BTA 29 were detected. The strongest ROH pattern was identified on BTA4 in the region from 65,400,608 bp to 80,706,119 bp that included QTLs affecting teat length (Ashwell *et al.*, 2001), longissimus muscle area and marbling score (Mizoshita *et al.*, 2004). The subset of biologically and economically most important quantitative trait loci located in each region is summarized in Table 3 and 4. Besides them, we identified in target regions of selection several genes involved in multiple signalling and signal transduction pathways in a wide variety of biological processes, including the genetic control of milk production and reproduction (LCT, CSN1S1, CSN1S2, CSN2, CSN3, BMPR1B), body conformation and meat quality (GHRHR, POMC, MYO1G), coat colour (MCR1, KIT) and immunity response (IGFBP, IGJ, MR1, TLR10, TLR6).

Generally, the breeding objectives of dual-purpose breeds are mostly similar, but environmental and management condition may vary, giving rise to slightly different selection pressures applied to given traits which can lead to different selection pressures to loci across the genome (Howard *et al.*, 2015). The comparison of ROH patterns from observed breeds could also provide an insight into the effect of selection on the genome over varying periods of time and determine if the direction of selection was similar (Kim *et al.*, 2013). The results of our study indicated that the regions

Table 3. Genomic region with extreme ROH frequencies in Slovak Spotted cattle

BTA	Start position (Mb)	End position (Mb)	Length (Mb)	No. of SNPs	No. of genes	QTL traits
1	57.17	57.91	0.74	10	12	Fat yield, Protein yield, Protein percentage
	102.57	105.75	3.19	40	9	Protein percentage, Resistance to BSE
2	71.21	71.66	0.45	11	7	Fat thickness, Birth weight, Marbling score, Slaughter weight, Functional herd life
4	44.51	48.82	4.31	82	45	Somatic cell score
5	60.85	69.96	9.10	111	90	Dressing percentage, Yearling weight, Birth weight, Longissimus muscle area, Protein yield, Twinning rate, Concentration of follicule stimulating hormone
6	21.59	23.78	2.19	37	27	Longissimus muscle area, Milk yield, Protein and Fat percentage, Protein and Fat yield, Pre-weaning average daily gain
	30.81	42.87	12.05	193	53	Birth weight, Milk yield, Protein and Fat yield, Protein and Fat percentage, Stature, Strength, Longissimus muscle area, Daily gain
	68.29	72.97	4.68	64	57	Stature, Strength, Body, Rump width, Suspensory ligament, Teat placement, Foot angle, Quality of udder, Quality of feet and legs, Udder depth, Protein percentage
7	41.03	46.35	5.32	84	226	
9	4.13	5.68	1.55	30	1	
11	92.69	95.64	2.95	37	85	
12	33.78	39.02	5.24	57	56	Milk yield, Protein and Fat yield, Protein percentage
21	20.89	23.76	2.86	63	3	Somatic cell score, Birth weight
	30.01	34.61	4.60	64	85	Birth weight
22	26.26	29.10	2.85	36	10	
	30.99	31.48	0.49	10	2	
	33.19	33.77	0.57	9	2	Somatic cell score
	34.88	35.63	0.75	10	5	
25	36.79	37.99	1.20	18	55	

displaying autozygosity in Slovak Spotted and Slovak Pinzgau cattle are linked mostly to milk production and muscle development, thus selection for dual-purpose performance.

CONCLUSION

In this study we provided the insight into the distribution of autozygosity islands across the genomes of two dual-purpose breeds with historical and national importance in Slovakia; Slovak Spotted and Slovak Pinzgau cattle. We found that the number of ROH segments as well as the length of genomes covered by them are considerably different depending on the studied animal and breed. Despite the fact that the overall ROH length indicated higher total level of autozygosity in the genome of Slovak Spotted cattle, the proportion of ROH segments > 16 Mb indicated higher risk of genetic diversity loss for Slovak Pinzgau cattle due to the recent inbreeding. For both breeds the regions of genome that were most commonly associated with ROH and therefore could reflect the impact of artificial selection were identified and described. Generally, our results

Table 4. Genomic region with extreme ROH frequencies in Slovak Pinzgau cattle

BTA	Start position (Mb)	End position (Mb)	Length of region (Mb)	No. of SNPs	No. of genes	QTL traits
2	49.81	51.90	2.09	32	6	Marbling score, Milk yield
	53.43	55.48	2.04	47	7	Marbling score, Milk yield
	60.09	63.78	3.70	52	20	Marbling score, Milk yield, Functional herd life
4	54.55	59.33	4.78	79	24	Somatic cell score
	60.92	61.73	0.81	20	9	
	65.40	80.71	15.31	235	163	Teat length, LMA, Marbling score
6	37.10	42.99	5.88	110	25	Birth weight, Milk yield, Protein and Fat yield, Protein and Fat percentage, Stature, Strength, Daily gain
	49.21	59.31	10.11	178	30	Birth weight, Yearling weight, Stature, Strength, Marbling score, Milk yield, Protein and Fat yield, Protein and Fat percentage
	65.34	69.88	4.54	77	53	Stature, Strength, Body, Rump width, Suspensory ligament, teat placement, Foot angle, Quality of udder, Quality of feet and legs, Udder depth, Milk yield, Protein and Fat yield, Protein and Fat percentage, Pre-weaning average daily percentage
	71.91	73.31	1.40	30	19	
	79.84	88.59	8.75	115	83	
	90.56	93.94	3.37	66	57	
9	53.77	57.95	4.18	85	15	Milk yield, Protein and Fat yield
11	71.34	77.56	6.22	84	108	Yield grade
	79.17	91.88	12.70	176	78	Fat yield, Pelvic and Heart fat
	93.29	103.62	10.33	160	249	
16	56.49	66.22	9.73	160	105	Fat depth, yield grade, Hot carcass weight
18	10.83	16.03	5.20	69	109	Dystocia (maternal effect), Hot carcass weight
20	62.05	68.20	6.15	123	36	Meat tenderness, Milk yield, Protein percentage
28	27.46	37.20	9.75	149	110	Protein and Fat percentage
29	6.69	15.49	8.80	140	54	Milk speed, temperament, Milk yield, Birth weight

confirmed that the regions displaying autozygosity in Slovak Spotted and Slovak Pinzgau cattle are linked mostly to milk production and muscle development, thus selection for dual-purpose performance. However, due to the common history of analysed breeds, some selection signatures were found in the same genomic regions located on seven autosomes directly or very close to the genes involved in the genetic control of milk production, carcass traits and coat colour. In addition, our study confirmed that the genome-wide scan for ROH segments in cattle can be used as an alternative strategy to identify the genomic regions as well as genes related to traits with biological and economical importance.

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PERFORMANCE, DIGESTIBILITY AND NITROGEN UTILIZATION OF WEST AFRICAN DWARF SHEEP FED *PANICUM MAXIMUM* WITH SUPPLEMENTAL LEGUME PELLETS

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ABSTRACT

This study was conducted to evaluate the effect of supplementing legume pellets in the diets of West African dwarf (WAD) rams. Twenty West Africa dwarf (WAD) rams of the average body weight of 12.43 ± 0.5 kg were allocated to four treatment: sole *Panicum maximum* (diet 1), *P. maximum* supplemented with *Lablab purpureus* pellets (diet 2), *P. maximum* supplemented with *Calopogonium mucunoides* pellets (diet 3) and *P. maximum* supplemented with *Mucuna pruriens* pellets (diet 4) arranged in a completely randomized design. *Panicum maximum* was offered to the animals *ad libitum* and legume pellets were fed at 5 % of their body weight. The feeding trial lasted 12 weeks and metabolic trial for two weeks. Nutrient intake, weight gain, nutrient digestibility and nitrogen utilization of the experimental animals were assessed. Obtained data were subjected to one-way analysis of variance. Among the rams supplemented with legume pellets, rams fed *M. pruriens* pellets had the highest ($P < 0.05$) nutrient intake (963.97 g). The highest ($P < 0.05$) weight gain was recorded for rams fed *L. purpureus* pellets (7.03 kg). Rams fed *L. purpureus* pellets had the highest ($P < 0.05$) dry matter (76.79 %) and crude protein digestibility (82.61 %) while the lowest ($P < 0.05$) was recorded for rams fed sole *P. maximum*. Nitrogen retention ranged from 38.90 % for rams fed sole *P. maximum* to 60.77 % for rams fed supplemented *L. purpureus* pellets. It can be concluded from this study that rams fed supplemented *L. purpureus* pellets gave the highest performance in weight gain, nutrient digestibility and nitrogen utilization.

Key words: legume pellets; *Lablab purpureus*; *Calopogonium mucunoides*; *Mucuna pruriens*

INTRODUCTION

Forage is the most widely available as low cost feed for ruminant animals during the wet season in the tropics and they rely on them almost exclusively for nutrition since it sustains their production (Aderinola *et al.*, 2008). Grasses are the most abundant forage species due to their aggressive growth and as they mature especially during the dry season, their productivity declines sharply as they tend to lose their nutrients (Aderinola, 2007). Their crude protein usually drop as low as 3 % which is below the critical level of 7 % recommended by Minson (1982) and this affects the productivity of the animals. However, efforts have been made in the past to augment low quality feeds for ruminant animals by supplementing grasses with concentrate feeds and agro industrial by-products, unfortunately, they are unavailable or in short supply and are expensive (Adjolohoun *et al.*, 2008; Ososanya *et al.*, 2013). Herbaceous legumes could be included in animal feed when the nutritive value of grasses is low so as to sustain and improve the performance of the animals. The use of forage legumes in livestock production systems has increased in the tropics

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in recent years since they are cheap feeds that are used as supplement to ruminant animal. However, seasonal fluctuations results in their low quality and unavailability, which poses a threat to livestock survival. To alleviate this problem, it becomes imperative to conserve and process during period of abundance for use during the period of scarcity. Legumes such as *Lablab purpureus*, *Calopogonium mucunoides* and *Mucuna pruriens* can be conserved as silage, hay and pellets to meet the nutritional needs of animals when there is low availability (Amole *et al.*, 2013). The major constraint in large scale hay production in the tropics is unreliable weather conditions and poor herbage quality for most of the year (Crowder and Chedda, 1982). Conservation of legumes as silage is not extensively practiced worldwide because of their high buffering capacity and low concentration of fermentable carbohydrates, which limits the quality of their silage (Tauqir *et al.*, 2009).

Pelletizing of forages is an alternative technology to solve the problem of decreasing dry matter consumption, total digestible nutrient content and energy inefficiency problem in ruminant productivity (Widiyanto *et al.*, 2011). Legumes pellets are also reported to be consumed much faster than long stemmed forages and this can increase the feed intake of animals and, hence their productivity. The present study was designed to assess the feed intake, digestibility and nitrogen utilization of WAD rams fed *P. maximum* grass supplemented with pellets of *L. purpureus*, *C. mucunoides* and *M. pruriens* at dry season.

MATERIALS AND METHODS

Location and climate of the study area

The experiment was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta (FUNAAB), located in the derived savannah zone of the South-Western Nigeria on latitude 7°13' 49.46'' N and longitude 3°25' 11.98'' E (Google Earth, 2015). It has an average annual rainfall of 1037 mm and temperature of about 34.7 °C and an average relative humidity of 82 %.

Legume pellets preparation

Lablab purpureus, *C. mucunoides* and *M. pruriens* were harvested at 12 weeks and sun dried. The dried legumes were milled and pelletized using a 6 mm die size to produce pelleted forage of average length of 40 mm. Cassava flour was used as binder (proportion of 1 kg of cassava flour to 100 kg of the milled sample) with addition of water to moisten. The pellets were warm and moist, when they came out of the mill. They were then cooled down to harden up so as to hold their form. The experimental diets were Sole *Panicum maximum*, *Panicum maximum* supplemented with *L. purpureus* pellet, *Panicum maximum* supplemented with *C. mucunoides* pellet, *Panicum maximum* supplemented with *M. pruriens* pellet. Table 1 shows the chemical composition of *P. maximum* and herbaceous forage legumes pellets.

Table 1. Chemical composition (g.kg^{-1}) of *P. maximum* and herbaceous forage legume pellets

Treatment	*Panicum maximum*	*Lablab purpureus* pellets	*Calopogonium mucunoides* pellets	*Mucuna pruriens* pellets	Standard Error of Means
Dry matter	256.00^{d}	855.20^{c}	902.07^{b}	915.23^{a}	1.13
Crude protein	94.50^{d}	120.80^{b}	105.00^{c}	126.00^{a}	3.79
Ether extract	40.00^{c}	50.00^{b}	50.00^{b}	70.00^{a}	3.30
Ash	120.00^{a}	95.00^{b}	70.00^{c}	70.00^{c}	6.26
Neutral detergent fibre	560.00^{b}	480.00^{d}	580.00^{a}	500.00^{c}	12.43
Acid detergent fibre	360.00^{a}	240.00^{c}	300.00^{b}	300.00^{b}	12.79
Acid detergent lignin	140.00^{a}	60.00^{d}	100.00^{b}	80.00^{c}	8.92

$^{a, b, c, d}$ Means on the same row with different superscripts are significantly different (P < 0.05)

Experimental animals and their management

A total number of twenty (20) West African dwarf (WAD) rams with average body weight of 12.43 ± 0.5 kg aged 10-12 months were used for the experiment. On arrival of the animals, they were acclimatized for 28 days during which they were given prophylactic treatment to ensure good health conditions. All the animals were fed *Panicum maximum* with groundnut haulms. Clean water was provided *ad libitum* to the animals on daily basis. After acclimatization, the animals were weighed and randomized into four treatment groups of five animals per treatment each balancing for body weight. A basal diet of fresh *Panicum maximum* grass was fed daily to the animals. The grass was harvested at 6 weeks of re-growth, wilted, chopped and offered to the animals *ad libitum* daily. The legume pellets were fed as supplements to the animals at 5 % of their body weight. The feeds were fed once daily at 8:00 am, with legume pellets served first with *Panicum maximum* after thirty (30) minutes in separate feeding troughs.

