Scientific Annals of Polish Society of Animal Production - Vol. 11 (2015), No 1, 9-19

Assessment of the impact of selection for improving the frequency of positive genetic predispositions in the PrP locus on selected traits in Zelaznenska sheep*

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The research was carried out on the WULS Sheep and Goat Research Farm on 256 Zelaznenska lambs. Blood samples were collected from the jugular vein into plastic tubes with EDTA. DNA was extracted and used to determine scrapie genotypes. The lambs were weighed at birth and on days 28, 56, 70 and 100 day of life, and daily weight gain was determined for these intervals. After slaughter, carcass and meat quality characteristics were estimated. The results showed no effect of scrapie genotype on growth and development traits. Among carcass characteristics, scrapie genotype affected only the spread of the hock joint, and among meat quality traits only component a* of meat colour. The results indicate that breeding work aimed at increasing genetic resistance to scrapie may be conducted without affecting the performance characteristics of lambs of this breed.

KEY WORDS: Zelaznenska sheep / scrapie / PrP / genotype

Scrapie is a prion disease affecting small ruminants. It is one of the Transmissible Spongiform Encephalopathies (TSE). The occurrence of scrapie is induced by prion proteins. Numerous polymorphisms have been identified in the *PRNP* gene encoding prion protein, three of which (in codons 136, 154 and 171) are considered determinants of susceptibility or resistance to the classical form of scrapie. The ARR allele has been shown to determine the lowest susceptibility to scrapie, while the VRQ allele determines the highest susceptibility [4]. Polymorphism in codon 141 (F/L) has been linked to the possibility of occurrence of the atypical form of scrapie [5]. In 2001 European Union authorities introduced a number of legal regulations [19] aimed at preventing, controlling and combating

***Study carried out under project no. N311 257036**

prion diseases. In 2003, decision no. 2003/100/EC [3] introduced the obligation to conduct selection for increased resistance to scrapie in all European breeds of sheep (i.e. increased frequency of the ARR/ARR genotype). In addition, the European Commission issued Regulation no. 260/2003 [18], concerning control of TSEs in sheep and goats and principles governing trade in live sheep and goats and bovine embryos.

Selection work aimed at increasing the frequency of positive genetic determinants of scrapie was conducted on a flock of Zelaznenska sheep at the Experimental Sheep and Goat Farm of the Agricultural Experimental Station of the Warsaw University of Life Sciences in Żelazna. In 2009 five genotypes were identified in a group of ewes from the foundation stock of Zelaznenska sheep: ARR/ARR, ARR/ARQ, ARR/AHQ, ARQ/ARQ and ARQ/AHQ, with frequencies of 22.8%, 46.7%, 5.4%, 20.7% and 4.3%, respectively. In 2011 the frequency of ARR/ARR was increased to 27.7% and that of ARR/ARQ to 52.5%, accompanied by a decrease in the unfavourable genotypes (ARQ/ARQ decreased to 11.9% and ARQ/AHQ to 3.0%) [10, 11].

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

Material and methods

The study was conducted on 256 lambs (122 ♀ and 134 ♂) of the Zelaznenska breed, born in 2009-2011, from a flock kept at the Experimental Sheep and Goat Farm of the Agricultural Experimental Station of the Warsaw University of Life Sciences in Żelazna. Detailed carcass analysis was conducted on 35 male lambs selected from this group. The selection criterion was the formation of genotype groups. The lambs were weaned at the age of 100 days. From the second week of life, in addition to their mothers' milk, the lambs received solid food—hay and crushed oats. After weaning they were fed in accordance with standards [13] using on-farm feeds—hay and a mixture of oat, barley and triticale meal.

Blood was collected from the jugular vein of the lambs into syringes containing EDTA as an anticoagulant. DNA was isolated using isolation kits from A&A Biotechnology (www.aabiot.com), based on the method of chromatography on minicolumns containing special silica membranes. The extracted DNA was used to determine the scrapie genotypes in the individuals tested. The genotype was determined by the KASP® system, using the single nucleotide polymorphism (SNP) method. The primers used in PCR are presented in Table 1.

