

MONITORING GENETIC VARIATION IN A CONSERVATION FLOCK OF COLOURED MERINO SHEEP BASED ON BLOOD GROUP AND PROTEIN POLYMORPHISM*

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Abstract

The aim of the study was to determine the level of genetic variability in the population of Coloured Merino sheep based on blood group and protein polymorphism, and to determine changes that have occurred in the genetic structure of the breed over 5 years. The study involved 312 sheep of the Coloured Merino breed. Animals were allocated to three groups according to year of birth. Blood samples were analysed for erythrocyte antigens and genetic variants of haemoglobin (HB) and transferrin (TF). For each group of animals and for the entire population, calculation was made of allele frequency at individual loci, mean degree of heterozygosity (\bar{H}) and effective number of alleles (\bar{E}) per locus. Analysis of genetic equilibrium based on the HB and TF system was performed. The total number of alleles decreased over the 5 years from 56 to 48. These alleles were lost in the EAB blood group system, where they decreased from 34 to 26. It was also found that the mean effective number of alleles (\bar{E}) was the lowest in group III (2.51) compared to groups I (2.72) and II (2.88). The mean degree of heterozygosity (\bar{H}) decreased to 0.4347 in group I, to 0.4402 in group II, and to 0.4060 in group III. The most frequent allele in the most polymorphic EAB blood group system was B^c (0.2228), whereas blood protein loci were dominated by HBB^B allele (0.8285) at the haemoglobin locus and TF^D allele (0.4936) at the transferrin locus. The most frequent genotypes were BB at the HBB locus (0.6827) and CD at the TF locus (0.2885). \bar{E} and \bar{H} values for the entire population of Coloured Merino sheep (2.78 and 0.4282, respectively) are one of the lowest when compared to those calculated for the other Polish sheep breeds under protection. The lower genetic variation in the analysed population may be due to the small size of the foundation stock. The studied population was found to be in genetic equilibrium, possibly indicating that the breeding work is appropriately managed.

Key words: sheep, blood groups, protein polymorphism, genetic variation

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Many efforts have been made in recent years to protect endangered breeds and small populations in order to preserve the genetic diversity of farm animals (Ingrassia *et al.*, 2005). Attention is drawn to the fact that these small and often endangered populations are a source of unique genes (Ruane, 2000). One of the strategic priorities of the “Global Plan of Action for Animal Genetic Resources”, adopted in Interlaken in 2007, is to inventory and characterize animal genetic resources, and to monitor trends and risks associated with them (FAO, 2007).

Preservation of these small populations is conditional on developing a plan of breeding work based not only on the evaluation of phenotypic variation and breeding documentation, but also on genetic variation. The latter is evaluated based on either blood group and protein polymorphism or the polymorphism of selected DNA sequences (Tapio *et al.*, 2003; Słota *et al.*, 2007).

Preliminary research on blood group and protein polymorphism and selected DNA markers in the population of Coloured Merino sheep was performed in 2006 (Rychlik *et al.*, 2007 b).

The aim of the present study was to determine the current degree of genetic diversity in the Coloured Merino population based on blood group and protein polymorphism, and to determine changes that have occurred in the genetic structure of the breed over 5 years.

Material and methods

The study involved 312 sheep from a genetic reserve flock of Coloured Merino sheep at the Kołuda Wielka Experimental Station of the National Research Institute of Animal Production. The analysed population of sheep was divided into three groups according to the date of birth of the lambs:

Group I – lambs born during November and December 2005 (50 sheep), their mothers (41 sheep) and fathers (11 flock rams),

Group II – lambs born during November and December 2007 (66 sheep), their mothers (30 sheep) and fathers (10 flock rams),

Group III – lambs born in December 2009 (69 sheep), their mothers (30 sheep) and fathers (15 flock rams).

Blood samples were collected from these animals in the years 2006 to 2010 to determine erythrocyte antigens and polymorphic variants of transferrin (TF) and haemoglobin (HB).