Feed intake and weight change

Feed refusal were estimated the following morning. The weight of individual animal was measured at the onset of the trial and subsequently on weekly basis. The difference between the initial and final weight was used to compute weight change (gain/loss) for rams in each dietary treatment.

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed (Feed intake)}}{\text{Weight change}}$$

Digestibility and nitrogen balance studies

At the end of the feeding trial which lasted for 84 days, the animals were transferred to individual metabolic cages with provision for separate collection of faeces and urine. Three rams per treatment were used for digestibility and nitrogen balance study. Animals were allowed for an adaptation period of seven days, followed by a seven-day of total faeces and urine collection period. The total voided faeces per animal were collected and weighed daily. Urine was collected daily into bottle fitted with plastic funnel containing two drops of 10 % H_2SO_4 to prevent loss of nitrogen from the urine. The total volume of urine of each animal was recorded daily and 10 % of the measured urine was stored in the refrigerator at 4 °C for nitrogen determination.

$$\text{Nutrient digestibility \%} = \frac{\text{Nutrient in feed consumed-Nutrient in faeces voided x 100}}{\text{Nutrient in feed consumed}}$$

Chemical analyses

Sub- samples of the grass, legume pellets and faeces were oven dried at 65 °C to constant weight. Proximate composition (dry matter, crude protein, ether extract and ash) were determined according to AOAC (2000) while determination of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was carried out according to the procedure of Van Soest *et al.* (1991).

Statistical analyses

All data obtained were arranged in a completely randomized design and subjected to one-way analysis of variance (ANOVA). Significant means were separated using Duncan's Multiple Range Test of SAS (2001).

RESULTS AND DISCUSSION

The performance characteristics of West African dwarf rams fed *P. maximum* supplemented with herbaceous forage legume pellets are given in Table 2. The total dry matter intake ranged from 758.84 g for rams fed sole *P. maximum* to 963.97 g for those fed supplemented *M. pruriens* pellets. Rams fed *M. pruriens* pellets had the highest ($P < 0.05$) legume pellet intake (466.80 g), while rams fed *C. mucunoides* pellets had the lowest (235.83 g). High nutrients intake were observed for rams fed *M. pruriens* pellet and *L. purpureus* pellet supplemented diets. The lowest dry matter intake for rams fed supplemented *C. mucunoides* pellets could be due to high fibre content. Rams fed *M. pruriens* pellets had the highest ($P < 0.05$) crude protein intake (105.80 g), which could be due to high protein content in the feed of the animals. Protein supplement brings about increase in protein content of the feed of the animal and this usually lead to increase in protein intake (Arigbede *et al.*, 2006). Neutral detergent fiber and ADF intakes were significantly higher for rams fed supplemented *M. pruriens* pellets (511.82 and 319.02 g) than others. Mtenga and Kitaly, (1990) reported a positive correlation between crude protein intake and dry

Table 2. Performance characteristics of West African dwarf rams fed *P. maximum* supplemented with herbaceous forage legume pellets

Parameters	Dietary treatments				Standard Error of Means
	Panicum maximum	*Lablab purpureus* + *Panicum maximum*	*Calopogonium mucunoides* + *Panicum maximum*	*Mucuna pruriens* + *Panicum maximum*	
Grass (g)	758.84^{a}	598.67^{b}	558.68^{bc}	497.17^{c}	31.34
Legume pellet (g)	265.25^{b}	235.83^{b}	466.80^{a}	37.80	
Total dry matter intake (g)	758.84^{c}	863.92^{b}	794.51^{c}	963.97^{a}	25.19
Crude protein intake(g)	71.71^{c}	88.67^{b}	77.65^{c}	105.80^{a}	4.00
Ether extract intake(g)	30.35^{d}	37.21^{b}	34.14^{c}	52.56^{a}	2.57
Ash intake (g)	91.06^{ab}	97.04^{a}	83.55^{b}	92.34^{a}	1.80
Neutral detergent fibre intake (g)	424.95^{b}	462.58^{b}	449.65^{b}	511.82^{a}	10.88
Acid detergent fibre intake (g)	273.18^{b}	279.18^{b}	271.88^{b}	319.02^{a}	6.71
Acid detergent lignin intake (g)	106.24	99.73	101.80	106.95	1.64
Total voided faeces (g)	369.67^{c}	537.44^{ab}	484.33^{b}	623.33^{a}	31.19
Initial weight (kg)	12.20	12.43	11.91	13.17	0.50
Final weight (kg)	15.72^{b}	19.46^{a}	17.14^{ab}	16.98^{ab}	0.58
Weight gain (kg)	3.52^{c}	7.03^{a}	5.23^{b}	3.81^{bc}	0.47
Feed conversion ratio	20.09^{a}	10.33^{b}	13.19^{ab}	21.28^{a}	1.78

$^{a, b, c}$ Means on the same row with different superscripts are significantly different (P < 0.05)

matter intake. Arigbede *et al.* (2006) stated that an increase in protein intake will enhance the intake of other nutrients, since high protein content will improve the rumen environment. Supplementing a low to medium quality forage with degradable protein in the form of forage legumes often results in improved growth performance of ruminants (Mupangwa *et al.*, 2000). Highest (P < 0.05) weight gain (7.03 kg) was recorded for rams fed supplemented *L. purpureus* pellets. This is reflected by high dry matter and crude protein digestibility recorded for the animals. Rams fed supplemented *M. pruriens* pellets had the highest feed conversion ratio (FCR) and this indicates that the feeds were not efficiently converted by the animals. This could be due to low digestibility in rams fed supplemented *M. pruriens* pellets compared to rams fed *L. purpureus* and *C. mucunoides* pellets.

Table 3 gives the nutrient digestibility of West African dwarf rams fed *P. maximum* supplemented with herbaceous forage legume pellets. The digestibility of DM was highest (P < 0.05) for rams fed supplemented *L. purpureus* pellets (76.79 %). The CP digestibility increased from 62.46 % for rams fed sole *P. maximum* to 82.61 % for rams fed supplemented *L. purpureus* pellets. Rams fed *L. purpureus* pellets had the highest NDF and ADF digestibility (81.14 and 70.61 %). Digestion in the rumen is dependent on the activity of micro-organisms. Processing of feeds such as pelletizing is conducted in an attempt to enhance digestibility (Faichney, 1986; Sarwar *et al.*, 1992). Rams fed legume pellets supplemented diets had improved dry matter digestibility compared to those fed sole *P. maximum*. This was in accordance with the findings reported by Abdel-Ghani *et al.* (2011) that dietary protein improves digestibility coefficient of many nutrients in sheep and lamb rations. Lower digestibility of CP, NDF and ADL were observed in rams fed sole P. maximum grass. Previous studies shows that addition of protein source to the diet of the animals enhanced digestibility (Oladotun *et al.*, 2003). Rams fed supplemented *L. purpureus* pellets had higher CP digestibility than rams fed supplemented *M. pruriens* pellets, which had higher crude protein intake. Rams fed legume pellets supplemented diets had higher NDF digestibility. The lower NDF digestion in rams fed sole grass could be due to insufficient protein for rumen microorganisms to improve the digestion of the feed. Rams fed *M. pruriens* pellets had the lowest NDF digestibility among supplemented diet feed.

Table 3. Nutrient digestibility of West African dwarf rams fed *P. maximum* supplemented with herbaceous forage legumes pellet

Nutrient (%)	Dietary treatments				Standard Error of Means
	Panicum maximum	*Lablab purpureus* + *Panicum maximum*	*Calopogonium mucunoides* + *Panicum maximum*	*Mucuna pruriens* + *Panicum maximum*	
Dry matter	60.58[c]	76.79[a]	70.11[b]	67.68[b]	1.84
Crude protein	62.46[c]	82.61[a]	73.49[b]	72.63[b]	2.38
Ether extract	54.27[c]	72.15[a]	64.13[b]	69.99[a]	2.20
Ash	43.34[b]	62.74[a]	42.35[b]	43.44[b]	2.73
Neutral detergent fibre	61.73[c]	81.14[a]	79.00[a]	72.20[b]	2.34
Acid detergent fibre	64.25[b]	78.72[a]	70.61[b]	65.52[b]	2.01
Acid detergent lignin	50.42[b]	66.55[a]	66.35[a]	62.35[a]	2.27

[a, b, c] Means on the same row with different superscripts are significantly different (P < 0.05)

The nitrogen utilization of West African dwarf rams fed *P. maximum* supplemented with herbaceous forage legume pellets are presented in Table 4. Among the rams fed supplemental diets, rams fed *L. purpureus* pellets (10.15 $g.d^{-1}$) had highest nitrogen balance and rams fed *C. mucunoides* pellets (6.84 $g.d^{-1}$) had the least. The nitrogen retention ranged from 38.90 % for rams fed *P. maximum* only to 60.77 % for rams supplemented *L. purpureus* pellets. The lower nitrogen intake observed in rams fed supplemented *C. mucunoides* pellets and sole *P. maximum* could be due to low level of protein intake and low crude protein content. The lower faecal N-output in rams fed supplemented *L. purpureus* pellets compared to those fed *M. pruriens* pellet supplemented diet could largely be a reflection of *L. purpureus* pellets that was well utilized. Nitrogen balance and retention were best in rams fed *L. purpureus* pellet supplemented diet. Higher nitrogen retention in rams supplemented *L. purpureus* pellets, indicated that protein requirements for maintenance were adequately met by the diets (Fadiyimu *et al.*, 2010).

CONCLUSION

Supplementing the legume pellets with *P. maximum* improved the nutrient intake, nutrient digestibility and growth performance of the experimental rams. *Lablab purpureus* pellets enhanced the performance of WAD rams better than *Mucuna pruriens* and *Calopogonium mucunoides* pellets.

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Table 4. Nitrogen utilization by the West African dwarf rams fed *P. maximum* supplemented with herbaceous forage legume pellets

Parameters ($g.d^{-1}$)	Dietary treatments				Standard Error of Means
	Panicum maximum	*Lablab purpureus* + *Panicum maximum*	*Calopogonium mucunoides* + *Panicum maximum*	*Mucuna pruriens* + *Panicum maximum*	
Nitrogen intake	11.47^{c}	14.19^{b}	12.41^{c}	16.93^{a}	0.64
Faecal nitrogen	4.67^{b}	2.99^{c}	4.31^{bc}	7.52^{b}	0.53
Urinary nitrogen	2.32^{a}	1.05^{b}	1.25^{b}	1.68^{ab}	0.19
Total nitrogen output	6.99^{b}	4.04^{d}	5.56^{c}	9.20^{a}	0.58
Nitrogen balance	4.48^{c}	10.15^{a}	6.84^{b}	7.73^{b}	0.63
Nitrogen retention (%)	38.90^{c}	60.77^{a}	51.23^{b}	39.78^{c}	2.86

$^{a, b, c}$ Means on the same row with different superscripts are significantly different ($P < 0.05$)

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THE EFFECT OF SILAGE ADDITIVES ON QUALITY OF THE MIXTURE SILAGES OF MAIZE AND DENDROMASS

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ABSTRACT

The aim of this work was to evaluate the influence of biological and chemical ensilaging additives on the quality of the fermentation process and nutrient content in silages produces from a mixture of maize plants (70 % in fresh matter) and oak and spruce twigs (dendromass) as a potential feed for wild ruminants. Mixture 1 was composed of 30 % fresh twigs of forest trees (50 % oak twigs, 50 % spruce twigs). The content of dry matter in Mixture 1 was 32.9 % of fresh matter. The nutrient content reached the crude protein concentration 7.8 % of dry matter, crude fibre 22.4 %, total sugar 9.4 % and starch 19.2 % of dry matter. Mixture 2 was composed of 30 % fresh twigs of forest trees (75 % oak twigs, 25 % spruce twigs). The content of dry matter in Mixture 2 was 32.1 % of fresh matter. The nutrient content reached the crude protein concentration 7.5 % of dry matter, crude fibre 23.1 %, total sugar 9.0 % and starch 16.7 % of dry matter. The fermentation process during ensilaging was observed in one control variant without treatment and two experimental variants, in which the ensilaged matter was treated with additives: T1 (*Lactobacillus buchneri* DSM 13573) and T2 (22.9 % sodium benzoate, 8.3 % sodium propionate). The influence of the treatment of the silage mixture on the nutrient content of the silage was low. Chemical additive applied to the ensilaged matter of both mixtures improved the quality of the fermentation process of the produced mixture silages for wild ruminants. This was confirmed by the highest lactic acid content, lowest content of volatile fatty acids and alcohol, but also the lowest losses of dry matter in the fermentation process. Positive effect of applying the biological inoculant based on heterofermentative bacteria of lactic fermentation was determined only in Mixture 1 silage produced from dendromass compound of 50 % oak twigs and 50 % spruce twigs. Our results indicate that application of ensilaging additives is beneficial in production of maize-dendromass mixture silages for wild ruminants and it improves the fermentation process as well as the quality of the produced silages.

