

Influence of PrP genotype on selected meat traits of Polish Heath Sheep*

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The research was conducted on 690 lambs (338 ♀, 352 ♂) of the Polish Heath Sheep breed. Samples of blood were collected from the jugular vein into plastic tubes with EDTA. Extracted DNA was used to determine scrapie genotypes. Lambs were weighed at birth and on days 28, 56, 70 and 100 of life, and daily weight gains in those intervals were estimated. After slaughter, carcass traits (carcass value, leg measurement, carcass cuts composition and tissue composition) and meat quality traits were determined. The results showed no effect of scrapie genotype on growth and development traits. Among carcass value traits, genotype affected only carcass fat colour, and among meat quality traits, only dry matter content. The results demonstrate the possibility of conducting breeding work aimed at increasing genetic resistance to scrapie without affecting the level of production traits of Polish Heath Sheep.

KEY WORDS: Polish Heath Sheep / scrapie / genotype / meat traits

Transmissible Spongiform Encephalopathies (TSEs) are degenerative brain diseases induced by prions. After bovine spongiform encephalopathy was diagnosed in cattle in the 1980s, the causes of prion diseases were thoroughly investigated and programmes for preventing and controlling them in individual species were developed.

The prion disease that attacks sheep and goats is scrapie, which is induced by the prion protein encoded by the gene *PRNP*, located on the 13th chromosome in cattle, sheep and goats [29]. This protein occurs in cells in a natural form (PrP^C), while its pathogenic form (PrP^{Sc}) is responsible for the disease [14]. Research has identified numerous polymorphisms in the *PRNP* gene, and three of these (in codons 136, 154 and 171) have been closely linked to susceptibility or resistance to the classical form of scrapie. The occurrence of the ARR allele has been shown to determine a high level of

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genetic resistance, while the VRQ allele determines the highest susceptibility to scrapie [34]. An F/L polymorphism in codon 141 has been recognized as a determinant of the atypical variant of scrapie [10].

Issues related to the problem of prion diseases, including scrapie in sheep, have been legally regulated in the European Union. A regulation of the European Parliament [26] has defined principles for the prevention and control of prion diseases. In 2003 a Commission Decision [8] introduced the obligation to conduct selection for increased resistance to scrapie in all European breeds of sheep. Also in 2003, Regulation no. 260/2003 [25] was issued, concerning control of TSEs in sheep and goats and principles governing trade in live sheep and goats and bovine embryos.

Many researchers have studied the relationship between the scrapie genotype and performance characteristics in various sheep breeds [1, 2, 9, 13, 22]. In Poland work in this area has been conducted only in regard to reproductive traits [12, 35]. At the Agricultural Experimental Station of the Warsaw University of Life Sciences in Żelazna, selection work was conducted in a herd of Polish Heath sheep in order to increase the level of genetic resistance to scrapie. In 2009-2011 an increase in the frequency of favourable genotypes was obtained in a group of ewes, while eliminating or reducing the occurrence of determinants conducive to scrapie (ARQ/AHQ and AHQ/AHQ). Following the identification of three genotypes in a group of breeding rams in 2009 (ARR/ARR, ARR/ARQ and ARR/AHQ), in 2010 the ARR/AHQ genotype was eliminated, and a year later a state of 100% frequency of the ARR/ARR genotype was achieved [16].

Due to the legal obligation to conduct selection aimed at increasing genetic resistance to scrapie in sheep, the question arose of how selection of animals with suitable genotypes for breeding affects the level of their performance traits in flocks, including those associated with meat content. The aim of the study was to attempt to determine the relationship between the scrapie genotype and selected performance characteristics of Polish Heath sheep, as well as the effect of breeding work based on elimination of animals with undesirable genotypes on meat characteristics in this breed.

Material and methods

A study evaluating body weight gain and development was conducted on a group of 690 lambs (338 ♀, 352 ♂) of the Polish Heath breed, born in 2009-2011, from a flock kept on the Experimental Sheep and Goat Farm of the Agricultural Experimental Station of the Warsaw University of Life Sciences in Żelazna. From this population 66 male lambs were selected for slaughter (selection criteria included the formation of genotype groups). After slaughter the carcasses were classified according to the EUROP system. As one of the groups was too small (5 lambs with genotype ALRQ/ALHQ), due to a lack of suitable material in the third year of the experiment, detailed carcass analysis was performed on a group of 61 lambs. The lambs were fed according to dietary standards [18] using on-farm feed (meadow hay, oat and barley meal, and triticale).