Blood antigens were determined by haemolytic and agglutination tests using 16 standardized test sera: anti-Aa, Ab, Bb, Bc, Bd, Be, Bf, Bg, Bi, PLB-17, Ca, Cb, Da, Ma, R, and 0. These sera were obtained at the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production and checked in international comparison tests. Polymorphic variants of transferrin and haemoglobin were determined by horizontal starch gel electrophoresis.

The frequency of alleles at individual loci was calculated for all animal groups using the direct gene counting method. Significant differences between the groups

were determined by Chi-square analysis (Stratil, 1970) for alleles that occurred in all the three groups with at least 1% frequency in one of the groups. The degree of heterozygosity (Nei and Roychoudhury, 1974) and the effective number of alleles per locus (Kimura and Crow, 1964) were calculated for all animal groups. Based on the observed and expected number of haemoglobin (HBB) and transferrin (TF) genotypes calculated, genetic equilibrium was evaluated for each group of animals and for the entire population of Coloured Merino in accordance with the Hardy-Weinberg law. Data obtained from these three groups were used to calculate allele and genotype frequencies at the analysed loci, the mean effective number of alleles, and the mean degree of heterozygosity for the entire population of Coloured Merino sheep. In each group, the frequency of alleles at the analysed loci was calculated for flock rams, and the frequency of homozygous animals was calculated in the F1 generation (offspring).

Results

Results of the present study on differences in selected blood genetic markers in the analysed population of Coloured Merino sheep are presented in Tables 1–6.

Table 1. Comparison of frequency of blood group (EA), haemoglobin (HB) and transferrin (TF) alleles between investigated groups of sheep

Locus	Alleles	Frequency			Chi-square	Frequency – all investigated groups	
		Group I n = 102	Group II n = 106	Group III n = 104			
1	2	3	4	5	6	7	
EAA	a	0.2549	0.2453	0.1779	5.78	0.2260	
	ab	0.0294	0.0377	0.0192	4.66	0.0288	
	b	0.1373	0.0849	0.1731	7.23	*	0.1314
	-	0.5784	0.6321	0.6298	1.59		0.6138
EAB	b	0.0833	0.0896	0.1250	2.35	0.0994	
	bc	0.0294	0.0094	0.0625	9.21	**	0.0337
	bcegiPLB-17	0.0098	-	-	+		0.0032
	bcefi	0.0098	0.0047	0.0096	+		0.0080
	bcegiPLB-17	0.0147	-	-	+		0.0048
	bceiPLB-17	0.0245	0.0330	0.0096	2.69		0.0224
	bcePLB-17	0.0098	0.0094	0.0048	+		0.0080
	bdfg	0.0049	0.0047	0.0000	+		0.0032
	bdfgiPLB-17	0.0539	0.0330	0.0144	5.05		0.0337
	bdfgPLB-17	0.0098	0.0377	0.0096	5.79		0.0192
	bdg	0.0098	-	-	+		0.0032
	befgPLB-17	0.0049	0.0047	0.0144	1.52		0.0080
	befiPLB-17	0.0098	-	0.0096	+		0.0064
	befPLB-17	0.0147	-	-	+		0.0048
bfi	0.0147	0.0047	0.0192	1.78		0.0128	

Table 2 – contd.