Key words: mixture of maize and dendromass; silage additives; fermentation process; quality of silages, wild ruminants

INTRODUCTION

The basis of the majority of wild ruminants' feeding systems is pasture, however, supplemental feeding is a very frequent form of management, especially during the winter season, and not only in Slovakia, but also in the northern parts of Europe and America. It has its significance especially in the areas where it is important to improve the conditions for survival of the animals. No less important is the prevention of damage from forest tree browsing by the animals (Smith, 2001; Peek *et al.*, 2002).

The botanical composition of red and roe deer and mouflon diet was studied by many authors. Kamler and Homolka (2016) focused on the proportion and quality of agricultural crops and natural forest plants and estimated quality of the herbivore diet. Red deer, roe deer and mouflon ingested all cultivated plants growing close to forest. The average proportion of corn for red deer was 40 %. Cultivated plants were well

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accessible for herbivores in the study area and during vegetation period formed an important part of their diet, but the importance of cultivated plants for herbivores was lower compared to natural food resources present in the forests during vegetation period. Although the main natural food sources had lower nutritional value, they formed the main part of herbivore diet.

In wild ruminant nutrition, new methods and systems for supplemental feeding are being implemented. One of the relatively new systems, which is spreading in practice, is the utilization of mixture feed rations. Mixture of feeds is preserved most often by ensilaging and after fermentation process it is offered to the animals. In livestock, the individual feeds are usually preserved first and only then combined to produce feed mixtures. For wild ruminants, this method is complicated from the management aspect and therefore there is emphasis on systems that will allow to first combine the feeds and then ensilage them.

The quality of the resulting silage mixture can then be evaluated in the same way as the quality of other silages. Supplementing silage mixtures to wild ruminants is a risk, because when silages are subjected to anaerobic conditions for an extended period of time, yeasts and fungi can be activated, which can then lead to undesirable fermentation processes, causing loss of nutrients and damage to the hygienic quality of the produced feed (Hrbek *et al.*, 2013). Vodňanský *et al.* (2007) pointed to the effect of the different silage quality feeding on the bark browsing intensity by red deer. According to Kung and Shaver (2001), production of silage is a complex process involving physiological, chemical and microbiological changes, which have to take place in the silage matter in order to achieve a stable product of high quality. Because the fermentation process is spontaneous and the conditions to ensure high quality silage are not always optimal, Bíro *et al.* (2014) recommend to apply ensilaging additives to improve the fermentation process in forage. Kaiser and Piltz (2004) consider quality silage to be that, which shows low losses of dry matter and nutrients with the concentration of crude protein the same or not significantly lower than in fresh unsilaged feed. Nijboer *et al.* (2003) determined that well-produced silages, when ensilaging dendromass for winter supplemental feeding of the wild ruminants, must have stable pH values. The pH value should be between 3.7 and 5.0 depending on the character and dry matter content of the ensilaged feed.

In our previous experiments (Rajský *et al.*, 2018), we explored the option of producing mixture silages of maize and dendromass composed of oak and spruce twigs in order to produce palatable feed rich in energy, which would also provide sufficient crude fiber content for wild ruminants. We found out that thanks to the high portion of easily ensilaged maize (70 %), the maize-dendromass mixture silages were of good quality.

The 30 % portion of dendromass in our experimental silages was created in two variants of different oak and spruce ratios. The first ratio variant was 50 : 50 %, which we considered to the default, resp. neutral. The second ratio variant was 75 % oak and 25 % spruce. Lower ratio of spruce was chosen because coniferous trees contain certain antinutritional and irritating components, therefore the higher ratio of broadleaf trees is preferable for the digestive system and metabolism of wild ruminants.

We investigated the use of coniferous and broadleaf trees as components in feed mixtures for game because trees represent a natural food source of wild ruminants. In Slovakia, mixed forests are common and the wild ruminants, depending on the tree species, vegetation phase and the percentage of the tree species, consumes broadleaf and coniferous trees with high intensity, often favouring them over pastures and meadows of lesser quality.

In this study, we focused on improving of mixture silages by applying ensilaging additives to the silage matter. Our objective was to evaluate the influence of biological and chemical ensilaging additives on the quality of the fermentation process and on the nutritional value of silages produced by combining twigs of oak and spruce with maize, with maize constituting the majority 70 % of the resulting mixture.

MATERIAL AND METHODS

In the experiment, two different mixtures were produced by combining whole maize plants and twigs of conifer and broadleaf trees. Maize was harvested at the stage of wax ripeness and it

represented 70 % of both mixtures (fresh matter content). In Mixture 1, the remaining 30 % were fresh twigs of forest trees compound of 50 % oak twigs and 50 % spruce twigs (Dendro 1). In Mixture 2, the remaining 30 % were fresh twigs of forest trees compound of 75 % oak twigs and 25 % spruce twigs (Dendro 2). The mixtures were without wilting chopped to 3 cm (maximally) and after homogenization ensilaged in 1.7 l glass laboratory silos. Measure for the equal intensity of compression of ensilaging mass was the equal weight of the laboratory silos. Anaerobic conditions were achieved by sealing the silos. Each variant was repeated 5 times.

The fermentation process during ensilaging was observed in one control variant without treatment of the mixture and two experimental variants, in which the ensilaged matter was treated with additives: T1 (biological silage additive, *Lactobacillus buchneri* DSM 13573; the application rate was 2 $l.t^{-1}$ feed) and T2 (chemical additive, 22.9 % sodium benzoate, 8.3 % sodium propionate; the application rate was 3.5 $l.t^{-1}$ feed). The filled experimental silos were stored in a dark room with stable temperature at 22 ± 1 °C. During the fermentation process, changes in weight were monitored and based on those, loses of dry matter weight were determined in %. The experiment finished 180 days after ensilaging.

Samples of feed components as well as silage samples were chemically analysed. The following parameters of organic analysis were determined: dry matter content (gravimetric analysis), crude protein content according to Kjeldahl, content of crude fibre, saccharides, ash, ether extract and starch according to Decree MP SR no. 2145 /2004-100 (2004), acid detergent and neutral detergent fibre according to Van Soest *et al.* (1991).

In addition to the basic parameters of organic analysis, parameters of fermentation process were also determined for the silage samples: pH in the aqueous extract was determined using electrometric method, lactic acid and volatile fatty acids (acetic, propionic and butyric acid) content was determined by gas chromatography and alcohol content by micro diffusion method. These chemical analyses were performed according to Decree MP SR no. 2145/2004-100 (2004), too.

Results were statistically processed by one-way analysis of variance, by the ANOVA multifactorial procedure and by the subsequent POST-HOC Tukey test.

Table 1. The nutrient content of ensilaged mixtures and their compounds (in $g.kg^{-1}$ dry matter)

Item	Mixture 1	Mixture 2	Maize	Dendro 1	Dendro 2
Dry matter in $g.kg^{-1}$ FW	329.01	321.48	287.32	457.69	485.98
Organic matter	946.49	951.93	952.51	940.39	947.81
Crude protein	78.28	75.02	78.37	76.29	63.50
Crude fibre	223.72	230.67	190.41	349.56	367.25
ADF	265.61	278,.04	213.47	429.86	447.63
NDF	564.02	573.41	512.64	528.99	602.00
Hemicelluloses	298.41	295.37	299.17	99.13	154.37
Nitrogen-free extract	613.65	619.83	659.14	474.28	489.37
Total sugars	94.44	90.45	110.25	39.82	39.78
Reduced sugars	83.95	88.36	106.32	38.84	31.78
Starch	192.31	167.43	245.50	0.00	0.00
Ether extract	30.84	26.41	24.59	40.25	27.68
Ash	53.51	48.07	47.49	59.61	52.19

FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre
Mixture 1: 70 % maize + 30 % Dendro 1, Mixture 2: 70 % maize + 30 % Dendro 2
Dendro 1 – dendromass mixture in rations of 50 % oak twigs and 50 % spruce twigs
Dendro 2 – dendromass mixture in rations of 75 % oak twigs and 25 % spruce twigs

RESULTS AND DISCUSSION

Table 1 presents the nutrient content in mixtures of whole maize plants with twigs of conifer and broadleaf trees. In addition to the nutritional value of the experimental mixtures it shows also the nutrient content of each feed components from which the mixtures were produced. The results indicate, that concentrations of nutrients in Mixture 1 and Mixture 2 reflect the composition of the individual feed components. Both mixtures can be characterised as feed with lower level of crude protein (78 and 75 g.kg^{-1} dry matter), ether extract (31 and 26 g.kg^{-1} dry matter) and ash (54 and 48 g.kg^{-1} dry matter), with average crude fibre content (224 and 231 g.kg^{-1} dry matter) and high content of neutral detergent fibre (564 and 573 g.kg^{-1} dry matter) as well as the reduced sugars (84 and 88 g.kg^{-1} dry matter). Starch concentration in the mixtures (192 and 167 g.kg^{-1} dry matter) corresponded to the ration of maize and dendromass. According to Weissbach (2003), concentrations of crude protein and saccharides soluble in water directly affect buffer capacity and acidification potential of feed, which determine its ensilagibility. Low crude protein content and high saccharide content in our experimental mixtures indicated their good ensilagibility.

Regarding the production of high quality silage and high production of usable nutrients, Juráček *et al.* (2012) recommend ensilaging maize of 30 – 35 % dry matter content. Considering that in our silages, maize represented 70 % of the silage, we aimed to produce silage mixture of dry matter content in this margin. The content of dry matter in the experimental silage mixtures at ensilaging was 32.9 and 32.1 %.

Table 2. The nutrient content and parameters of fermentation process in silages from Mixture 1 (in g.kg^{-1} dry matter)

Item	Control		T1		T2	
	x̄	SD	x̄	SD	x̄	SD
Dry matter v g.kg^{-1} FW	299.75^{a}	5.09	301.54^{a}	4.21	320.35^{b}	6.35
Dry mater losses in %	9.98^{a}	1.58	9.45^{a}	1.29	3.00^{b}	1.69
Organic matter	946.71	2.34	947.96	3.18	949.75	2.23
Crude protein	80.30	0.89	80.94	3.48	78.75	5.36
Crude fibre	272.19^{a}	5.84	250.87^{b}	6.67	258.43^{a,b}	10.79
ADF	330.71	6.12	325.68	10.30	322.87	13.27
NDF	558.65	8.75	560.05	15.98	545.74	18.09
Hemicelluloses	227.94	3.27	234.37	13.87	222.87	15.97
Nitrogen-free extract	561.78^{a}	4.56	582.89^{b}	11.86	582.35^{b}	9.70
Starch	203.53	23.16	223.86	9.77	205.81	22.25
Total sugars	3.82^{a}	0.55	7.14^{a}	2.37	14.33^{b}	1.23
Reduced sugars	2.43^{a}	0.95	5.46^{a}	2.09	12.20^{b}	1.55
Ether extract	32.44	0.70	33.26	1.17	30.22	2.67
Ash	53.29	2.34	52.04	3.18	50.25	2.23
pH	4.27^{a}	0.06	4.12^{b}	0.01	3.92^{c}	0.02
Acids						
– lactic	36.26^{a}	3.21	52.34^{b}	1.20	60.15^{c}	1.32
– acetic	19.69^{a}	1.76	26.18^{b}	3.06	13.74^{c}	1.61
– propionic	1.18^{a}	0.24	0.78^{a,b}	0.29	0.62^{b}	0.20
– butyric	1.69^{a}	0.20	1.12^{a,b}	0.35	0.77^{b}	0.20
Alcohol	7.89^{a}	0.64	8.16^{a}	0.52	2.34^{b}	0.28

n = 5, FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre, T1 – biological additive (*Lactobacillus buchneri* DSM 13573), T2 – chemical additive (22.9 % sodium benzoate, 8.3 % sodium propionate), Different superscripts within a row mean statistical difference (P ≤ 0.01); ^{ab} vs ^{a,b} is not different

In order to improve the fermentation process and the silage quality, the mixtures were treated with one biological ensilaging additive based on heterofermentative bacteria of lactic fermentation (T1) and one chemical additive based on salts of organic acids (T2). Control was a silage without treatment by ensilaging additives. The results of nutritional content and fermentation process of the produced mixtures Mixture 1 and Mixture 2 are presented in Tables 2 and 3.

In silages of Mixture 1, which contained dendromass in rations of 50 % oak twigs and 50 % spruce twigs, only low influence of ensilaging additives on nutrition value was determined. Highly significant differences were determined between T2 silage and the other silages in the content of dry matter (320 compared to 300 and 302 $g.kg^{-1}$ dry matter) and sugars, but also in the crude fibre content between the control and T1 silage. The dry matter losses during the fermentation process were from 3.00 % to 9.98 %. The conclusively lowest losses were determined in the silage treated with the chemical ensilaging additive. Low losses of dry matter prove the lowest degree of nutrient degradation and the highest effect of conservation during the fermentation process. In the silage treated with biological ensilaging additive fermentation losses were determined to be only 0.53 % lower compared to the untreated control silage, which indicates low effect of this additive. The differences in pH values and lactic acids and acetic acid concentrations between the experimental Mixture 1 silages were highly statistically significant. In the untreated control silage we determined the highest pH (4.27), highest butyric acid (1.69 $g.kg^{-1}$ dry matter) and propionic acid (1.18 $g.kg^{-1}$ dry matter) concentrations and the lowest concentration of lactic acid (36.26 $g.kg^{-1}$ dry matter). This confirms that the lowest quality of fermentation process of all Mixture 1 silages was in the untreated silage control. The highest content of acetic acid was determined in the silage treated with the biological additive (26.18 $g.kg^{-1}$ dry matter) and the highest lactic acid content was determined the silage treated with the chemical additive (60.15 $g.kg^{-1}$ dry matter).

