GENETIC RESISTANCE AGAINST SCRAPIE DISEASE RELATED TO LAMB GROWTH PERFORMANCE TRAITS*

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The objective of the study was to identify the impact of selection on genetic resistance against scrapie disease related to lamb growth performance attributes for Suffolk, Kent, Charollais, and Texel lambs. The allelic genotypes were grouped according to the presence of scrapie resistant allele (*ARR*): *ARR* homozygotes, *ARR* heterozygotes, no presence of *ARR* allele. The influence of these groups on lamb live weight (LW), *musculus longissimus lumborum et thoracis depth* (MLLT), and backfat thickness at 100 days of age was investigated using SAS software. No significant differences for Suffolk and Charollais breeds were detected. Significantly highest LW (34.41 kg) and MLLT (27.80 mm) were noticed for Kent *ARR* homozygotes lambs, while significantly lowest values were estimated at lambs with absent *ARR* allele (LW = 33.42 kg, MLLT = 26.68 mm). Significantly lower muscle depth (-0.69 mm) was detected for Texel *ARR* homozygote compared to *ARR* heterozygote lambs. As a result, we were unable to detect a consistent evidence for reduced growth performance traits in relation to genetic resistance against scrapie disease. However, the number of animals in some groups caused as a limiting factor. This can be a potential reason of opposed trends in Texel and Kent lambs.

prion protein, allele, live weight, musculus longissimus lumborum et thoracis depth, backfat thickness



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INTRODUCTION

The Czech sheep production sector has been primarily oriented towards meat production, from practically 90% (B u c e k et al., 2016). The breeding program of the Czech Republic is primarily aimed at improving growth and meat characteristics for meat breeds or for Kent sheep (representative of dual-purpose breed with a strong meat orientation in the Czech Republic). Lamb live weight (LW, kg) determination and ultrasound measurements of *musculus longissimus lumborum et thoracis* depth (MLLT, mm) and backfat thickness (BT, mm) are performed in an official manner at 100 days of age (M i l e r s k i, 2005, 2007). Animal biosecurity and foot safety are other points of interest in animal production. One of serious health risks in sheep flock is scrapie disease (transmissible spongiform encephalopathies; TSE), which is usually manifested by behavioral changes such as aggression, failure to herding response or rubbing and biting on specific body parts 'scratch reflex' (P u g h, B a i r d, 2012) (Fig. 1). The importance of TSE is underlined by its close resemblance to bovine spongiform encephalopathy in cattle or Creutzfeldt Jakob disease in man (P r i t c h a r d et al., 2008). Prion protein (*PrP*) coding polymorphisms at codons 136 (*A/V*), 154 (*R/H*), and 171(Q/R/H) give rise to five major alleles, sometimes referred to as haplotypes (*ARR*, *ARQ*, *AHQ*, *ARH*, and

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VRQ) encoding a total of 15 genotypes (B a y lis, Goldmann, 2004). The ARR allele is significantly associated with resistance to natural and experimental infections with scrapie (J effrey et al., 2002), while the ARQ, VRQ, and ARH alleles are the most susceptible to this disease (Sweeney et al., 2007). Therefore Ram Genotyping Scheme was established as a part of National Scrapie Plan in Great Britain (Arnold et al., 2002; Gubbins, Webb, 2005; Roden et al., 2006). In the Czech Republic, preventive monitoring of TSE was launched in 1991 by establishing the National Reference Laboratory for TSE. Active TSE monitoring (including checkup of clinically healthy animals) has been performed since 2000 (E A G R I, 2017). Since that time, information about genotype has become a necessary part of breeding programs. It is also a criterion when selecting animals in the subsequent breeding. Therefore, previous studies investigated the association of PrP genotype and sheep reproductive or productive traits as well as relationships between *PrP* genotype and lamb growth performance attributes. S w e e n e y et al. (2007) demonstrated no effect of PrP genotype on ovulation rate or litter size of Belclare ewes. Alexander et al. (2005) detected a greater frequency of multiple births in Suffolk ewes having no ARR allele in comparison with ARR heterozygous animals. However, no such evidence was noticed for Columbia, Rambouillet, and Hampshire sheep. As Brandsma et al. (2005), Hanrahan et al. (2008), and Sweeney, Hanrahan (2008) have concluded; there is predominantly only minor influence of PrP allele on lamb weaning weight (usually at 90–135 days of age), despite of some significant association detected by Brandsma et al. (2004), de Vries et al. (2004) or Man et al. (2006). Hanrahan et al. (2008) and Moore et al. (2009a, b) found no consistent association of PrP genotype for muscle depth or backfat thickness, suggesting no or only minor influence.