1	2	3	4	5	6	7	
	bfiPLB-17	0.0098	0.0189	0.0048	2.23	0.0112	
	bfPLB-17	0.0098	0.0047	0.0048	+	0.0064	
	bg	0.0147	0.0142	0.0096	0.24	0.0128	
	bgiPLB-17	0.0098	0.0047	-	+	0.0048	
	bi	0.0588	0.0849	0.0769	1.07	0.0737	
	biPLB-17	0.1127	0.0943	0.0529	5.05	0.0865	
	c	0.2500	0.1698	0.2500	4.61	0.2228	
	cePLB-17	0.0049	0.0047	0.0144	1.70	0.0080	
	ciPLB-17	0.0049	0.0047	0.0337	8.49	*	0.0144
	cPLB-17	0.0637	0.0425	0.0577	0.97	0.0545	
	dfg	0.0098	0.0142	0.0096	0.29	0.0112	
	fgiPLB-17	0.0147	0.0519	0.0096	9.02	*	0.0256
	fi	0.0049	0.0047	-	+	0.0032	
	fPLB-17	0.0196	0.0519	0.0625	4.78	0.0449	
	g	0.0049	0.0377	0.0096	7.79	*	0.0176
	i	0.0147	0.0189	0.0481	5.24	0.0272	
	iPLB-17	0.0098	0.0142	0.0000	+	0.0080	
	PLB-17	0.0245	0.0330	0.0337	0.37	0.0304	
	-	0.0539	0.0991	0.0434	6.06	*	0.0657
EAC	a	0.0147	0.0283	0.0192	0.96	0.0208	
	ab	0.1225	0.1462	0.1010	1.99	0.1234	
	b	0.7942	0.7736	0.8077	0.75	0.7917	
	-	0.0686	0.0519	0.0721	0.83	0.0641	
EAD	a	0.6275	0.5943	0.6298	0.71	0.6170	
	-	0.3725	0.4057	0.3702	0.61	0.3830	
EAM	a	0.2794	0.2264	0.1731	6.53	*	0.2260
	-	0.7206	0.7736	0.8269	6.65	*	0.7740
EAR	R	0.8824	0.8491	0.8750	1.09	0.8686	
	0	0.1176	0.1509	0.1250	1.13	0.1314	
SI	I	0.9608	0.9717	0.9760	0.89	0.9696	
	i	0.0392	0.0283	0.0240	0.87	0.0304	
HBB	A	0.1569	0.2028	0.1538	2.26	0.1715	
	B	0.8431	0.7972	0.8462	2.22	0.8285	
TF	A	0.2304	0.2500	0.2115	0.88	0.2308	
	B	0.0343	0.0377	0.0144	2.41	0.0288	
	C	0.2794	0.2217	0.2404	1.94	0.2468	
	D	0.4559	0.4906	0.5337	2.53	0.4936	

*P<0.05; **P<0.01.

+ Chi-square not calculated.

Table 2. Frequency of blood group (EA), haemoglobin (HB) and transferrin (TF) alleles in the group of flock rams

Locus	Allele	Frequency			Total
		Group I n = 11	Group II n = 10	Group III n = 15	
1	2	3	4	5	6
EAA	a	0.1818	0.2000	0.1000	0.1528
	ab	-	0.0500	-	0.0139
	b	0.2273	0.1500	0.3333	0.2500
	-	0.5909	0.6000	0.5667	0.5833
EAB	b	0.1364	0.1000	0.2000	0.1528
	bcgiPLB-17	0.0454	-	-	0.0139
	bciPLB-17	0.0451	0.0500	0.0333	0.0417
	bdfgiPLB-17	-	0.0500	0.0333	0.0278
	befPLB-17	-	-	0.0333	0.0139
	bfi	0.0454	0.0500	-	0.0278
	bfiPLB-17	0.0454	0.0500	-	0.0278
	biPLB-17	0.1364	0.1500	0.1000	0.1250
	c	0.3182	0.2500	0.0667	0.1944
	ce	-	-	0.0333	0.0139
	ciPLB-17	-	-	0.0333	0.0139
	cPLB-17	-	0.0500	0.2000	0.0972
	dfg	0.0454	-	-	0.0139
	fgiPLB-17	0.0454	0.0500	0.0333	0.0417
	fPLB-17	-	0.0500	0.0667	0.0417
	g	0.0454	-	-	0.0139
	i	0.0454	0.0500	0.0333	0.0417
iPLB-17	-	0.0500	-	0.0139	
PLB-17	-	0.0500	0.0667	0.0417	
-	0.0454	-	0.0667	0.0417	
EAC	a	-	-	0.0333	0.0139
	ab	0.0454	0.1000	0.1667	0.1111
	b	0.9092	0.9000	0.7333	0.8333
	-	0.0540	-	0.0667	0.0417
EAD	a	0.6818	0.4500	0.5333	0.5556
	-	0.0454	-	0.0667	0.4444
EAM	a	0.0909	0.1000	0.1333	0.1111
	-	0.9091	0.9000	0.8667	0.8889
EAR	R	1.0000	0.9000	0.9333	0.9444
	-	-	0.1000	0.0667	0.0556
EASI	I	0.8182	0.9500	0.9667	0.9166
	i	0.1818	0.0500	0.0333	0.0834