In silages produced using Mixture 2, which contained dendromass in rations of 75 % oak twigs and 25 % spruce twigs, the lowest dry matter losses during the fermentation process were determined in the silage treated by chemical additive T2 (6.07 %), which were highly statistically significant differences ($P \leq 0.01$) compared to other silages (9.23 and 10.54 %). The influence of ensilaging additives on the majority of nutrient content was insignificant. Highly significant differences were determined only between T2 silage and control in crude fibre content (242 compared to 260 $g.kg^{-1}$ dry matter) and between all silages in the content of total and reduced sugars. Fermentation process was adequate. The pH values were determined to be between 3.96 and 4.13, with the lowest values determined in the silage treated using chemical additive and the highest in the untreated control silage. Highly statistical significant differences in acid content were determined between T2 silage and the other silages in lactic acid (55.51 compared to 45.31 and 45.75 $g.kg^{-1}$ dry matter, acetic acid (12.76 compared to 18.10 and 19.08 $g.kg^{-1}$ dry matter) and alcohol (2.87 compared to 6.26 and 7.20 $g.kg^{-1}$ dry matter). T1 silage showed the lowest butyric acid content (0.55 $g.kg^{-1}$ dry matter), in which there was a highly statistical significant difference only in comparison to the untreated silage (1.34 $g.kg^{-1}$ dry matter).

Silage production is process resulting in physiological, chemical and microbiological changes, which need to take place in the feed being ensilaged in order to produce a quality product. In order to achieve the expected quality of the produced silage, a number of measures is necessary to control the fermentation process and to minimize the losses during fermentation. In addition to following the technological process, it is possible to also apply ensilaging additives of biological and chemical character (Spörndly and Pauly, 2008). Silage additives encompass a wide range of products including bacterial inoculants, fermentable substrates and enzymes, all of which are designed to promote growth of desirable organisms and appropriate fermentation products, as well as to inhibit growth of undesirable organisms and prevent poor quality silage (Schroeder, 2004).

Ward (2000) as well as Kung and Shaver (2001) consider the most helpful analyses for the determination of good silage composition to be the dry matter, acidity, crude protein, acid detergent fibre, neutral detergent fibre, lignin, calcium and phosphorus. The use of a fermentation profile including organic acids such as lactic,

acetic, propionic and butyric acids is also a helpful tool. The monitored parameters of the nutrient composition and fermentation process correspond to their recommendations. Though lignin, calcium and phosphor were not monitored in ours experimental silages, the focus was on sugars, starch, ether extract, ash and alcohol, which are also important parameters determining the quality of the produced silages.

Based on the results, we have determined that the influence of treatment by biological and chemical additives on nutrient content of Mixture 1 and Mixture 2 silages was low. Notable is the decrease of crude fibre content compared to untreated silage. Fermentation process had the most significant influence on utilizability of total and reduced sugars. The highest content of reduced sugars was determined in the silage treated by chemical additive and the lowest in the untreated control silage, in both mixture silages.

One of the parameters to evaluate the quality of fermentation process is the losses of dry matter during fermentation. The lower the losses, the lower are also the losses of nutrients and therefore the fermentation process was better. The lowest losses were determined in silages treated with chemical additive, in both types of mixtures (Mixture 1 and Mixture 2).

As known, the preserving effect in silage is obtained by suppressing the aerobic microbes by exclusion of air and eliminating the remaining harmful anaerobic microbes, which require no oxygen, through reducing the pH value by means of enrichment of lactic acid. Whether the harmful acid – sensitive microbes can be eliminated, particularly the *Enterobacteria* and

Table 3. The nutrient content and parameters of fermentation process in silages from Mixture 2 (in g.kg^{-1} dry matter)

Item	Control		T1		T2	
	x̄	SD	x̄	SD	x̄	SD
Dry matter v g.kg^{-1} FW	295.71	4.38	291.52	6.96	304.24	6.16
Dry mater losses in %	9.23^{a}	1.36	10.54^{a}	2.15	6.07^{b}	1.27
Organic matter	948.66	1.61	946.41	2.04	949.59	3.56
Crude protein	78.05	2.09	80.38	3.04	76.77	2.21
Crude fibre	260.16^{a}	3.55	247.51^{a,b}	10.84	241.95^{b}	8.26
ADF	304.08	8.77	296.57	13.61	291.45	8.68
NDF	534.16	10.52	537.65	10.75	550.87	18.45
Hemicelluloses	230.08	9.94	241.08	11.24	259.42	15.85
Nitrogen-free extract	580.65	17.82	584.53	15.12	601.66	9.21
Starch	231.63	11.33	227.76	14.35	217.51	7.77
Total sugars	1.46^{a}	0.01	5.47^{b}	1.66	11.52^{c}	0.73
Reduced sugars	1.01^{a}	0.01	3.53^{b}	0.60	8.60^{c}	1.39
Ether extract	29.80	0.91	33.99	2.28	29.21	1.49
Ash	51.34	1.61	53.59	2.04	50.41	3.56
pH	4.13^{a}	0.08	4.04^{a,b}	0.04	3.96^{b}	0.02
Acids						
– lactic	45.31^{a}	3.12	45.75^{a}	1.71	55.51^{b}	3.68
– acetic	18.10^{a}	1.23	19.08^{a}	1.59	12.76^{b}	1.47
– propionic	0.52^{a}	0.14	1.33^{b}	0.41	0.59^{a}	0.11
– butyric	1.34^{a}	0.38	0.55^{b}	0.19	0.83^{a,b}	0.23
Alcohol	6.26^{a}	0.56	7.20^{a}	0.71	2.87^{b}	0.13

n = 5, FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre, T1 – biological additive (*Lactobacillus buchneri* DSM 13573), T2 – chemical additive (22.9 % sodium benzoate, 8.3 % sodium propionate), Different superscripts within a row mean statistical difference ($P \leq 0.01$); ^{ab} vs ^{a,b} is not different

Clostridia, depends on the green fodder's potential for acidification (Weissbach, 2003). Bíro *et al.* (2014) considers the decrease of the pH values during the fermentation process to be one of the main preservation factors, which inhibits the multiplication of undesirable microorganisms in silages. For easily ensilaged forage, decrease of pH < 4.3 is recommended. Mitrík (2010) also highlights the importance of silage acidification and suggests that for evaluation of the quality of silages of maize and other easily ensilaged forages, the following formula should be used to determine the maximum pH value of the silage:

pH ≤ 0.0026 x Dry matter in % + 3.694

Mixture 1 and Mixture 2 silages reached in our experiment pH values corresponding to the recommendations of the aforementioned authors. In both mixture types, the highest pH value was determined in the untreated control silage and the lowest pH value in the silage treated by chemical additive.

Few authors engage in the research on silages containing dendromass. Jeon *et al.* (2003) determined in detailed research of such silages, that forest by-product silage had a fermentative quality of 4.1 pH and 8.9 % lactic acid (DM basis). In the silages in our experiment, the same pH values but significantly lower lactic acid concentrations (3.6 – 6.0 %) were determined.

It is essential to have a good microbial fermentation process to produce high quality silage. A good fermentation process is not only dependent on the type and quality of the forage crop, but also on the harvesting and ensiling technique. Many additives have been used to improve fermentation quality of made silages (Oude Elferink *et al.*, 2011).

Quality of fermentation process in silage is characterised by the content of fermentation products. Favourable is a high lactic acid content and low volatile fatty acids and alcohol content. The results of the fermentation process in our silages show that the effect of biological additive application to Mixture 2 silage was very low. The only demonstrably positive influence was from the chemical additive, which was manifested in the highest concentration of lactic acid and the lowest concentrations of acetic acid, propionic acid and alcohol. In Mixture 1 silages, more significant differences were determined between the silages treated with additives and the untreated control, which confirm the positive influence of treatment of the silage mixture. Fermentation process was the most successful in silages treated with the chemical additive, which is confirmed by the highest lactic acid content and the lowest content of volatile fatty acids and alcohol.

From this it can be determined that the chemical additive applied to the silage mixture composed of 70 % whole maize and 30 % fresh twigs shoots of forest trees conclusively improved the quality of the produced silage mixtures for wild ruminants. Positive influence of applying the biological inoculant based on heterofermentative bacteria of lactic fermentation had only partial effect, determined only in Mixture 1 silage, which was produced using the dendromass compound of 50 % oak twigs, 50 % spruce twigs.

The gathered data indicate that application of ensilaging additives has its purpose in production of maize-dendromass mixture silages for wild ruminants and it improves the fermentation process as well as the quality of the produced silages.

CONCLUSION

In this study, we evaluated the influence of selected biological and chemical ensilaging additives on the quality of fermentation process and nutritional value of silage produced from mixture of dendromass and maize, with 70 % maize. Two mixtures were created, which differed in the ratios of oak and spruce twigs in the dendromass. The ensilaging additives were more effective in case of Mixture 1 silage, which was produced using dendromass compound of 50 % oak and 50 % spruce twigs. Of the applied silage additives, the chemical additive, based on salts of organic acids, was determined to be more effective in improving the quality of the produced silages.

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RELATIONSHIP BETWEEN INBREEDING AND THE MAJOR HISTOCOMPATIBILITY COMPLEX: A REVIEW

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ABSTRACT

Inbreeding is a phenomenon common to a number of scientific areas. In recent years, inbreeding research has focused on immunology, which is providing valuable results and broader understanding of the subject. The aim of this article was to review research of the relationship between inbreeding and the major histocompatibility complex. Though the main focus is farm animals, the results for other animal species are presented for comparison.

Key words: animal species; inbreeding; major histocompatibility complex

INTRODUCTION

Discovery of the major histocompatibility complex (MHC) is playing an important role in our understanding of the function of the immune system in animals and humans. The characteristics of the MHC, in particular, high polymorphism, have also had a significant impact on other areas of research (not only immune research), such as transplantation medicine and population genetic studies. The genetic aspects of the MHC also led to research on the relationship of the MHC to a significant phenomenon in the field of animal population genetics, namely, inbreeding. The aim of this paper is to review major studies on the MHC's relationship to inbreeding, increase in homozygosity and also to some important traits, especially in relation to cattle and other farm animals. For comparison, research on other animal species is also presented.

Inbreeding in animal populations

Animal breeding (mainly farm animals) is currently focused on a number of areas, such as production (milk and meat), reproduction, exterior, fitness and other traits. In addition, the health of the animal plays a key role at this time too. The right combination of parent pairs is crucial for achieving optimal mating results and can confer heterosis effects to offspring (by mating of unrelated animals) or inbreeding depression (by mating of related animals). Increased inbreeding is associated with a number of adverse characteristics, including: 1). decline in quantitative traits (production, reproduction, exterior, etc.); 2). higher probability of lethal allele manifestation in a homozygous state; 3). increasing homozygosity of individual genes.

The negative effect on production, reproduction and exterior traits has been studied in great detail in recent years in relation to cattle breeding in different breeds and in various very large populations (Beller and Plesník, 1974; Kadlečík *et al.*, 2004, 2013; Sørensen *et al.*, 2005; Bezdíček *et al.*, 2005; Bezdíček *et al.*, 2012; Kasarda *et al.*, 2014; Bezdíček, 2017; Moravčíková *et al.*, 2017). Specifically, in Holstein inbred cattle (F_X = 9 %) Thompson *et al.* (2000a) reported reduced

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milk production by 327.6, 12.27 and 8.67 kg (milk, fat and protein in kg). In Jersey cattle, the same authors found a decline of 163.7, 8.99 and 8.16 kg (Thompson *et al.*, 2000b). Similar conclusions were drawn by Croquet *et al.* (2006), who monitored the Wallonischen Region Belgiens. In the Czech Republic, Bezdíček *et al.* (2008) studied the effect of inbreeding and reported that in cows with F_X = 4.0 - 12.5 %, there was a reduction in milk production of 472.24 kg compared to non-inbred contemporaries. Decrease in milk production due to inbreeding was also demonstrated in a Canadian population of Holstein and Jersey cattle (Miglior 1995; Miglior 1992), similar to the Montbeliard breed (Dezetter *et al.*, 2015), Ayrshire, Guernsey, Milking-Shorthorn (Wiggans *et al.*, 1995) and other breeds. Also, crucial is monitoring at the inbreeding level in small cattle populations, where the risk of mating between relatives is greater and there is also a higher average inbreeding in the population. These studies have been conducted, for example, in Deutsches Gelbvieh and Braunvieh (Krogmeier *et al.*, 1997), in a Schweizer Braunvieh population (Casanova *et al.*, 1992) and in Pinzgau cattle in Slovakia (Kasarda and Kadlečík, 2007). E.g. in the Pinzgau cattle population in Slovakia, Kasarda and Kadlečík (2007) reported an average inbreeding coefficient of 4.225 %.