The lambs were weighed at birth and on days 28, 56, 70 and 100 of life. The data were used to calculate daily weight gains 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.

Table 1
Primers and SNP sites of the prion protein locus

Locus	Primers 3'- 5'	SNP	Changes	Localization
PrP prion protein	CACAGTCAGTGGAACAAGCC/ CTTTGCCAGGTTGGGG	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

Carcass analysis was performed on a group of 35 male lambs. The lambs were slaughtered when they had attained an average body weight of 40 kg (±2 kg), at an average age of 10-11 months. The carcasses were chilled 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 evaluation of the carcasses according to the EUROP classification, determining the following: meat content (E, U, R, O, P), fat cover (1, 2, 3, 4, 5), fat colour (white or coloured) and fat consistency (very soft, soft, cohesive, very cohesive). The following measurements were made of the carcasses: spread of the hock joint, leg length, leg depth, leg circumference, and leg index [8].

Next the carcasses were divided into half-carcasses. The left half-carcass was used for further analysis (with the tail left on the right half-carcass). 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 cuts were weight and their percentage share of the carcass was calculated. The total weight of the primal cuts, i.e. the tenderloin, rack, loin, and leg, were calculated as well. 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). The width and length of the cross section and the thickness of the fat layer over this section were measured. The contour of the cross section of the muscle was determined and used to determine its area. The final element of the evaluation performed on the mld was measurement of the colour of the meat. Colour was measured using a Konica-Minolta CR-400 colorimeter, taking into account the components L*, a* and b*. From the same site where these measurements were made a meat sample was collected for further analysis.

The final step of the slaughter stage was dissection of the leg, which was used to determine the tissue composition of the carcass [8].

The following analyses were performed in the meat samples:

– dry matter content (by the oven-dry method, according to the standard [15])

– water holding capacity (by the method of Grau and Hamm [6] as modified by Pohja and Niinivaary [16])

– protein content in the meat (Kjeldahl method, according to PB 11, edition 5 of 7 March 2012)

– fat content in the meat (Soxhlet method, according to PB 19, edition 5 of 18 June 2007)

– fatty acid profile in the meat (Röse-Gottlieb method [1]).

The fatty acid profile was determined by gas chromatography according to Polish standard PN-EN ISO 5508 [14], using an Agilent 7890A gas chromatograph with a Varian Select FAME column (length 100 m, inner diameter 0.25 mm, polar liquid phase film thickness 0.25 μm).

Statistical calculations were performed using the IBM SPSS Statistics 21.0 software package. The effect of scrapie genotype on daily weight gain and body weight was evaluated by multi-factor analysis of variance, taking into account scrapie genotype, sex, type of birth and year of birth. The effect of genotype on the carcass characteristics of the lambs was determined by analysis of covariance, with scrapie genotype, year of birth, and type of birth as sources of variance, and body weight at slaughter as the concomitant variable. The effect of scrapie genotype on the carcass evaluation traits according to the EUROP classification was analysed using the chi-squared test, creating contingency tables.

Results and discussion

Four scrapie genotypes were identified in the group of Zelaznenska lambs: ALRR/ALRR, ALRR/ALRQ, ALRR/ALHQ and ALRQ/ALRQ. Sex distribution in the genotype groups was similar, and twin births were dominant (Tab. 2). Carcass characteristics and meat quality were analysed in a group of 35 male lambs, which were characterized by three scrapie genotypes: ALRR/ALRR, ALRR/ALRQ and ALRQ/ALRQ. The results are presented in Table 3.