Table 2 – contd.

1	2	3	4	5	6
HBB	A	0.2273	0.2000	0.2666	0.2361
	B	0.7727	0.8000	0.7334	0.7639
TF	A	0.1818	0.3000	0.1667	0.2083
	B	-	-	0.0333	0.0139
	C	0.2273	0.2000	0.3000	0.2500
	D	0.5909	0.5000	0.5000	0.5278

Table 3. Frequency of haemoglobin (HB) and transferrin (TF) genotypes in investigated groups of sheep

Locus	Genotype	Frequency			Total population investigated
		Group I	Group II	Group III	
HBB	AA	0.0294	0.0283	0.0192	0.0256
	AB	0.2549	0.3491	0.2692	0.2917
	BB	0.7157	0.6226	0.7116	0.6827
TF	AA	0.0490	0.0377	0.0289	0.0385
	AB	0.0000	0.0189	0.0000	0.0064
	AC	0.1667	0.1132	0.0962	0.1250
	AD	0.1961	0.2925	0.2692	0.2532
	BC	0.0000	0.0377	0.0096	0.0160
	BD	0.0686	0.0189	0.0192	0.0353
	CC	0.0490	0.0189	0.0288	0.0321
	CD	0.2941	0.2547	0.3173	0.2885
	DD	0.1765	0.2075	0.2308	0.2051

Tables 1 and 2 compare the frequency of blood group alleles in the A, B, C, D, M, R and SI systems and the frequency of the alleles of blood plasma proteins (transferrin) and erythrocytes (haemoglobin) in the analysed groups of animals and flock rams.

In the A blood group system, A⁻ allele was the most frequent in all three groups of animals (0.5784, 0.6321 and 0.6298 in groups I, II and III, respectively; 0.6138 in the whole population). Statistically significant ($P < 0.05$) differences were established between the groups in A^b allele frequency.

A total of 34 alleles were found in the B system, with the most frequent allele in all animal groups being B^c (0.2500, 0.1698 and 0.2500 in groups I, II and III, respectively; 0.2228 in the whole population). High frequencies (above 5%) were also noted for alleles B^{bi} (0.0588), B^{biPLB-17} (0.1127) and B⁻ (0.0539) in group I; B^{bi} (0.0849), B^{biPLB-17} (0.0943), B^{figiPLB-17} (0.0519), B^{fiPLB-17} (0.0519) and B⁻ (0.0991) in group II; and B^{bi} (0.0769), B^{biPLB-17} (0.0529) and B^{fiPLB-17} (0.0625) in group III. In addition, there were significant differences in the B system in the frequency of 5 alleles, i.e. B^{bc} ($P < 0.01$), B^{ciPLB-17}, B^{figiPLB-17}, B^g and B⁻ ($P < 0.05$).