A negative effect of inbreeding on cattle reproduction has also been demonstrated. The first studies were carried out in animal husbandry (effects of inbreeding on service-period, calving interval, number of inseminations) and later on, gametogenesis (male and female) as well as embryo production. A longer service-period due to inbreeding was described, for example, by Bjelland *et al.* (2013) and Bezdíček *et al.* (2007). The effect of inbreeding on the 70-Day nonreturn rate was described by Cassell *et al.* (2003), the impact of inbreeding on calving ease and stillbirth were studied by Hinrichs and Thaller (2011) and McParland *et al.* (2007). The impact of inbreeding on ovarian activity and embryo gain after superovulation was reported by Bezdíček *et al.* (2015), Alvarez *et al.* (2005) and other authors. In particular, Bezdíček *et al.* (2015) found a higher proportion of degenerated embryos in inbred than in noninbred animals (21.48 vs. 17.54) and a higher proportion of transferable embryos (4.29 vs. 2.72). Inbreeding depression has been studied in relation to the sperm production too. In inbred bulls (F_X = 10 %) a lower volume of ejaculate by 1.5 ml was found, and there was also a lower total sperm count (by 2.4×10^9) and reduced sperm vitality (Maximini *et al.*, 2011).

Some authors also calculated the total financial loss associated with inbreeding in cows (Smith *et al.*, 1998; Croquet *et al.*, 2006). For estimation of inbreeding intensity an important role is also played by completeness of pedigree and the number of ancestor generations. This has been described not only in cattle (Cassell *et al.*, 2003; Lutaaya *et al.*, 1999), but also in horses (Pjontek *et al.*, 2009). An important aspect of inbreeding is its relation to genomic selection and genomic models (VanRaden, 2016) which, apart from inbreeding, include other effects (heterosis, dominance, interaction).

In addition to large (Holstein, Fleckvieh) and small populations of cattle (including Gelbvieh, Braunvien and Pinzgauer), monitoring of inbreeding in other animal populations is important (Jafari, 2014). A valuable source of information on inbreeding is the breeding of the Przewalski and Old Kladrub horse. This is a significant genetic resource with few animals at the outset (the value of gene banks was reviewed in Chrenek *et al.*, 2017). With good animal husbandry these breeds have been successful and inbreeding was gradually reduced. In the case of the Przewalski horse, less than twenty horses were imported into Czechoslovakia in 1899-1902. This close knit animal group became the basis of today's population of about 2,000 individuals (Kůs, 2006; Jakubec *et al.*, 2012). Inbreeding in the Przewalski horse was observed in 1901-2004 with a mean value of 9.4 % (Wolc *et al.*, 2008). With progressive population growth inbreeding was reduced. The latter authors report that inbreeding of the Przewalski horse increased in particular until 1940, when its level was 37 %. During this period, it was difficult to avoid inbreeding due to the small number of animals. Increase in the population occurred in the 1960s, which was also associated with a decrease in average inbreeding in this horse population (Wolc *et al.*, 2008).

Similarly, in the Old Kladrub horse, there was a decline in inbreeding between 1993 and 2003 in stallions (from 5.65 to 5.57) and in mares

(from 7.75 to 4.88 %) as well (Jakubec *et al.*, 2009). The Old Kladrub horse and the Przewalski horse had to overcome the danger of inbreeding, especially at a time when the number of active animals was low. In general, horses are reported to be less susceptible to inbreeding depression. Tatin *et al.* (2008), for example, studied a natural herd of Przewalski's horses in France. These authors reported that the F_X coefficient for dead foals was not higher than that for living foals (F_X = 0.183 vs. 0.181; n= 11 vs. 51). In contrast, an effect of inbreeding on horse reproduction was found by Seving *et al.* (2004). The authors reported a partial association between incidence of retained placenta and inbreeding level in Friesian horses. Also, the impact of inbreeding on the sperm quality of stallions in the Shetland pony was investigated (Van Eldik *et al.*, 2006). The coefficient of inbreeding ranged from 0 to 25 %. The authors showed that inbreeding above 2 % was significantly associated with lower sperm motility and less proportion of morphologically normal sperm. Specifically, in the case of animals with low (F_X = 0-1 %) vs. animals with high (F_X above 12 %) inbreeding there was an increase in sperm motility from 59.8 % to 67.4 %, resp. Although, less susceptiblity to inbreeding was found in horses, as mentioned, overall studies have shown its negative effect on reproduction for other traits.

The negative consequences of inbreeding on a number of traits have been demonstrated in studies on various animal species. One serious consequence of inbreeding is increasing homozygosity in the entire DNA of the inbred individual. The unanswered question was, why heterozygosity was better or, oppositely, why increased homozygosity was detrimental. The answer to this question must await research findings in immunology, especially understanding of lymphocyte maturation (gene rearrangement) and the function of the major histocompatibility complex (MHC).

The major histocompatibility complex (MHC)

The main function of the MHC complex in cells is the presentation of endogenous antigens to enable the immune system to recognise foreign substances. The MHC I complex has been found on nuclear cells of nearly all vertebrates. It presents the antigens from pathogens to cytotoxic T lymphocytes (co-receptor CD8). MHC II occurs in the immune cells (e.g., monocytes, macrophages, dendritic cells, B-lymphocytes) and represents the antigen to helper T-lymphocytes (co-receptor CD4). Each individual has a specific combination of major histocompatibility complex molecules, which are inherited from the parents in a codominant manner (except for the so-called zero allele). In humans, the major histocompatibility complex is called the HLA complex (Human Leukocyte Antigens). This expresses its first appearance on leukocytes. In bovine it is called BoLA (Bovine leukocyte antigen; Fries *et al.*, 1986).

Characteristic for the major histocompatibility complex is its high polymorphism. In case of the HLA complex, the discovery of new alleles is regularly updated at the site (http://hla.alleles.org). For example, up to December 2017 a total of 17,695 HLA (and related) alleles (HLA I Alleles 12,893, HLA II Alleles 4,802) were described. The highest polymorphism for HLA Class I was found in genes A (genes/proteins: 4,081 / 2,853), B (4,950/3,582) and C (3,685/2,550), while in other genes (E, F, G) only several tens of alleles are known. In HLA Class II, the highest polymorphism is the DRB gene (2,440/1,779), a high polymorph is the DQB1 gene (1,178/795) too - http://hla.alleles.org. This very high HLA I and II polymorphism enables use of these genes for research in a number of areas.

In animals, there is an interesting database IPD-MHC (https://www.ebi.ac.uk/ipd/mhc/), that catalogues Major Histocompatibility Complex sequences from a number of species organized into taxonomic groups. The database was established in 2003, and in 2017 it included 7,000 alleles from 70 species (Maccari *et al.*, 2017). For example, the MHC system is reported in bovines (BoLA), equines (ELA - equine leukocyte antigen), swine (SLA - Swine Leukocyte Antigen), sheep (OLA – Ovine leukocyte antigen), in salmonid fish, felines and other animals. As noted, the major histocompatibility complex is found on nuclear cell surfaces in nearly all vertebrates. However, there are differences between animals in terms of chromosome localization (BoLA – on 23rd chromosome, SLA – on 7th chromosome, ELA – on 20th chromosome), polymorphism of individual loci, relation to disease and other factors. For example, the bovine MHC consists of three gene classes

(Class I; IIa and IIb; III.). The most studied gene is BoLA-DRB3 (Class IIa), which is highly polymorphic (Takeshima *et al.*, 2003; Miyasaka *et al.*, 2005). At present, over 130 alleles have been detected at this locus.

Relationship of major histocompatibility complex to selected traits and inbreeding

Research on the major histocompatibility complex is generally focused on specific areas (traits), especially in relation to various diseases. The specificity of its high polymorphism in individual loci has led to MHC evaluation from the viewpoint of homo- and heterozygosity and its relation to inbreeding. Research of the MHC has been carried out around the world, in a variety of vertebrate species living in diverse conditions and in various large populations.

The *BoLA-DRB3* gene mentioned above is currently studied in cattle breeding, for example, in relation to bovine leukemia virus (BLV) disease. In particular, the *BoLA-DRB3*0902* allele appears to be associated with this infection (Juliarena *et al.*, 2016). The authors point out that due to increase in the prevalence of BLV in a number of countries around the world and the current lack of vaccine or treatment, a viable strategy is the selection of infected cattle carrying the *BoLA-DRB3*0902* allele (Juliarena *et al.*, 2016; 2017). Association between *BoLA-DRB3* and embryonic mortality during *in vitro* fertilization was studied by Kulaj *et al.* (2015). In Holstein-Friesian cattle, embryonic mortality was connected with homozygosity at the *Bola-DRB3* locus. The *BoLA-DRB3* gene was also examined in relation to resistance to mastitis and somatic cell count in cattle (Rupp *et al.*, 2007; Nandedkar *et al.*, 2017; Sharif *et al.*, 2000; Starkenburg *et al.*, 1997). The relation of mastitis and the major histocompatibility complex in cattle was evaluated in relation to the gene *BoLA-DQA* (Takeshima *et al.*, 2008) in cattle as well. In this study, homozygous *BoLA-DQA1*0101/0101* and *BoLA-DQA1*10011/10011* genotypes were found to be associated with higher susceptibility to mastitis in cows, caused by *Streptococci* and *Escherichia*, respectively. The authors conclude that heterozygosity of the *BoLA-DQA1* gene is connected with resistance to mastitis in cows (Takeshima *et al.*, 2008). Similarly, in sheep, Hui *et al.* (2012) showed that polymorphism in MHC-DQB1 may be connected with immune protection. The polymorphism was assessed in Chinese Merino sheep and the authors found a relation of this gene to *Cystic echinococcosis* disease. In the Suffolk breed, Sayers *et al.* (2005) identified seven alleles in the Ovar-DRB1 gene and also found that this gene plays a major role in resistence to sheep nematode infection. An important association of the MHC class II DRB1 gene was found with resistance to two chronic respiratory diseases in sheep - Ovine pulmonary adenocarcinoma and Maedi–Visna (Larruskain *et al.*, 2010). The major histocompatibility complex in sheep was the focus of one review aimed at genetic and epigenetic factors influencing mastitis in ewes (Tančin *et al.*, 2016).

The advantages of heterozygosity (MHC genetic diversity) have likewise been demonstrated. For example, Agudo *et al.* (2012) found a positive correlation between genetic diversity in MHC in birds and breeding fitness. In fish (Atlantic salmon) increase in heterozygosity in offspring was shown by the preference for mating between fish with dissimilar MHCs (reviewed by Bernatchez and Landry, 2003; Landry and Bernatchez, 2001).

In addition to MHC research, from the standpoint of different traits and the advantages of heterozygosity, a number of studies have focused on inbreeding and mating between related animals. Some key experiments were carried out in mice. In one study, the rationale was that it is an attempt to select a partner according to different MHCs, thereby avoiding inbreeding (Potts *et al.*, 1991; Yamazaki *et al.*, 1976). Demonstration of the importance of MHC was also found in laboratory *in vitro* fertilization procedures with two inbred mouse strains (Wedekind *et al.*, 1996). In addition to the MHC, mouse research has also focused on major urinary proteins (MUP), which can be labeled by genetically-encoded pheromones and which can be applied to species behavior (Logan *et al.*, 2008; Mudge *et al.*, 2008). Studies have shown that MUP (major urinary proteins) is involved in olfactory communication in mice and that MUP profiles vary between inbred mice (Robertson *et al.*, 1996). Similar conclusions were drawn by Sherborne *et al.* (2007), who studied the relationship between inbreeding in house mice (*Mus musculus domesticus*) and two genetic factors – MHC and MUP. In their research, the authors found that there was no relation between mating in mice and MHC. However, the animals found kinship

on the basis of MUP (Sherborne *et al.*, 2007). In this context it is important to note the protein component of urine is very low in most mammals. Mice are unique in the excretion of proteins in their urine (Vandenbergh *et al.*, 1975).

Inbreeding avoidance through MHC has been observed in non-human primates. One interesting study was performed by Schwensow *et al.* (2008a; 2008b). These authors evaluated the role of MHC diversity in terms of choice of mate in a promiscuous primate (mouse lemur – *Microcebus murinus*) and in an obligate pair-living primate (dwarf lemur – *Cheirogaleus medius*). They found that in mating, promiscuous primate males with dissimilar MHC were preferred for fertilisation. In non-humans primates this was the first study showing the importance of the MHC in post-copulatory mate choice (Schwensow *et al.*, 2008a). In another study, the same authors focused on an obligate pair-living primate. The importance of MHC-genes for mate choice and partner selection was confirmed. Females preferred males who would guarantee higher heterozygosity and larger number of MHC-alleles in their offspring (Schwensow *et al.*, 2008b).

The major histocompatibility complex has been studied by a number of authors in horses including the Przewalski (Hedrick *et al.*, 1999) and in Old Kladrub horses (Hořín *et al.*, 1998; Janova *et al.*, 2013), in donkey (Arbanasić *et al.*, 2013) and in a plains zebra population (Kamath and Getz, 2012). Most papers focused on polymorphism of the major histocompatibility complex and its relation to the selected traits. Some researchers also studied inbreeding avoidance in horse. Duncan *et al.* (1984) found that by random mating in a natural (isolated) herd of Camargue horses the inbreeding coefficient was lower than expected according to random mating. There was also reduced sexual behaviour with members of their own harem. Regarding an involvement of the MHC in female choice, Burger *et al.* (2010) found interesting results from monitoring 19 mares aged 5-17 years (different breeds). They found a tendency ($P < 0.063$) for estrous mares to select stallions with a different major histocompatibility complex (ELA). All ovulations in the mares in this test were induced by 1500 IU hCG i.v. In another study, Andersson *et al.* (2012) focused on the relationship between MHC II and insect bite hypersensitivity (a serious chronic allergic dermatitis in horse). They found that homozygosity in MHC II was connected with a greater danger of developing this disease.

An important source of information on inbreeding and MHC research is specific animal populations such as bottleneck populations. For example, O'Brien and Evermann (1985; 1988) reported that the Cheetah population (*Acinonyx jubatus*) lost its polymorphism and is extremely genetically monomorphic, which includes its MHC genes. The authors emphasized that the loss of variability leads to increased sensitivity in the gepard population to a viral pathogen (O'Brien and Evermann, 1985).