Table 2
Scrapie genotypes determined in the study population taking into account the lambs' sex and type of birth

Genotype	Sex			Type of birth			
	♀	♂	total	singles	twins	triplets	total
ALRR/ALRR	44	41	85	29	50	6	85
ALRR/ALRQ	70	82	152	29	119	4	152
ALRR/ALHQ	3	3	6	2	4	0	6
ALRQ/ALRQ	5	8	13	3	9	1	13
Total	122	134	256	63	182	11	256

Table 3
Genotype groups in relation to the type of birth of rams selected for carcass analysis

Genotype	Type of birth			
	singles	twins	triplets	total
ALRR/ALRR	2	10	1	13
ALRR/ALRQ	2	11	3	16
ALRQ/ALRQ	1	4	1	6
Total	5	25	5	35

Scrapie genotype was not found to affect body weight or daily weight gain during the periods analysed. This is confirmed in studies conducted on various breeds of sheep [2, 7, 9, 20, 22, 23]. The mean body weight of the lambs at birth was 3.96 kg, and the mean weight gain (in the period from 0 to 100 days) was 0.174 kg/day.

Carcass evaluation according to EUROP produced results similar to those obtained in a previous study [12]. In the assessment of meat content, 25 of the 35 carcasses were assigned to class R, 6 to class U, 3 to class O and one to class P. As a result of the fat cover evaluation 30 carcasses were assigned to classes 1 and 2 (9 and 21, respectively), and 5 to class 3. Cohesive fat was observed in 80% of the carcasses, the fat of 6 carcasses was described as soft, and in the case of one carcass the fat consistency was described as highly cohesive. The evaluation of the colour of the fat did not reveal any clear distinctions: 19 carcasses had white fat and 16 coloured fat. Scrapie genotype was not found to influence any of the elements of the EUROP evaluation described above.

Dressing percentage in the group of lambs was 38.42% on average, with a mean carcass weight of 15.79 kg. No effect of genotype was noted on these values. Analysis of the relationships between scrapie genotype and carcass measurements revealed a highly significant influence of genotype on the spread of the hock joint. The mean spread of the hock in individuals with the ALRR/ALRR genotype was 3.61 cm, as compared to 3.40 cm in ALRQ/ALRQ lambs. Detailed results are presented in Table 4.

Scrapie genotype was not found to influence either the percentage share of individual cuts or the tissue composition of the carcass. Despite the greater weight of the half-carcasses of lambs with the ALRQ/ALRQ genotype, the highest percentage share of valuable cuts was noted in the ALRR/ALRQ individuals. The lambs were similar in terms of the share of individual tissues in the leg, determined on the basis of dissection. In the case of lambs with genotype ALRR/ALRR a slightly higher percentage of meat and lower percentage of fat were observed in comparison with the other individuals,

Table 4
Carcass measurements depending on scrapie genotype (n=35)

Trait		Genotype		
		ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRQ
Spread of hock joint (cm)	LSM	3.61^{A}	3.53^{b}	3.40^{Ab}
	SE	0.04	0.03	0.05
Depth of leg (cm)	LSM	20.38	20.29	19.68
	SE	0.37	0.34	0.46
Length of leg (cm)	LSM	25.43	25.42	24.23
	SE	0.44	0.41	0.56
Circumference of leg (cm)	LSM	38.47	37.18	37.40
	SE	0.49	0.45	0.62
Index of leg (%)	LSM	151.45	146.68	154.39
	SE	3.41	3.15	4.30

Statistical significance: A, B, C… – P≤0.05; a, b, c… – P≤0.01

but these differences were not statistically significant. The results are presented in Table 5.

Table 5

Proportions of selected cuts in the half-carcass and tissue composition of the leg in relation to scrapie genotype (n=35)