Blood was collected from the jugular vein of the lambs into syringes containing EDTA as an anticoagulant. DNA was extracted from the samples. Genetic material

was isolated from the leukocytes using isolation kits from A&A Biotechnology (www.aabiot.com), which employ the method of chromatography on minicolumns containing special silica membranes. The extracted DNA was used to determine scrapie genotypes. The genotype was determined by the KASP® system (www.lgcgenomics.com), which employs the single nucleotide polymorphism (SNP) method, using the primers presented in Table 1.

Table 1
Primers and SNP sites of the prion protein locus

Locus	Primers 3' - 5'	SNP	Changes	Localization
<i>PrP</i> prion protein	CACAGTCAGTGGAACAAGCC/ CTTGCCAGGTTGGGG	AY909542:g.385A>G	A/G	exon 3
		AY909542:g.386G>T	G/T	exon 3
		AY909542:g.479C>T	C/T	exon 3
		AY909542:g.493C>T	C/T	exon 3
		AY909542:g.534G>A	G/A	exon 3

To evaluate body weight development the lambs were weighed at birth and on days 28, 56, 70 and 100 of life. Then daily weight gains were calculated for the following periods: 0-28 days, 0-56 days, 0-70 days, 0-100 days, 28-56 days, 28-70 days, 28-100 days, 56-70 days, 56-100 and 70-100 days.

A group of 66 male lambs was selected for carcass analyses. The lambs were slaughtered when they had attained a body weight of about 40 kg. The carcasses were refrigerated for 24 h at 4°C. Then the pH of the meat was measured using a Cp-411 pH meter with a spear tip electrode. The carcasses were weighed and the data were used to calculate the dressing percentage. This was followed by subjective evaluation of the carcasses according to the EUROP classification, determining the following: meat content (E, U, R, O, P), fat content (1, 2, 3, 4, 5), fat colour (white or coloured) and fat consistency (very soft, soft, cohesive, very cohesive). Further analysis, as mentioned above, was conducted on a group of 61 individuals. The following measurements were made of the carcasses: spread of the hock joint, leg length, leg depth, leg circumference, and leg index [15].

The carcasses were divided into half-carcasses and the left half-carcass was used for further analysis. It was weighed and divided into the following cuts [15]: neck, chuck, rack, loin, tenderloin, leg, shoulder, kidney with flare fat, flank steak with brisket and ribs, foreshank, and hindshank. The weight and percentage of the cuts in the carcass were calculated, as well as the total weight of the prime cuts, i.e. the tenderloin, rack, loin, and leg. After the half-carcass had been divided into cuts the 'eye' of the tenderloin was measured, i.e. the cross section of the *longissimus dorsi* muscle (*mld*). Measurements of the loin made with an electronic calliper included the width and length of the cross section of the *mld* and the thickness of the fat layer over this muscle. The contour of the cross section of the muscle was used to determine its area [15]. The final element of the evaluation performed on the *mld* was measurement of the colour of the meat, using a Konica-Minolta CR-400

colorimeter, taking into account the components L^* , a^* and b^* [30]. From the site where these measurements were made a meat sample was collected for further analysis. The evaluation of carcass characteristics also included dissection of the leg, which was used to determine its tissue composition [15].

Laboratory analysis of the meat samples included determination of dry matter content by the oven-dry method, according to PN-ISO 1442 [20]; water holding capacity by the method of Grau and Hamma [11] as modified by Pohja and Niinivaara [21]; protein content in the meat by the Kjeldahl method; and fat content by the Soxhlet method. The fatty acid profile in the meat was determined using the method described by R6se-Gottlieb [3], by gas chromatography (Agilent 7890A gas chromatograph, Varian Select FAME column: length 100 m, inner diameter 0.25 mm, polar liquid phase film thickness 0.25 μm), according to Polish standard PN-EN ISO 5508 [19].