CONCLUSION

The studies outlined in this review confirm a substantial amount of research of the major histocompatibility complex in various vertebrate species. Although a number of relations have been found between the genes of the major histocompatibility complex and major production traits (including animal health), the influence appears in some cases to have a multifactorial character with MHC the only one of several factors.

Loss of genetic variation (for different reasons – including inbreeding), can pose a significant risk to the population or individual. In a number of studies, it has been shown that increased inbreeding is associated with decline in production, reproduction and other traits in a number of animal species (mainly in farm animals). Research in recent years also shows that inbreeding can lead to greater susceptibility to disease (weakened immunity) based on lower genetic diversity (MHC; lymphocyte receptors – TcR, BcR). In general, in the case of inbred animals there is a greater homozygosity. This corresponds to a lower number of variants in the gene rearrangement in lymphocytes (the gene rearrangement of T and B lymphocytes) and thus a lower number of antibody variants in inbred animals.

Inbreeding is generally a phenomenon that we can study at various levels in different animal species. It is also an important means for studying the biological process, especially in terms of reproduction and genetics. Research in the area of inbreeding consequences (inbreeding depression)

has made a significant contribution to a knowledge from the standpoint of the major histocompatibility complex, which is an important component of the immune system in vertebrates.

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GENETIC EVALUATION OF CALVING DIFFICULTY IN CATTLE: A REVIEW

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ABSTRACT

Advantages and drawbacks of different approaches of genetic evaluation of calving difficulty are described and discussed in the review. Calving difficulty is a complex trait, which affects the economics of cattle breeders. The main factors affecting calving difficulty include calf size, pelvic measures of the cow and, more importantly, their compatibility, breed, parity of the calving, sex of the calf, gestation length and the season of the calving. Scoring scales of calving difficulty differing in the number and description of the categories are applied across the countries. Among the various statistical approaches, preference is given to threshold and linear models for genetic evaluation of calving difficulty. It seems that linear models are more suitable for the use of field data. An improvement of predictions can be obviously achieved by the application of correlated traits.

Key words: prediction; genetic parameters; linear model; threshold model; calving difficulty; dystocia

INTRODUCTION

Dystocia or calving difficulty is a complex reproductive trait. Some sources also define it as delayed or difficult parturition. The impact of difficult calving can be identified directly through higher costs of labour and costs of veterinary assistance during the calving. The economic impact of the dystocia on production, fertility, cow and calf morbidity and mortality in dairy cattle was shown during a long time period across different countries (Dematawewa and Berger, 1997; López de Maturana *et al.*, 2007; Ghiasi *et al.*, 2011). Consequent problems, like retained placenta and longer calving period, also indirectly contribute to lower productivity of animals (Gaafar *et al.*, 2010; Bujko *et al.*, 2018)

The incidence of dystocia is different across breeds and countries. In beef cattle, Phocas and Laloë (2003) reported 8 % of the calvings in Charolais population to need mechanical assistance. Eriksson *et al.* (2004) reported 6.6 % and 6.2 % incidence of dystocia in Charolais and Hereford primiparous cows, respectively. De Amicis *et al.* (2018) reported 5.6 % incidence of dystocia in local beef breeds. Jamrozik and Miller (2014) reported 3.7 % incidence of difficult calving in Canadian Simmental.

Despite low frequency of dystocia in the beef herds, its large impact on the economics pushes farmers to avoid difficult calvings. In the past, birth weight was used as an indicator to avoid these problems (Eriksson *et al.*, 2004). Later, evaluation of calving difficulty was included into the recording scheme. This approach is based on subjective evaluation by the farmer or by the trained personnel from a breeding company. Calving difficulty became a part of the routine genetic evaluation programs. Consequently, an increasing number of countries that record calving ease were reported (Mark *et al.*, 2005) and international genetic evaluation started (Jacobsen and Fikse, 2005).

Calving difficulty is a part of the performance recording in dairy and beef cattle in the Slovak Republic. However, while these data are used in the genetic evaluation of the dairy cattle, they are not used in the genetic evaluation of the beef cattle

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so far. This review gives examples of systems of calving difficulty recording and genetic evaluation of this economically important trait.

Recording of calving difficulty

Due to its nature, calving ease is recorded as a discrete trait, with no strictly defined limits. In general, however, the categories have a linear ascent. Scoring systems are used to describe calving ease or calving difficulty depending on the country (Mee, 2008). In the Slovak Republic, a four-point scale is used with 1 referring to no assistance needed and 4 referring to caesarean section, and with additional category 0 referring to unknown calving. In Norway, a three-point scale with additional category of unobserved calving was used (Holmøy *et al.*, 2017). Four-point scales were used in France (Phocas and Laloë, 2003), Germany (Fuerst and Egger-Danner, 2003) and Ireland (Berry and Evans, 2004). Five-point scales were used in USA (Cole *et al.*, 2005), Austria (Fuerst and Egger-Danner, 2003), Canada (Jamrozik and Miller, 2014) and South Korea (Alam *et al.*, 2017). The scales have a slight differences and definitions of categories depending *e.g.* on the number of personnel needed or the level of assistance during the calving. In Sweden, Eriksson *et al.* (2004) described Swedish beef-recording scheme as having 7 categories of calving. In respect to the use of the data, Mee (2008) summarized that in some countries unknown calvings are excluded from analyses, while in some countries these are included in unassisted category (easy calvings). In some countries, more detailed information on reasons of difficult calving is recorded. These recordings can be further used for the analyses of foetal and maternal causes of dystocia. Other approaches to calving difficulty exist. In particular, De Amicis *et al.* (2018) studied incidence of dystocia in Italy and used the classification of maternal and foetal dystocia, and thus avoided the classification based on severity degree. In some countries, like Iran, the calving difficulty is recorded as a binary trait (Ghiasi *et al.*, 2014). This means that only easy calving (with no assistance needed) and difficult calving with assistance are distinguished and assigned as 0 or 1. This approach seems quite effective, especially in populations where animals are calving on the pasture, and the only concern of the farmer is the assistance needed but not its extent. Treating the calving ease as a binary trait can also help in avoiding common problems of categorical trait (occurrence of extreme categories) or continuous variable (deviation from normal distribution).

Factors affecting calving difficulty

Since dystocia is a complex trait, statistical modelling and estimation of breeding values require identification of the number of factors that affect its incidence. The most important factors can be divided into groups including factors of calf, factors of cow and environmental factors. Obviously, the model predicting the calving ease should, therefore, involve direct and maternal effects. The consequences of including solely the direct effect were shown by Ghiasi *et al.* (2014), who concluded that this approach was not sufficient and there has to be selection applied using both direct and maternal effect.

Calf weight and sex

Although one of the main factors leading to dystocia is incompatibility between the size of calf and the pelvic measurements of cow, De Amicis *et al.* (2018) reported that in the local Italian breeds most of dystocia occurred due to foetal causes, from which almost 93% were due to foetal malposition and foetal macrosomia. Similarly, Strapák *et al.* (2000) reported the influence of birth weight (and sex) of the calf on the calving ease. This can be supported by findings of Mujibi and Crews (2009) and Jamrozik and Miller (2014). However, the effect of calf weight may be confounded with the effect of calf sex (Nix *et al.*, 1998), since the male calves are born heavier than female calves. This suggestion can be supported by findings of Lombard *et al.* (2007), Atashi *et al.* (2012) and McHugh *et al.* (2014), who observed higher incidence of dystocia in cows giving birth to young males. On the other hand, Piwczyński *et al.* (2013) considered the body weight of the calf a more important factor of calving difficulty than the sex of the calf. In relation to the sex of the calf, it was shown that the incidence of dystocia may be decreased when sexed semen is used (Norman *et al.*, 2010). The increased risk of the dystocia, when twins are considered (Mee *et al.*, 2011), will not be discussed here, since these cases are often excluded from the genetic evaluations. In the prediction models, the sex of the calf can

be treated as a single trait (Phocas and Laloë, 2004) or in combination with the age of the dam (Jamrozik and Miller, 2014). On the other hand, the weight of the calf is often used as a correlated trait in multivariate models (Varona *et al.*, 1999b; Matilainen *et al.*, 2009). The use of birth weight in prediction models can be questionable in situations where farmers estimate the weight and not truly measure it (as showed by Phocas and Laloë, 2004). The problem can be more visible in cases, when farmers only report constant birth weight, which lead to deviations from normal distribution or even getting the features of categorical one.

Gestation length, calving parity, body condition score

The gestation length can also affect the incidence of the difficult calvings (Eaglen *et al.*, 2013; Uematsu *et al.*, 2013). Higher incidence of the dystocia was recorded in cows with gestation length higher than 301 days and lower than 280 days. Very high incidence was recorded in the group of cows with gestation length lower than 270 days. Positive genetic correlations between calving ease and gestation length in multiparous cows can suggest that the calf that gestates longer before birth to a multiparous dam is genetically prone to a difficult birth (Eaglen *et al.*, 2012).

It is well known and proved that the incidence of dystocia differs according to the parity of the calving (Berglund, 2008; Atashi *et al.*, 2012; De Amicis *et al.*, 2018). Especially, the difference between the first and further calvings is emphasized in the literature sources. The problems with dystocia in primiparous cows can be explained by the fact, that young heifers have smaller pelvic size, which lead to calving difficulties (Fuerst and Egger-Danner, 2003). For older cows, malpresentation of the calf, weak labour and insufficient dilatation of the cervix are more likely to lead to calving difficulty (Meijering, 1984). According to older literature sources, the size of the cow expressed by her weight is not a good predictor of calving difficulty, since heavier cows tend to have heavier calves (Luo *et al.*, 2002). The area measurements solely used to predict the calving difficulty are not sufficient, because the compatibility of calf size with pelvic area size is important. Olson *et al.* (2009) added that the parity of calving can affect the dystocia occurrence through shorter gestation and lower calf weights in heifers. According to higher incidence of dystocia in primiparous cows, the herd management is an important factor (Holmøy *et al.*, 2017) and the emphasis should be put to adequate service weight of the heifers at the first mating. The trend of decreasing incidence of the dystocia with increasing parity of the calving was shown by several authors. Oppositely, Mõtus *et al.* (2017) reported higher incidence of dystocia in the third and later parities compared to the second one. This can be supported by Juozaitiene *et al.* (2017), who reported extremely difficult calvings in primiparous cows and also cows at the 6^{th} – 8^{th} lactation. According to their observations, most of the cows that experienced difficult calving had also consecutive calving scored as difficult.

The correlation between body condition score 10 days before calving and maternal calving ease was studied, emphasizing the relation between fat cows before calving and dystocia incidence (Bastin *et al.*, 2010). The positive genetic correlation between average daily gain and the calving performance can suggest, that animals that grow faster, tend to produce progeny with more problems at calving (Albera *et al.*, 2004).

The gestation length is mostly used as a correlated trait in the models (López de Maturana *et al.*, 2009), while the parity of the calving is always included as an explaining factor, or the single models are designated for the different parities. The use of the gestation length and parity in the genetic evaluation of calving difficulty puts higher demands to the data. While the parity of the calving can be assumed from the age of dam and previous calvings, lack of information on date of matings in the extensive farming systems leads to exclusion of the gestation length from the model.

Season of calving

The season of calving was identified as the factor affecting the incidence of the dystocia (Meyer *et al.*, 2000; Fuerst and Egger-Danner, 2003; Matilainen *et al.*, 2009). Although the seasons are not strictly defined across the countries, studies showed higher rates of dystocia in the winter and spring (Uematsu *et al.*, 2013; Mekonnen and Moges, 2016) and lower rates in the summer and autumn. The possible explanation of these differences is that cows calving in winter and spring experience the last part of gestation in the winter

period with changed and maybe improved feeding regime, thus more intensive foetal growth leading to problematic calvings. Another explanation may be hostile environmental conditions during the parturition in the winter period. In fact, the increased temperature during the calving month (and 2 preceding months) reduced the need for the assistance during parturition (Colburn *et al.*, 1997).

Significant effect of the season is reflected in all statistical models for calving difficulty prediction. Mostly the joint year-season effect is used, but also single effect of season can be found (Eriksson *et al.*, 2004), or joint herd-year-season (Ramirez-Valverde *et al.*, 2001; Luo *et al.*, 2002). Including the season into joint effects is reasonable and helps saving computational costs. Treatment of this effect is important and needs more insight. Distribution of calvings in dairy herds is continuous and more or less regular over the year. However, beef cattle farmers try to manage all calvings during one or two seasons. Therefore, the definition of a season has to be adequate to reflect this fact. In case of joint effects (herd-year-season) in combination with field data, the attention should be also put on the number of records in the groups, in order to avoid too many groups with too few records. Including the herd effect can be considered as covering the effects of the management and nutrition, which are mentioned later.

Other factors

Differences in dystocia incidence between dairy and beef cattle (Mekonnen and Moges, 2016; De Amicis *et al.*, 2018) are generally known, and the difference can be also found among the breeds (Cole *et al.* 2005; Olson *et al.* 2009; El-Tarabany *et al.*, 2015). In some cases, differences among the breeds should be reflected in the matings and suitable combinations of the sire and dam breed should be chosen (Vallée *et al.*, 2013; Ahlberg *et al.*, 2016). This especially applies in beef cattle population, where many breeds with different exterior characteristics and measures are reared and their crossbreds are used for production. It is obvious that mating sires of large breeds to dams of small breeds can lead to the incompatibility between the size of calf and the pelvic measures of the dam. In this respect, not only the breed is relevant but also the effect of sire (Holm *et al.*, 2014; Mekonnen and Moges, 2016).