Trait			Genotype		
			ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRQ
Half-carcass	kg	LSM	7.92	7.70	8.24
		SE	0.19	0.17	0.24
Share of selected cuts in half-carcass					
Rack	kg	LSM	0.53	0.51	0.56
		SE	0.02	0.02	0.03
	%	LSM	6.69	6.68	6.81
		SE	0.27	0.25	0.34
Loin	kg	LSM	0.45	0.46	0.49
		SE	0.02	0.02	0.03
	%	LSM	5.69	5.91	5.92
		SE	0.25	0.23	0.31
Tender loin	kg	LSM	0.16	0.14	0.16
		SE	0.01	0.01	0.02
	%	LSM	2.01	1.87	2.01
		SE	0.13	0.12	0.17
Leg	kg	LSM	2.09	2.06	2.11
		SE	0.05	0.04	0.06
	%	LSM	26.46	26.76	25.55
		SE	0.52	0.48	0.65
Prime cuts	kg	LSM	3.07	3.03	3.16
		SE	0.08	0.07	0.10
	%	LSM	38.84	39.35	38.29
		SE	0.73	0.67	0.92
Tissue composition of leg					
Lean	kg	LSM	1.61	1.52	1.57
		SE	0.04	0.04	0.05
	%	LSM	77.05	74.15	74.70
		SE	1.07	0.99	1.35
Fat	kg	LSM	0.21	0.22	0.25
		SE	0.02	0.02	0.02
	%	LSM	10.05	10.90	11.39
		SE	0.87	0.80	1.09
Bone	kg	LSM	0.27	0.29	0.28
		SE	0.02	0.01	0.02
	%	LSM	12.84	13.88	13.39
		SE	0.79	0.73	0.99

The acidity (pH) of the meat (5.78 ±0.43) and the percentage of free water in the meat (18.57 cm^2/g ±1.55), like the content of protein and fat, proved to be independent of the scrapie genotype (Tab. 6). The mean protein content in the meat of the Zelaznenska lambs, according to research, is about 20-22%, and fat content is about 5% [12, 21]. The results obtained (20.71% protein and 4.81% fat) confirm previous results.

The genotype was found to affect the colour of the meat. The ALRR/ALRR lambs were characterized by meat with greater saturation of the colour red (a higher value for the a* component; Table 7). This result may indicate a certain tendency, but further experiments using a larger group of animals should be conducted in this area.

The percentages of individual groups of fatty acids in the meat of the Zelaznenska lambs are presented in Table 8. The content of saturated fatty acids in the group of lambs was 47.12 g/100 g fat. A similar result for this breed was obtained by Radzik-Rant et al. [17]. The scrapie genotype did not affect the content of individual groups of acids in the lamb meat. The highest content of PUFA was noted in the ALRR/ALRQ lambs. It is worth

Table 6

Physical and chemical characteristics of *mld* muscle in relation to scrapie genotype

Trait		Genotype		
		ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRQ
pH_{24}	LSM	5.72	5.83	5.80
	SE	0.06	0.06	0.08
Free water (cm^2/g)	LSM	18.07	18.79	18.86
	SE	2.17	2.00	2.74
Dry matter (%)	LSM	26.30	25.44	25.74
	SE	0.56	0.51	0.70
Crude protein (%)	LSM	20.86	20.58	20.69
	SE	0.33	0.31	0.42
Fat (%)	LSM	5.43	4.21	4.80
	SE	0.60	0.55	0.75

Table 7

Meat colour in relation to scrapie genotype (n=35)

Trait		Genotype		
		ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRQ
L*	LSM	35.92	38.11	37.66
	SE	1.22	1.13	1.54
a*	LSM	18.03[a]	16.56[a]	16.20[a]
	SE	0.53	0.49	0.67
b*	LSM	2.87	3.71	2.94
	SE	0.46	0.52	0.58

Statistical significance: a, b, c… – $P \leq 0.01$

Table 8
Proportions of selected fatty acid groups in meat (g/100 g) in relation to scrapie genotype (n=35)