Statistical calculations were performed using the IBM SPSS Statistics 21.0 software package. The effect of scrapie genotype on growth characteristics in the lambs was evaluated by multi-factor analysis of variance with a model taking into account genotype, year of birth, and type of birth. Correlations between genotype and carcass characteristics were determined by analysis of covariance, taking into account genotype, year of birth, and type of birth, with body weight at slaughter as the concomitant variable. The occurrence of links between carcass evaluation characteristics according to the EUROP classification (meat content and fat cover, fat consistency, and fat colour) and the scrapie genotype were determined using the chi-square test, creating contingency tables [27].

Results and discussion

Five scrapie genotypes were identified in the group of lambs: ALRR/ALRR, ALRR/ALRQ, ALRR/ALHQ, ALRQ/ALRQ and ALRQ/ALHQ (Tab. 2). The ALRR/ALRQ genotype was most frequently represented, identified in 343 of 690 individuals (49.71%). The ALRR/ALRR genotype was identified in 192 lambs, while the ALRQ/ALRQ genotype was noted in only 30 individuals (4.35%). The sex distribution was similar in all genotype groups. The lambs were mainly from twin or triplet births.

Table 2

Size of genotype groups determined in the study population, taking into account sex and type of birth of lambs

Genotype	Sex			Type of birth			
	♀	♂	total	singles	twins	triplets	total
ALRR/ALRR	90	102	192	15	127	50	192
ALRR/ALRQ	173	170	343	29	222	92	343
ALRR/ALHQ	40	48	88	11	59	18	88
ALRQ/ALRQ	13	17	30	3	21	6	30
ALRQ/ALHQ	22	15	37	6	21	10	37
Total	338	352	690	64	450	176	690

Table 3 presents data concerning the genotype and type of birth of the male lambs selected for detailed carcass analysis.

Table 3

Size of genotype groups in relation to type of birth of rams selected for slaughter analysis

Genotype	Type of birth			
	singles	twins	triplets	total
ALRR/ALRR	3	9	6	18
ALRR/ALRQ	2	15	1	18
ALRR/ALHQ	1	15	3	19
ALRQ/ALRQ	0	6	0	6
Total	6	45	10	61

The mean body weight of the lambs at birth was 2.61 (± 0.03) kg and had increased to 16.96 (± 0.25) kg after 100 days. The mean daily weight gain during this period was 0.143 (± 0.002) kg (Tab. 4). The body weight of the lambs and their daily weight gain proved to be independent of the scrapie genotype.

The body weight of the lambs before slaughter was 38.9 (± 1.9) kg (Tab. 5). Analysis of the carcass evaluation according to the EUROP system showed a correlation between fat colour and the scrapie genotype ($\chi^2 = 12.628$; $P \leq 0.05$). In three of the five genotype groups carcasses with white fat were clearly predominant. In the case of lambs with the ALRR/ALHQ genotype the division was more even: 11 carcasses with white fat and 8 with coloured fat. Only the carcasses of the ALRR/ALRQ lambs were more frequently had coloured fat. Overall the fat colour was defined as 'white' on 40 carcasses and 'coloured' on 26.

The remaining components of the EUROP evaluation proved to be independent of the scrapie genotype. In the meatiness evaluation, 32 carcasses were assigned to class R, 19 to class O, 14 to class U and one to class P. In terms of fat cover, the carcasses were classified as 1, 2 and 3 (23, 42 and 1 carcass, respectively). Soft fat was observed on 34 carcasses, cohesive fat on 26, and very fat and very cohesive on three carcasses each.

The dressing percentage was 38.17% (± 0.52) and the carcass weight was 14.82 kg (± 0.20) – Table 6. This result is similar to values previously obtained for lambs of this breed [7, 17]. The scrapie genotype did not affect the weight of individual cuts or their percentage in the carcass, or the content of tissues (meat, fat and bone) in the leg. The lack of correlation confirms the results of an experiment by Sawalha et al. [28] evaluating the effect of scrapie genotype on the tissue composition of the carcasses of Scottish Blackface lambs on the basis of computer tomography.