Table 4. Frequency of homozygous genotypes in F1 generation

Locus	Genotype	Frequency			Total F ₁ generation
		Group I n = 50	Group II n = 66	Group III n = 69	
A	a/a	0.1800	0.1060	0.0289	0.0973
	b/b	0.0200	0.0151	-	0.0108
	-/-	0.3600	0.6969	0.3043	0.4594
B	b/b	0.0200	0.0151	0.0290	0.0216
	c/c	0.0400	0.0151	0.0435	0.0324
	-/-	-	0.0454	-	0.0162
C	a/a	-	-	-	-
	ab/ab	0.2000	0.0151	0.0289	0.0216
	b/b	0.6600	0.6364	0.6956	0.6649
	-/-	0.0800	0.0454	0.0145	0.0432
D	a/a	0.7600	0.4394	0.4348	0.5243
	-/-	0.1200	0.3939	0.1304	0.2216
M	a/a	0.2600	0.1060	0.0580	0.1297
	-/-	0.6400	0.8030	0.6522	0.7027
R	R/R	0.9000	0.8485	0.8985	0.8811
	O/O	0.0800	0.1515	0.1015	0.1135
	-/-	0.0200	-	-	0.0054
HBB	A/A	0.0200	-	-	0.0054
	B/B	0.7600	0.6363	0.5507	0.6378
TF	A/A	0.0200	0.0303	0.0145	0.0216
	C/C	0.0600	0.0151	0.0145	0.027
	D/D	0.1800	0.1969	0.2319	0.2054

In the other blood group systems, i.e. C, D, M, R and SI significant ($P < 0.05$) differences in allele frequency were found for the M system.

The most frequent of the two alleles observed at the haemoglobin locus was HBB^B allele (0.8431, 0.7972 and 0.8462 in groups I, II and III, respectively; 0.8285 in the whole population). The most frequent of the 4 transferrin alleles identified was TF^D allele (0.4559, 0.4906 and 0.5337 in groups I, II and III, respectively; 0.4936 in the whole population). In the analysed protein systems, there were no statistically significant differences in allele frequency.

The most frequent alleles at the analysed loci in the whole population of sheep studied were also the most frequent in the group of flock rams (Table 2).

Table 3 shows the frequency of haemoglobin and transferrin genotypes. In the sheep groups under study, the most frequent genotypes were BB at the HBB locus (0.7157, 0.6226 and 0.7116 in groups I, II and III, respectively; 0.6827 in the whole population) and CD (0.2941, 0.2547 and 0.3173 in groups I, II and III, respectively; 0.2885 in the whole population) at the TF locus.

Table 5. Number of alleles (N), effective number of alleles (E) and degree of heterozygosity (hk) in the investigated groups of sheep

System	Group I			Group II			Group III			Total population investigated		
	N	E	h _k	N	E	h _k	N	E	h _k	N	E	h _k
EAA	4	2.38	0.5808	4	2.13	0.5316	4	2.18	0.5414	4	2.24	0.5541
EAB	34	10.44	0.9043	29	12.03	0.9169	26	9.44	0.8941	34	11.26	0.9112
EAC	4	1.53	0.3495	4	1.60	0.3768	4	1.49	0.3318	4	1.55	0.3535
EAD	2	1.87	0.4674	2	1.93	0.4822	2	1.87	0.4664	2	1.90	0.4726
EAM	2	1.67	0.4026	2	1.54	0.3504	2	1.39	0.2816	2	1.54	0.3498
EAR	2	1.26	0.2076	2	1.34	0.2791	2	1.28	0.2188	2	1.30	0.2283
EASI	2	1.08	0.0754	2	1.05	0.0550	2	1.05	0.0468	2	1.06	0.0590
HBB	2	1.35	0.2646	2	1.48	0.3240	2	1.35	0.2603	2	1.40	0.2841
TF	4	2.93	0.6598	2	2.82	0.6462	4	2.58	0.6125	4	2.79	0.6414
Total	56			51			48			56		
\bar{E}		2.72			2.88			2.51			2.78	
\bar{h}_k			0.4347			0.4402			0.4060			0.4282

Table 6. Observed and expected distributions of haemoglobin (HB) and transferrin (TF) genotypes in the investigated population of Coloured Merino