Beside obvious factors that are considered in various statistical models, other factors also directly or indirectly influence the incidence of dystocia. These can include nutrition, management, infection and exercise of animals (Zaborski *et al.*, 2009; Mato *et al.*, 2015; Mekonnen and Moges, 2016). It is commonly known, that overfeeding of the dam can lead to rapid intrauterine growth of the calf, while underfeeding can lead to poor condition of dam during the parturition. The type of husbandry system can also play a role in the incidence of calving difficulty (Mee *et al.*, 2011; Piwczyński *et al.*, 2013). The other effects are included in models through the effect of herd, which is mostly joined with the season or/and the year of the calving.

Models used for evaluation of calving difficulty

Since the calving ease in its nature is recorded as a discrete trait, it should be most suitable to use the threshold model to predict genetic parameters and breeding values. Although according to Gianola (1982), the threshold model should be superior over the linear model, due to different reasons linear models are widely applied in practice. The application of the linear model can be preceded with Snell transformation (Snell, 1964) of discrete variable into continuous (Mujibi and Crews, 2009; Alam *et al.*, 2017), which is based on the premise that there is exists an underlying continuous distribution of calving ease scores of which the Snell scores represent class interval midpoints.

Latest methods including multinomial regression models, decision trees, random forests and neural networks were studied by Fenlon *et al.* (2017) in order to provide decision support and simulation modelling for calving difficulty.

Genetic parameters

Generally, low heritability of calving performance is reported in the literature. Koots *et al.* (1994) reported that heritability for calving ease may be higher in beef breeds compared to dairy breeds. Low direct (up to 0.14) and maternal (up to 0.06) heritability of calving ease was reported in recent studies (Mujibi and Crews, 2009; Jamrozik and Miller, 2014; Alam *et al.*, 2017). Higher values of direct (0.40) and maternal (0.23) heritability were also reported (Lee, 2002b; Vostrý *et al.*, 2014). When studying and using heritability of the calving difficulty, it is important to consider

what kind of model was used for their calculation. Although low estimates of heritability can be found in recent studies with threshold models (Ghiasi *et al.*, 2011; Vanderick *et al.*, 2014), it was shown that the estimates obtained from linear models are lower compared to the estimates obtained from threshold models (Alday and Urgabte, 1998; Varona *et al.*, 1999a). Lower heritabilities obtained by linear models were explained as underestimated (Abdel-Azim and Berger, 1999), when comparing with heritabilities obtained by threshold models. It was also shown that with increasing number of categories and frequency of records in the categories, the estimates from linear models were closer to estimates from threshold models. Estimates were similar for linear models using raw and transformed data, suggesting that the transformation of calving ease scores is not necessary. Differences among the breeds are also manifested in different direct and maternal heritabilities (Roughsedge *et al.*, 2005). When separate models for heifers and multiparous cows were applied, higher heritabilities were calculated for calving ease in heifers (Carnier *et al.*, 2000; Jamrozik *et al.*, 2014), showing the calving difficulty is more related to the primiparous cows.

Most of the authors reported negative correlations between direct and maternal heritability. This is explained by the fact that small calf born easily and becoming cow (with smaller pelvic dimensions) is prone to have difficult calvings (Eaglen *et al.*, 2012). It has to be pointed out, that negative correlations can be found mostly in the studies using linear models. These correlations are lower with increasing parity of calving. Positive correlations between these effects were reported when threshold model was used (Luo *et al.*, 2002). Based on older literature sources, it was shown that no or very weak correlation exists between cow birth weight and dystocia. Recent findings also showed that heifers, which experienced dystocia during their own birth, did not tend to experience dystocia during their first calving (Holm *et al.*, 2014). Differences between studies were attributed to different populations, genetic progress, treatment of the calving ease and type of statistical model used. Despite a linear model being used (Jamrozik *et al.*, 2014; Vanderick *et al.*, 2014), positive correlations between direct and maternal genetic effects were reported. Additionally, Vanderick *et al.* (2014, 2017), according to very low positive correlations, suggested application of the model with no correlation between maternal and direct additive genetic effects.

Threshold model

The choice of the threshold model is intuitive due to the nature of calving difficulty. Indeed, superiority of the threshold model over the linear model was reported many times. However, authors reported substantial requirements for computer hardware in order to use this type of the model in the past (Lee *et al.*, 2002a). Even nowadays, with more powerful computers, several authors (Matilainen *et al.* 2009; Vostrý *et al.*, 2014) reported practical problems (more time needed compared to linear model), when threshold model was applied.

Beside practical issues, some studies experienced other drawbacks of application of the threshold models. Problems with convergence may occur (Luo *et al.*, 2001). These problems may result from fitting the herd-year as fixed effect in the threshold model. On the other hand, treating the herd-year effect as random would result in incorrect ranking of animals based on their estimated breeding values. Eriksson *et al.* (2004) also reported failure to use threshold model in case of small contemporary groups and limited use of artificial insemination (only few offspring per sire). Many authors including Jamrozik *et al.* (1991) did not show advantages of threshold model over the linear model applied to calving ease as categorical trait.

Linear model

Many studies can be found which preferred the linear model over the threshold model based on the findings of Misztal *et al.* (1989) and Hoeschele (1988). The limiting factor of using the threshold model can be the number of progeny per sire. Ramirez-Valverde *et al.* (2001) showed that for bulls with at least 50 calving records, the threshold and linear models give similar results. Mujibi and Crews (2009) summarized that when the field data are used, the differences among linear and threshold models are decreasing and the rankings of animals by both types of model are almost similar. This trend was proven in the study on calving rate and calf survival (Guerra *et al.*, 2006). Linear models are more suitable than the threshold models

in the situations where the populations with small sized herds (small size of herd-year groups) are considered (Phocas and Laloë, 2003). Similar findings were reported in the study where the linear model showed higher stability in predicting breeding values of animals whose records were randomly set to missing (Vanderick *et al.*, 2014). Vostrý *et al.* (2014) reported satisfying results with the linear model and Snell transformation, which were approximating to the results of the threshold model. When comparing the use of the original and normalized categories of calving, only slight differences between predictive ability of linear models were found (Matilainen *et al.*, 2009).

Although not ideal, the use of the linear model for the categorical trait has been shown to work for practical purposes in many studies. Especially, from the point of routine evaluation of animals, the preference of the linear model is obvious (Phocas and Laloë, 2004; Vanderick *et al.* 2013; Forutan *et al.*, 2015).

Multivariate models

Since most of calvings are scored as normal calving, i.e. recorded in one category, and other categories include only few records, there is a tendency of joining the records from extremely difficult calvings into joint category (Cole *et al.*, 2005; Alam *et al.*, 2017). This correction can lead to slight improvement of predictions (López de Maturana *et al.*, 2009). Some studies suggested that the first and later parities are genetically different but correlated traits (Carnier *et al.*, 2000; Steinbock *et al.*, 2003; Eriksson *et al.*, 2004) and, thus, they are sometimes treated as correlated traits in multivariate models.

From another point of view, the application of multivariate model is driven by the low heritability of the calving difficulty and efforts to use an information on other correlated traits in order to increase the accuracy of the prediction. Although, inclusion of birth weight and gestation length, as correlated traits, improved accuracy of prediction (Matilainen *et al.*, 2009; Jamrozik and Miller, 2014), addition of only the gestation length has no effect on the accuracy (López de Maturana *et al.*, 2009). The use of the gestation length in the multivariate model depends also on the importance of this trait, since its inclusion as an indicator trait for the calving ease has only limited effect (Hansen *et al.*, 2004). The advantage of bivariate linear-threshold model with inclusion of birth weight was shown by Varona *et al.* (1999b). The advantage of including the birth weight into analysis was explained by gaining the stabilizing effect of the continuous trait.

Anyhow, a higher improvement of the accuracy can be achieved when the multivariate model is preferred over the univariate in comparison to preference of the threshold model over the linear ones (Ramirez-Valverde *et al.*, 2001). They also showed that the preference of the animal model over the sire model should be made in cases where limited number of progeny per sire is expected.

The use of multi-breed models in genetic evaluation of the calving difficulty was shown by the Vanderick *et al.* (2017). Their study proved that this approach benefits from using the crossbreds and thus improving the accuracy of the estimates of purebred animals. They also showed increased values of heritability estimates and values of direct-maternal genetic correlations, when compared to single-breed approach.

In the last fifteen years, already known and newly identified SNPs were associated with calving ease and other calving traits (Fortes *et al.*, 2013; Purfield *et al.*, 2015; Abo-Ismail *et al.*, 2017). These can be used in the marker-assisted or genomic selection in order to improve the prediction accuracy, selection of animals and, thus, to decrease the incidence of calving difficulties.

CONCLUSION

Experiences show that, from the practical point of view, linear models are optimal choice for routine genetic evaluation. The main arguments for this choice may be the decreasing difference between models, when field data are used, and better suitability of the linear model for situations with small groups (herd-year-season). Higher impact on the predictive ability of the model can be achieved by the inclusion of correlated trait, *e.g.* birth weight. But there may be a risk of confusing results if the data on birth weight of the calves are only estimated by the farmers but not measured. The choice of other effects has to be done according to the availability of data. The sex of the calf, herd, season and year of the calving are routinely recorded, however the use of gestation length and parity can be limited in case of extensive farming

systems. From the point of view of practical farmers it is worth to consider recording and treating the calving difficulty as a binary trait. Here, however, more research and discussion with farmers has to be done.

In case of actual data, primary analysis of the recorded data on calving difficulty in beef cattle in the Slovak Republic is required in order to decide on the next steps.

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RODENT ANIMAL MODEL FOR RESEARCH IN DIABETES: A MINI-REVIEW

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ABSTRACT

Diabetes mellitus (DM) is classified into two groups: type 1 diabetes (T1DM) and type 2 diabetes (T2DM). T1DM requires insulin treatment. T2DM is characterized by insulin resistance, and it can be treated with variety of pharmacological and other compounds to alleviate or delay diabetes complications. The primary factors in the onset of DM are hyperglycaemia and hyperlipidaemia. Diabetic complications are grouped as macrovascular (heart disease, stroke and others) and microvascular (diabetic nephropathy, neuropathy, and retinopathy). For diabetes research several models have been used. In this review we provide an introduction to *diabetes mellitus* and its complications, currently used rodent animal models in diabetes research, the main results concerning therapeutical agents and the main targets.

Key words: diabetes; complications; animal model; ZDF rats; therapy

INTRODUCTION

Diabetes mellitus (DM) is a serious disease noted for its typical symptoms as hyperglycaemia and relative or complete insulin deficiency (King and Bowe, 2016). It was estimated that the prevalence of diabetic patients worldwide will reach 380 million by 2025 (Ramachadran and Snehalatha, 2010). DM is classified into two types. Type 1 DM or insulin-dependent diabetes mellitus (IDDM) is autoimmune disease caused by T cell-mediated damage of pancreatic β-cells of pancreas. This condition results in total insulin deficiency (Bluestone *et al.*, 2010; Daneman, 2009). It is unclear what triggers the autoimmune response but environmental factors as viral infections, toxins, psychosocial factors are though to play an important role (Akerblom and Knip, 1998). At least 20 genes of the major histocompatibility complex (MHC) are implicated in type 1 diabetes (Adorini *et al.*, 2002). Type 2 DM or non-insulin-dependent *diabetes mellitus* (NIDDM) is characterized as the progressive worsening of insulin resistance, hyperglycaemia (Adeghate *et al.*, 2006; Kleinert *et al.*, 2018) and lack of adequate compensation by pancreatic beta cells (Khan, 2003). There is a strong hereditary component, but obesity and a sedentary lifestyle play an important role in the development of this disease (Ali, 2013; King and Bowe, 2016). Insulin resistance is connected with decrease in insulin receptors and insulin receptor kinase activity, resulted in decreased glucose transporter 4 (GLUT4) translocation due to impaired signalling (Lencioni *et al.*, 2008). While T2DM is a multifactorial and complex disorder, it is clear, that obesity-induced insulin resistance accelerates pancreatic islet destruction and thus the onset of T2DM (Khan *et al.*, 2006). In T2DM overweight and obesity contribute to insulin resistance through several pathways, including an imbalance in the concentrations of hormones (increased leptin and glucagon, reduced adiponectin), increased

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concentrations of cytokines (tumour necrosis factor α, interleukin 6), suppressors of cytokine signalling, other inflammatory signals, and possibly retinol-binding protein (Wellen and Hotamisligil, 2005).

Glucose and insulin metabolism

Generally, hyperglycaemia is a primary factor in the onset of DM. It is unable to efficiently transport glucose from the blood into tissue. Thus, the measurement of plasma glucose level is important in diagnosis (Min and Park, 2010). The lipid profile of T2DM is defined by increased triglycerides level, decrease in high-density lipoproteins and increased very low-density lipoproteins (Therond, 2009). Insulin promotes anabolic processes and inhibits catabolic processes in pancreas, liver, skeletal muscle, adipose tissue and intestines. When glucose concentration exceeds the upper limit of normal range, glucokinase and glucose transporter 2 (GLUT 2) are activated in the pancreas followed by increasing intracellular ATP level. Consequently, ATP-sensitive K+ channels in the membrane of β-cells close, and the plasma membrane depolarizes what opens voltage-dependent Ca^{2+} channels. Then Ca^{2+} ions influx and induce exocytosis of insulin vesicles from pancreatic β-cells into portal circulation (Prentki, 1996). After reaching the liver, insulin stimulates glycogen and triglyceride synthesis, but inhibits glycogenolysis, ketogenesis and gluconeogenesis (Capeau, 2008). Higher insulin concentration suppresses hepatic glucose output and stimulates its uptake by the skeletal muscle and adipose tissue (Khan and Pessin, 2002). The dysfunction of insulin signalling in hepatocytes results in overall insulin resistance in the liver (Valverde *et al.*, 2003).