The values for the physical characteristics of the meat proved to be independent of scrapie genotype (Tab. 7). The pH of the meat was 5.90 (± 0.08). Measurement of meat colour confirmed previous results [7]. The value for the L^* component (34.81) indicates

Table 4
Effect of the study factors on body weight and daily weight gain in lambs (n=690)

Trait	Factor				\bar{x}	SE
	genotype	sex	type of birth	year of birth		
Body weight:						
birth weight	NS	**	**	**	2.61	0.03
on day 28	NS	*	**	**	6.95	0.09
on day 56	NS	*	**	**	11.29	0.15
on day 70	NS	**	**	**	13.52	0.18
on day 100	NS	**	**	**	16.96	0.25
Daily weight gain:						
0-28	NS	NS	**	**	0.155	0.002
0-56	NS	NS	**	**	0.155	0.002
0-70	NS	*	**	**	0.156	0.002
0-100	NS	*	**	**	0.143	0.002
28-56	NS	NS	**	**	0.155	0.002
28-70	NS	**	**	**	0.156	0.003
28-100	NS	**	**	**	0.139	0.003
56-70	NS	**	NS	**	0.159	0.005
56-100	NS	*	NS	**	0.129	0.004
70-100	NS	NS	NS	**	0.115	0.005

Statistical significance : *P≤0.05; **P≤0.01; NS – no significance

Table 5
Fat cover colour depending on scrapie genotype (n=66)

Trait	Genotype					Total	Statistical significance
	ALRR/ ALRR	ALRR/ ALRQ	ALRR/ ALHQ	ALRQ/ ALRQ	ALRQ/ ALHQ		
Fat colour							
white	n	13	6	11	6	4	40
	%	32.5	15.0	27.5	15.0	10.0	100.0
coloured	n	5	12	8	0	1	26
	%	19.2	46.2	30.8	0.0	3.8	100.0
Total	n	18	18	19	6	5	66
	%	27.3	27.3	28.8	9.1	7.5	100.0

Statistical significance: *P≤0.05; **P≤0.01; NS – no significance

Table 6
Effect of the study factors on carcass traits of lambs (n=61)

Trait	Factors			Regression	\bar{x}	SE
	genotype	type of birth	year of birth	slaughter weight		
Carcass value (%)	NS	NS	NS	NS	38.17	0.52
Carcass weight (kg)	NS	NS	NS	**	14.82	0.20
Half-carcass cuts composition						
Half-carcass weight (kg)	NS	NS	NS	**	7.45	0.10
Rack (%)	NS	NS	NS	NS	7.03	0.22
Loin (%)	NS	NS	NS	NS	6.05	0.22
Tender loin (%)	NS	NS	**	NS	1.57	0.09
Leg (%)	NS	NS	NS	NS	25.22	0.35
Valuable cuts (%)	NS	NS	NS	NS	39.86	0.54
Tissue composition of leg						
Lean (kg)	NS	NS	NS	**	1.46	0.028
Fat (kg)	NS	NS	*	NS	0.18	0.010
Bones (%)	NS	NS	NS	NS	0.24	0.013

Statistical significance: *P<0.05; **P<0.01; NS – no significance

that meat obtained from Polish Heath sheep is considerably darker than that of other sheep breeds [6, 24, 37]. This is because Polish Heath is a primitive breed, and its meat is similar in colour to game. The mean protein content in the meat of Polish Heath lambs is about 20-22%, while fat content is over 3% [17, 31]. The results presented in Table 7 confirm the literature data. Differences were found in the content of dry matter in the meat of the lambs depending on the scrapie genotype. Detailed results are presented in Table 8. The meat of the lambs with genotype ALRR/ALRR had a statistically confirmed higher percentage of dry matter (26.52%) than ALRR/ALHQ individuals (24.49%).