Locus	Genotype	Group I			Group II			Group III			Entire population investigated		
		observed genotype distribution	expected genotype distribution	chi-square	observed genotype distribution	expected genotype distribution	chi-square	observed genotype distribution	expected genotype distribution	chi-square	observed genotype distribution	expected genotype distribution	chi-square
HBB	AA	3	2.5	0.14	3	4.4	0.68	2	2.5	0.13	8	9.2	0.22
	AB	26	27.0		37	34.3		28	27.1		91	88.7	
	BB	73	72.5		66	67.3		74	74.4		213	214.1	
TF	AA	5	5.4	11.63	4	6.6	8.71	3	4.6	6.36	12	16.6	12.88
	AB	0	1.6		2	2.0		0	0.6		2	4.1	
	AC	17	13.1		12	11.7		10	10.6		39	35.6	
	AD	20	21.5		31	26.0		28	23.5		79	71.2	
	BC	0	2.0		4	1.8		1	0.7		5	4.4	
	BD	7	3.2		2	3.9		2	1.6		11	8.9	
	CC	5	8.0		2	5.2		3	6.0		10	19.0	
	CD	30	26.0		27	23.2		33	26.7		90	76.1	
	DD	18	21.2		22	25.6		24	29.7		64	76.1	

The frequency of homozygous genotypes at the blood group and protein loci of the F1 generation is presented in Table 4. The following genotypes were the most frequent: -/- in the A system, c/c in the B system, b/b in the C system, a/a in the D system, -/- in the M system, and R/R in the R system. The most frequent homozygous genotypes were B/B at the haemoglobin locus and D/D at the transferrin locus.

The mean values of both the effective number of alleles (\bar{E}) and the degree of heterozygosity (\bar{H}) were the highest in group II (2.88 and 0.4402, respectively) (Table 5). In the whole population analysed, these values were $\bar{E} = 2.78$ and $\bar{H} = 0.4282$.

Analysis of genetic equilibrium was performed on the basis of HBB and TF systems (Table 6). No significant differences between the observed and expected number of genotypes were found in any of the systems.

Discussion

The Coloured Merino line, created at the Koluda Wielka Experimental Station of the National Research Institute of Animal Production (Osikowski and Pakulski, 1992), is the only population of this Merino type in Poland, with just about 100 ewes of the foundation stock. This line is one of several lines of sheep raised in Poland to have been included in the genetic resources conservation programme by the Ministry of Agriculture and Rural Development. Because of the dwindling population of sheep and long-term selection work involving the elimination of coloured animals and their parents from pedigree breeding, there is little scope to obtain coloured rams for mating from their random births in "white" Merino flocks. Such populations run a high risk of increasing genetic relatedness and unification (Norberg and Sørensen, 2007). It appears appropriate, therefore, to find possible ways of prevention through research.

To ensure breeding progress, it is necessary to record genetic variation, which is estimated using the total number of alleles (N), the effective number of alleles (E), and the degree of heterozygosity (h_k) within breeds. High values of these parameters are indicative of greater genetic diversity of a given breed.

The total number of alleles, which in the analysed population amounted to 56 in group I, decreased over 5 years to 48 (group III). Alleles were lost in the most polymorphic blood group system (EAB), decreasing from 34 in group I, to 29 in group II and to 26 in group III. Four alleles, i.e. $B^{bcgiPLB-17}$, $B^{bcgiPLB-17}$, B^{bdg} and $B^{befPLB-17}$ occurred only in group I, and another four alleles (B^{bdfig} , $B^{bgiPLB-17}$, B^{fi} and $B^{iPLB-17}$) were found only in groups I and II.

It was also demonstrated that the mean effective number of alleles (\bar{E}) was the lowest in group III (2.51) compared to groups I (2.72) and II (2.88). There was also a decrease in the mean degree of heterozygosity (\bar{H}), from 0.4347 in group I, to 0.4402 in group II and to 0.4060 in group III.

The mean effective number of alleles (\bar{E}) calculated for the entire population of Coloured Merino ($\bar{E} = 2.78$) is the lowest among all the native Polish breeds of sheep

that have been investigated to date, i.e. Olkuska (4.44) (Rychlik et al., 1997), Kamienniecka (3.81) (Kaczor and Rychlik, 2004), Corriedale (3.44) (Kaczor and Rychlik, 2005), Wrzosówka (2.90) (Rychlik et al., 2007 a), Świniarka (3.37) (Rychlik et al., 2009) and Coloured Polish Mountain Sheep (3.32) (Rychlik and Krawczyk, 2009).