Insulin stimulates glucose uptake, protein and glycogen synthesis in the skeletal muscle, but inhibits protein degradation and glycogenolysis (Turcotte and Fisher, 2008). The dysfunction of insulin signalling pathways in the skeletal muscle is a factor in the diabetes progression (Assano *et al.*, 2007).

Adipose tissue is responsible for the glucose utilization. Adipocytes secrete pro-inflammatory cytokines as interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α) and anti-inflammatory cytokines (adiponectin) (Sowers, 2008). A reduced level of adiponectin and increase in IL-6 and TNF-α may induce or worsen insulin resistance in the adipose tissue. Dysfunction in the adipose tissue or adipocytes is associated with T2DM (Bluher, 2009).

Diabetes and its complications

Diabetic complications are acute (ketoacidosis, ketoacidic coma) and chronic (macrovascular, microvascular) (Min and Park, 2010). Macrovascular complications include mainly myocardial infarction, congestive cardiac failure and stroke. These complications account for more than 70 % of diabetic mortality (Hyvarinen *et al.*, 2009). Microvascular complications include diabetic neuropathy, nephropathy and retinopathy (Basit *et al.*, 2004). The most common diabetic complication is diabetic neuropathy (Basit *et al.*, 2004). It is characterized by progressive nerve fibre loss, clinical signs and symptoms as paraesthesia, pain, loss of sensation (Silva *et al.*, 2009). Diabetic retinopathy is a neurodegenerative state resting in structural and functional changes in retina cells (Silva *et al.*, 2009). Diabetic nephropathy is defined by superfluous accumulation of extracellular matrix with thickening of glomerular and tubular basement membranes and an increase in the mesangial matrix, which ultimately progresses to glomerulosclerosis and tubule-interstitial fibrosis (Kanwar *et al.*, 2008). T2DM is closely associated with obesity and it is the main pathological cause of insulin resistance (Khan and Flier, 2000). Abnormalities in other hormones, such as reduced secretion of the incretin glucagon-like peptide 1 (GLP-1), hyperglucagonaemia and raised concentrations of other counter-regulatory hormones, also contribute to insulin resistance, decreased insulin production and hyperglycaemia in T2DM (Stumvoll *et al.*, 2005; Kahn *et al.*, 2006).

Both, T1DM and T2DM ultimately lead to pancreatic β-cells dysfunction (Bonner-Weir *et al.*, 1983). They are associated with long-term complications raised after long exposure to elevated blood glucose concentration. The pathogenesis of the development of this complication can be often more important for the study than the manner in which the animals become hyperglycaemic (King and Bowe, 2016). The uncontrolled hyperglycaemia has harmful impacts on the organs that are pivotal in the homeostasis control and results in the development of diabetes (Fig. 1).

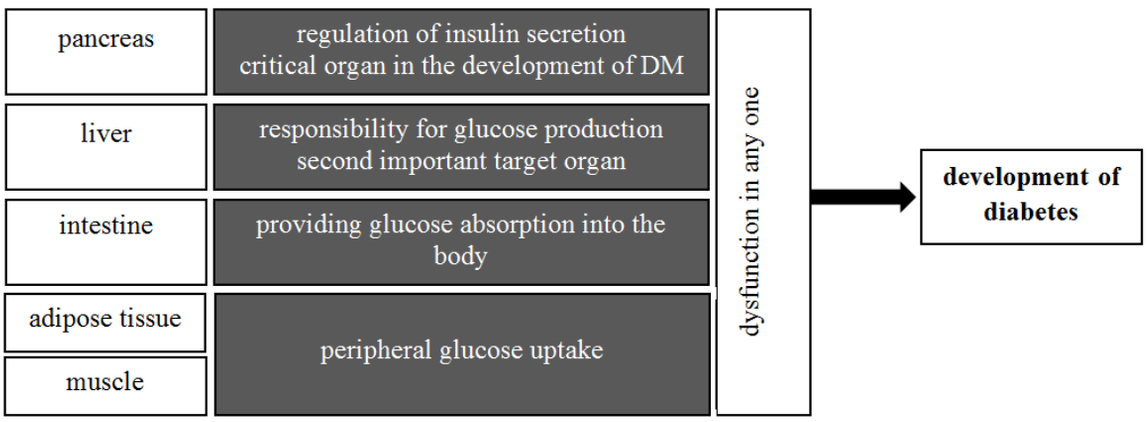

Figure 1. Target organs in development and treatment of DM
(Modified according to Min and Park, 2010)

Current therapeutic strategies for T2DM are limited and include insulin and oral antidiabetic agents that stimulate pancreatic secretion, reduce hepatic glucose production, delay digestion and absorption of intestinal carbohydrates or improve insulin action. These agents, however, suffer from inadequate efficacy and number of adverse effects (Bailey, 2005). In the scientific community the interest is raised to evaluate raw or isolated natural products used in the experimental diabetes study (Table 1). Natural supplements are widely used around the world to treat diabetes (Fröde and Medeiros, 2008).

Rodent animal models of *diabetes mellitus*

An animal model for biomedical research is one in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon is one or more respects resembles the same phenomenon in humans or other animal species (Chatzigeorgiou *et al.*, 2009). Diabetes research on humans is not possible, because provocation of DM is strictly impermissible. Therefore, animal models of DM are greatly useful and advantageous in biomedical studies. They promise new insights into human diabetes, new methods of treatment (Srinivasan and Ramarao, 2007) and the utility of therapeutic agents (Chen and Wang, 2005). The existing therapeutic approaches to treat *diabetes mellitus* and obesity, which are saving many lives every day, were discovered, validates and optimized on animal models (Kleinert *et al.*, 2018). There are many different animal models of diabetes available including spontaneous, induced and transgenic models (King and Bowe, 2016). Most appropriate model for diabetes research is rodent model. Rodents are easy to handle, small, economically effective and have a short generation interval (Min and Park, 2010). Animal models used for investigation of T1DM are: alloxane-induced, streptozotocin-induced, non-obese diabetic (NOD) mouse models, and bio-breeding (BB) rat model (Kim *et al.*, 1998). Alloxane, a uric acid derivate, which selectively destroys pancreatic β-cells through induction of oxidative stress, what causes insulin deficiency and hyperglycaemia (Rerup, 1970). A nitrosureas derivative isolated from *Streptomyces achromogenes* - streptozotocin (STZ) destroys pancreatic β-cells similarly as alloxane (Yamamoto *et al.*, 1981). Animal models, where the animals spontaneously develop T1DM are NOD mice and BB rats (Makino *et al.*, 1980). STZ is favoured over alloxane because it is more stable and less toxic (Kleinert *et al.*, 2018). Although both methods (alloxane and STZ) continue to be used in diabetes research, they are often criticized as not accurately reflecting the human T2DM phenotype. Thus, many investigators rely on specific rodent strains that model key features of T2D. These genetic models have been widely used to explore the pathophysiology of obesity and T2D, as well as in preclinical drug

development (Bedow and Samuel, 2012).

Rodent model for T2DM includes the genetically altered Zucker diabetic fatty (ZDF) rats, Otsuka Long Evans Tokushima fatty (OLETF) rats, Kuo Kondo (KK) mice, Goto Kakizaki (GK) rats, spontaneously diabetic Tori (SDT) rats, *ob*/*ob*$^{+}$/$^{+}$ mice, and *db*/*db*$^{+}$/$^{+}$ mice (Kim *et al.*, 1998). OLETF rats develop diabetes at around 18-25 weeks of age, mostly males. They suffer from polyphagia, mild obesity, hypertriglyceridemia, hyperinsulinemia and impaired glucose tolerance in 16 weeks of age (Kawano *et al.*, 1992). KK mice exhibit hyperphagia, insulin resistance and hyperinsulinemia. It is a polygenic model of obesity and T2DM (Reddi and Camerini-Davalos, 1988). The *ob*/*ob*$^{+}$/$^{+}$ mice are characteristic by a mutation in the leptin gene, manifested as obesity, hyperglycaemia, impaired glucose intolerance and hyperinsulinemia (Dubuc, 1976). The *db*/*db*$^{+}$/$^{+}$ mice have a leptin receptor mutation and are spontaneously hyperphagic, obese, hyperglycaemic, hyperinsulinemic and insulin resistant within the first month of life (Shariff, 1992). GK rat is a non-obese Wistar sub-strain, which develops type 2 *diabetes mellitus* early in life (Bedow and Samuel, 2012). SDT is inbred strain of Sprague-Dawley rat. Male SDT rats show high plasma glucose levels by 20 weeks, pancreatic islet histopathology, including haemorrhage in pancreatic islets and inflammatory cell infiltration with fibroblasts. Prior to the onset of diabetes, glucose intolerance with hypoinsulinemia is also observed (Sasase *et al.*, 2013). Generally, rats are more appropriate model when compared to the mice as many traits, the genetics and pathophysiology in rats has proven more relevant to human disease (Betz and Conway, 2016).

ZDF rats

In our laboratory we use the Zucker diabetic fatty rat (ZDF) as animal model for the research (Capcarova *et al.*, 2017; Kalafova *et al.*, 2017; Capcarova *et al.*, 2018). ZDF rat is commonly used as a model for the study of diabetes (Cefalu, 2006). ZDF rat was derived through selective breeding of hyperglycaemic obese Zucker rats. Zucker fatty (ZF) rats have spontaneous mutation “obese” (fatty) and it was found in the rat stock of Sherman and Merck, by Zucker, Harriet Bird Memorial Laboratory, Stow, Massachusetts, USA in 1961. ZF rats are resulted from the simple autosomal recessive (fa) gene on chromosome 5 (Srinivasan and Ramarao, 2007). These animals have a mutated leptin receptor leading to hyperphagia and obesity at 4 weeks of age (Philips *et al.*, 1996) along with increased growth of subcutaneous fat depot (Durham and Truett, 2006). It is associated with mild hyperglycaemia, insulin resistance, mild glucose intolerance, hyperlipidaemia, hyperinsulinemia and moderate hypertension (Durham and Truett, 2006). They have impaired glucose tolerance rather than apparently diabetes (Wang *et al.*, 2014). Consequently, a mutation in this strain leads to a sub-strain with an overtly diabetic phenotype - the Zucker diabetic fatty (ZDF) rats (Wang *et al.*, 2014). ZDF rats are less obese than ZF rats having a decreased beta cell mass leading to inability to compensate for severe insulin resistance (Pick *et al.*, 1998). ZDF rats carry an autosomal recessive defect in the β-cell transcription machinery that is inherited independently from the mutation in leptin receptor (*Lepr*). This animal model develops obesity with a severe diabetic syndrome, with sustained and early-onset hyperglycaemia and progression to β-cell death, hyperinsulinemia and premature death (Peterson *et al.*, 1990). ZDF rats appear to develop diabetes because of an inability to increase β-cell mass (Tomita *et al.*, 1992, Cefalu, 2006). This strain is highly useful for the investigation of mechanism of T2DM (Srinivasan and Ramarao, 2007).

There are sex differences in ZDF rats for phenotypes of diet-induced insulin resistance and glucose intolerance. Male rats are the most affected (Nadal-Casellas *et al.*, 2012). On normal chow diet, male ZDF rats develop severe hyperglycaemia and hypoinsulinemia by 4 month of age. Female ZDF rats maintain normal level of glucose and insulin throughout their life, despite developing obesity to a similar extent as the males (Kleinert *et al.*, 2018).

ZDF model is used for diabetic studies. There is no evidence or validation that a natural plant material can serve as a complete replacement for insulin. However, several plants and plant products have been reported to mimic the effect of insulin partially or enhance the effects of very low endogenous insulin concentrations (Eddouks *et al.*, 2012).

CONCLUSION

The investigations of *diabetes mellitus* have a long history. The prevalence of DM increased dramatically over the recent past, and therefore, the further research is required. Animal models for study of DM are needed to uncover and understand pathophysiology of the disease. This is the key to the development of new therapies and treatment. There are many various animal models simulating T1DM or T2DM, and each model is specific and has its own value. However, none of the models completely represents the pathophysiology of diabetes. The use of particular animal model depends on the study scheme.

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The 6th International Scientific Conference "Animal Biotechnology"

ANIMAL BIOTECHNOLOGY 2018

December 6th, 2018

NPPC – Research Institute for Animal Production Nitra
Hlohovecká 2, Lužianky, Slovak Republic
and
Slovak University of Agriculture Nitra - Faculty of Biotechnology and Food Science, Nitra, Slovak Republic

Dear Participants and Colleagues

We are very pleased to welcome you on the 6th International Scientific Conference "Animal Biotechnology 2018". The conference is organized by the Research Institute for Animal Production Nitra, National Agricultural and Food Centre in cooperation with the Faculty of Biotechnology and Food Science, Slovak University of Agriculture Nitra.

The aim of the conference is presentation of an actual research from the field of animal biotechnology, with a special focus on *Laboratory, farm and wild animals as a biological model*.

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

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

Organizing Committee of the Conference

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