Trait		Genotype			X	SE
		ALRR/ALRR	ALRR/ALRQ	ALRQ/ALRQ		
SCFA	LSM	0.332	0.403	0.296	0.344	0.039
	SE	0.055	0.051	0.069		
MCFA	LSM	28.797	28.864	31.182	29.614	0.539
	SE	0.752	0.695	0.949		
LCFA	LSM	64.437	63.306	62.996	63.579	0.726
	SE	1.014	0.937	1.279		
SFA	LSM	46.213	46.836	48.312	47.120	0.692
	SE	0.967	0.893	1.220		
MUFA	LSM	42.652	40.790	41.295	41.579	0.687
	SE	0.959	0.886	1.210		
PUFA	LSM	4.701	4.947	4.867	4.838	0.258
	SE	0.360	0.333	0.454		
UFA	LSM	47.352	45.737	46.162	46.417	0.752
	SE	1.050	0.970	1.325		
PUFA *n-3*	LSM	0.513	0.655	0.559	0.576	0.045
	SE	0.063	0.058	0.080		
PUFA *n-6*	LSM	4.188	4.291	4.309	4.263	0.234
	SE	0.327	0.302	0.412		
n-6/n-3	LSM	10.761	7.832	9.084	9.226	0.883
	SE	1.233	1.139	1.555		
UFA/SFA	LSM	1.027	0.986	0.961	0.991	0.030
	SE	0.042	0.039	0.054		
MUFA/SFA	LSM	0.102	0.107	0.102	0.104	0.007
	SE	0.010	0.009	0.013		
PUFA/SFA	LSM	0.925	0.878	0.859	0.888	0.026
	SE	0.036	0.033	0.045		
C14:1/C14:0	LSM	0.075	0.083	0.076	0.078	0.008
	SE	0.012	0.011	0.015		
AI	LSM	0.649	0.683	0.731	0.688	0.026
	SE	0.037	0.034	0.046		

SCFA – short-chain fatty acids (<6 carbons in chain)
MCFA – medium-chain fatty acids (6-12 carbons in chain)
LCFA – long-chain fatty acids (>12 carbons in chain)
SFA – saturated fatty acids
MUFA – monounsaturated fatty acids
PUFA – polyunsaturated fatty acids
UFA – unsaturated fatty acids (MUFA + PUFA)
AI – atherogenic index (C12:0 + 4 x C14:0 + C 16:0 / MUFA +PUFA)

noting the high *n-6/n-3* ratio of 9.226. This was a markedly higher value than that obtained in previous studies. Wood et al. [24] (citing Enser et al., 1996) report a *n-6/n-3* ratio of 1.3 for sheep meat, and Radzik-Rant et al. [17] obtained a value of 2.63-2.65. The percentages

of individual groups of fatty acids are mainly a result of diet. The lambs used in the experiment were from births from a period of three years, and their diet was mainly dominated by concentrate feeds with high content of linoleic (C18:2 *n-6*) and arachidonic (C20:4 *n-6*) acids and low content of α-linolenic acid (C18:3 *n-3*). This may explain the high values for the *n-6/n-3* ratio.

The study conducted indicates that the body weight gain and development of the Zelaznenska lambs were independent of the scrapie genotype. Among carcass evaluation characteristics the genotype factor differentiated the lambs only in the case of the spread of the hock joint ($P \leq 0.01$). The carcass characteristics, despite noticeable differences in the case of certain values, proved to be independent of the genetic factor. The pH of the meat and the percentage of free water were not found to depend on the scrapie genotype. Among the physical characteristics tested, an effect of genotype was noted only in the case of the a* component of meat colour. ALRR/ALRR individuals had significantly more red colour than animals with the other genotypes. The protein and fat content, as well as the proportion of dry matter and water in the meat of the lambs, showed no association with the genetic factor. The content of selected fatty acid groups in the meat was found to be independent of the animals' genotypes.

To sum up, despite a few relationships noted among the characteristics analysed, the scrapie genotype was not found to affect the growth and development traits, carcass characteristics, or meat quality of the Zelaznenska lambs raised on the Experimental Sheep and Goat Farm in Żelazna. No similar experiments had previously been conducted on this breed of sheep. It can be concluded from the results obtained that the scrapie genotype does not influence the characteristics analysed in Zelaznenska sheep, and 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 the sheep and can be carried out independently of breeding work in the flock.

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