The mean effective degree of heterozygosity (\bar{H}) for all the animal groups studied was $\bar{H} = 0.4282$. It was lower than 0.5, a value considered the most beneficial in terms of genetic diversity of a given population.

The mean degree of heterozygosity (\bar{H}) calculated for the Coloured Merino was slightly higher than the value obtained for the Corriedale breed (0.414) (Kaczor and Rychlik, 2005). The other native sheep breeds that had been studied previously were characterized by a higher mean degree of heterozygosity: 0.594 in Olkuska sheep (Rychlik et al., 1997); 0.537 in Kamienniecka sheep (Kaczor and Rychlik, 2004); 0.495 in Wrzosówka (Rychlik et al., 2007 a); 0.4608 in Świniarka (Rychlik et al., 2009); and 0.5440 in Coloured Polish Mountain Sheep (Rychlik and Krawczyk, 2009).

In the sheep population under study, no discrepancy was found between the expected and observed number of HBB and TF genotypes, thus indicating that it is in genetic equilibrium. This may indicate that breeding work is appropriately managed and rams are properly selected for reproduction from the small population available. The study showed that selection of flock rams has a clear effect on the genotypes and on the frequency of blood group and protein alleles in the offspring.

On the other hand, the decrease in the number of alleles, the low effective number of alleles and the low heterozygosity all point to decreasing genetic variability in the Coloured Merino population. This may be due to the small size of the foundation flock, which runs a high risk of inbreeding.

References

- FAO (2007). Interlaken Declaration on Animal Genetic Resources. Annex 1. The First International Technical Conference on Animal Genetic Resources for Food and Agriculture. 3–7 September, Interlaken, Switzerland.
- Ingrassia A., Martyniuk E., Manzella D. (2005). The legal framework for the management of animal genetic resources. *FAO Legislative Study*, 89: 2–6.
- Kaczor U., Rychlik T. (2004). The immunogenetic characteristics of the Kamienniecka sheep variety of Polish Longwool sheep. *Scientific Messenger of Lviv National Academy of Veterinary Medicine named after S. Z. Gzhytskyj*, 6 (2): 114–119.
- Kaczor U., Rychlik T. (2005). Evaluation of differences in the genetic structure of Corriedale and Polish Lowland sheep. *Scientific Messenger of Lviv National Academy of Veterinary Medicine named after S. Z. Gzhytskyj*, 6 (2): 111–118.
- Kimura M., Crow J.F. (1964). The number of alleles that can be maintained in finite population. *Genetics*, 49: 725–738.
- Nei M., Roychoudhury A.K. (1974). Sampling variances of heterozygosity and genetic distance. *Genetics*, 76: 379–390.
- Norberg E., Sørensen A.C. (2007). Inbreeding trend and inbreeding depression in the Danish populations of Texel, Shropshire and Oxford Down. *J. Anim. Sci.*, 85: 299–304.
- Osikowski M., Pakulski T. (1992). Wstępne wyniki badań nad wytworzeniem linii barwnego merynosa. *Rocz. Nauk. Zoot.*, 31: 107–126.