Table 9 presents the effect of the factors tested on the percentage of selected groups of fatty acids in the lamb meat. Genotype was not shown to affect the percentage of these fatty acid groups in the meat. The content of short-chain and polyunsaturated fatty acids was shown to be correlated with the type of birth. The year of birth mainly influenced the content of short-chain and medium-chain fatty acids and mono- and polyunsaturated fatty

Table 7
Effect of the study factors on the chemical and phisycal characteristics of lamb meat (n=61)

Trait	Factor			Regresion	\bar{x}	SE
	genotype	type of birth	year of birth	slaughter weight		
Meat colour						
L^*	NS	NS	NS	NS	34.81	0.75
a^*	NS	NS	NS	NS	19.44	0.56
b^*	NS	NS	NS	NS	3.81	0.41
Phisycal and chemical characteristics of <i>mld</i>						
pH ₂₄	NS	NS	NS	NS	5.90	0.08
Crude protein (%)	NS	NS	NS	NS	20.13	0.60
Fat (%)	NS	NS	NS	NS	3.32	0.31
Dry matter (%)	*	NS	NS	NS	25.49	0.473

Statistical significance: *P≤0.05; **P≤0.01; NS – no significance

Table 8
Dry matter content of lamb meat depending on scrapie genotype (n=61)

Trait	LSM	Genotype			
		ALRR/ALRR	ALRR/ALRQ	ALRR/ALHQ	ALRQ/ALRQ
Dry matter (%)		26.52 ^a	25.50	24.49 ^a	25.44
	SE	0.548	0.602	0.570	0.921

Statistical significance: aa – P≤0.05

acids. In comparison with previous results [23, 36], a high *n-6/n-3* ratio of 6.980 was obtained. Fatty acid content is mainly a consequence of diet. The animals were fed concentrate feed rich in linoleic (C18:2 *n-6*) and arachidonic (C20:4 *n-6*) acids, with low content of α -linolenic acid (C18:3 *n-3*) [4, 32]. This may explain the unfavourable, high values for the *n-6/n-3* ratio.

The following conclusions were drawn from the results obtained:

- The growth traits in the lambs were independent of their scrapie genotype.
- Among carcass evaluation traits the genotype variable influenced only fat colour (P≤0.05).
- Many carcass traits and physical traits were not found to be affected by genotype.
- A significantly higher percentage of dry matter was noted in the meat of the lambs

Table 9
Effect of the study factors on content of fatty acid groups in lamb meat (n=61)

Trait	Factor			Regression	\bar{x}	SE
	genotype	year of birth	type of birth	slaughter weight		
SCFA	NS	**	*	NS	0.396	0.045
MCFA	NS	**	NS	NS	27.961	0.400
LCFA	NS	NS	NS	NS	63.358	0.711
SFA	NS	NS	NS	NS	39.533	0.667
MUFA	NS	**	NS	NS	45.320	0.701
PUFA	NS	NS	NS	NS	6.862	0.426
UFA	NS	**	NS	NS	52.183	0.802
PUFA n-3	NS	**	*	NS	0.978	0.083
PUFA n-6	NS	*	NS	NS	5.884	0.379
n-6/n-3	NS	**	NS	NS	6.980	0.522
UFA/SFA	NS	NS	NS	NS	1.330	0.039
MUFA/SFA	NS	NS	NS	NS	0.176	0.012
PUFA/SFA	NS	NS	*	NS	1.154	0.033
C14:1/C14:0	NS	NS	NS	NS	0.087	0.007
AI	NS	NS	NS	NS	0.553	0.021

SCFA – short-chain fatty acids (<6 carbons in chain)

MCFA – medium-chain fatty acids (8-12 carbons in chain)

LCFA – long-chain fatty acids (14-24 carbons in chain) [5]

SFA – saturated fatty acids

MUFA – monounsaturated fatty acids

PUFA – polyunsaturated fatty acids

UFA – unsaturated fatty acids

AI – atherogenic index ($C12:0 + 4 \times C14:0 + C16:0 / MUFA + PUFA$) [33]

Statistical significance: * $P \leq 0.05$; ** $P \leq 0.01$; NS – no significance

with the ALRR/ALRR genotype in comparison with individuals with the ALRR/ALHQ genotype.

No similar experiments have been conducted on this breed of sheep. The results obtained suggest that scrapie genotype does not influence the characteristics analysed, and also confirm that selection aimed at increasing genetic resistance to scrapie (increasing the frequency of the ALRR allele and the ALRR/ALRR genotype) does not affect the level of important performance characteristics in sheep and can be carried out independently of breeding work in the flock.

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