- Ruane J. (2000). A framework for prioritizing domestic animal breeds for conservation purposes at the national level: a Norwegian case study. *Conserv. Biol.*, 14 (5): 1385–1393.
- Rychlik T., Kaczor U., Wierzchoś E., Marchwica E. (1997). Characteristics of populations of prolific Olkuska sheep and selected sheep breeds with regard to blood groups and polymorphism of hemoglobin and transferrin. *Rocz. Nauk. Zoot.*, 24: 23–34.
- Rychlik T., Krawczyk A. (2009). Class I marker polymorphism in Polish Mountain sheep of coloured and white varieties. *Ann. Anim. Sci.*, 9 (4): 383–391.
- Rychlik T., Krawczyk A., Sikora J. (2007 a). Blood group and blood protein polymorphism in a conservation flock of Wrzosówka sheep. *Ann. Anim. Sci.*, 2: 227–235.
- Rychlik T., Natonek-Wiśniewska M., Pakulski T. (2007 b). Characteristics of the genetic structure of a Coloured Merino genetic reserve flock based on the polymorphism of class I and II genetic markers. *Ann. Anim. Sci., Suppl.*, 1:59–62.
- Rychlik T., Radko A., Krawczyk A. (2009). Preliminary evaluation of the genetic structure of Świniarka sheep based on blood groups and polymorphic protein variants. *Ann. Anim. Sci.*, 9 (1): 35–42.
- Słota E., Rejduch B., Bugno M., Rychlik T., Ząbek T. (2007). Characterization of animal genetic resources using molecular genetics methods. *Ann. Anim. Sci. Suppl.*, 1: 33–44.
- Stratil A. (1970). Genetic polymorphisms of proteins in different breeds and different populations of chickens. *Anim. Blood Groups Biochem. Genet.*, 1: 117–122.
- Tapio M., Miceikienė I., Vilkki J., Kantanen J. (2003). Comparison of microsatellite and blood protein diversity in sheep: inconsistencies in fragmented breeds. *Mol. Ecol.*, 12: 2045–2056.

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**Monitorowanie zmienności genetycznej w stadzie zachowawczym merynosa barwnego
na podstawie polimorfizmu grup i białek krwi**

STRESZCZENIE

Celem badań było określenie stopnia zróżnicowania genetycznego populacji merynosa barwnego na podstawie polimorfizmu grup i białek krwi oraz określenie zmian, jakie zaszły na przestrzeni 5 lat w strukturze genetycznej tej rasy. Badaniami objęto 312 owiec rasy merynos barwny. Zwierzęta podzielono na trzy grupy, obejmujące ściśle określone lata. W próbkach krwi oznaczono antygeny erytrocytarne oraz genetyczne warianty HBB i TF. Dla każdej z grup zwierząt, jak również dla całej badanej populacji wyliczono częstości występowania alleli w poszczególnych loci, średnie wartości stopnia heterozygotyczności (\bar{H}) i efektywnej liczby alleli (\bar{E}) w locus oraz przeprowadzono analizę równowagi genetycznej opartej na układzie HBB i TF. Ogólna liczba alleli zmalała w ciągu 5 lat z 56 do 48. Ubytek alleli nastąpił w układzie krwi EAB z 34 do 26. Wykazano ponadto, że średnia wartość efektywnej liczby alleli (\bar{E}) była najniższa w grupie III (2,51), w porównaniu do wartości tego parametru w grupie I (2,72) i w grupie II (2,88). Zmniejszeniu uległy także średnie wartości stopnia heterozygotyczności (\bar{H}) z 0,4347 w grupie I, poprzez 0,4402 w grupie II, do 0,4060 w grupie III. Najczęstszym allelem w najbardziej polimorficznym układzie grupowym EAB był B^c (0,2228), natomiast w loci białek krwi przeważały allele: HBB^B (0,8285) w locus hemoglobiny i TF^D (0,4936) w locus transferyny. Najczęstszym genotypem hemoglobiny był BB (0,6827), a w locus TF – CD (0,2885). Wartości \bar{E} i \bar{H} dla całej populacji merynosa barwnego wyniosły odpowiednio 2,78 i 0,4282 i są jednymi z najniższych w porównaniu do tych parametrów wyliczonych w innych chronionych polskich rasach owiec. Mniejsze zróżnicowanie genetyczne badanej populacji owiec może być spowodowane małą liczebnością stada podstawowego. Wykazano natomiast, że badana populacja znajduje się w stanie równowagi genetycznej, co może świadczyć o dobrze prowadzonej pracy hodowlanej.