Fig. 1. Suffolk sheep with clinical signs of scrapie disease (Pugh, Baird, 2012)

Table 1. Frequency of PrP genotypes and PrP genotype groups detected in particular sheep breeds

	Suffolk	Kent	Texel	Charollais				
PrP genotypes								
AHQ/AHQ	0	2	0	0				
ARQ/AHQ	0	17	1	0				
ARQ/ARQ	36	180	0	11				
ARQ/ARH	2	0	0	0				
ARQ/VRQ	0	2	2	0				
ARR/AHQ	7	49	52	0				
ARR/ARH	14	0	70	0				
ARR/ARQ	851	762	89	104				
ARR/ARR	3146	790	770	327				
ARR/VRQ	2	2	0	5				
PrP allelic groups								
ARR/ARR	3147	790	770	327				
$ARR/\times\times\times$	874	813	211	109				
×××/×××	38	201	3	11				

The aim of the present study was to evaluate the influence of PrP genotype variations on growth performance traits of Suffolk, Kent, Texel, and Charollais lambs in the Czech Republic.

MATERIAL AND METHODS

Data collection and evaluated traits

The study lasting over 3 years (2013–2015) included Suffolk (SF), Kent (K), Texel (T), and Charollais (CH) purebred lambs. All lambs were examined for growth traits during their performance recording at 100 days of age (Milerski, 2005, 2007). This examination included lambs' live weight (LW, kg) determination and ultrasound measurements of *musculus longissimus lumborum et thoracis* depth (MLLT, mm) and backfat thickness (BT, mm) in the area of the last *thoracis* vertebra. The analysis of *PrP* allelic genotype was performed in a certified laboratory (National Reference Laboratory for TSE monitoring, Jihlava, Czech Republic).

The five *PrP* allelic haplotypes (*ARR*, *ARQ*, *AHQ*, *ARH*, *VRQ*) encoded totally ten genotypes (Table 1). For the evaluation, three *PrP* genotypes groups were formed (according to L i p s k y et al. (2008) and M o o r e et al. (2009a, b), modified): *ARR* homozygotes (*ARR*/*ARR*), *ARR* heterozygotes (*ARR*/×××), and absence of *ARR* allele (×××/×××). This classification virtually corresponded with the five genetic risk groups against scrapie disease defined by R o d e n et al. (2006)

		Litter	size	Sex o	f lambs			Grou	ups of dam	age at lamb	ing		Pr	P allelic grou	dn
	singles	twins	triplets and quadruplets	males	females	1 year	2 years	3 years	4 years	5 years	6 years	7 years and older	ARR/ARR	ARR/×××	***/***
Suffolk	992	2658	409	2395	1664	140	991	1063	750	579	283	253	3147	874	38
Kent	414	1180	210	536	1268	40	408	438	361	264	157	136	790	813	201
Texel	194	682	108	508	476	22	311	274	155	103	66	53	770	211	I
Charollais	96	271	80	321	126	11	87	123	102	58	30	36	327	109	11

or P r i t c h a r d et al. (2008); therefore, the evaluation of five genetic risk groups against scrapie disease was not included in the trial as an individual assessment. Lamb growth performance traits, PrP allelic genotype as well as information about flock, year of lambing, season of lambing, lamb litter size, sex of lamb, and age of dam at lambing were provided by the Sheep and Goat Breeders Association in the Czech Republic.

Statistical analysis

The influence of *PrP* genotype group on lamb growth performance traits was investigated separately in particular sheep breeds (CH, K, SF, T). Statistical analyses were conducted using MIXED procedure of SAS software (Version 9.3, 2011). Models were adapted to explain the variability of dependent variables (LW, MLLT, BT). The model for each breed (SF, *n* = 4059; K, *n* = 1804; T, *n* = 984; CH, *n* = 447) included combined flock- year-seasonal effect as random and fixed effects of litter size (3 classes), sex of lambs (2 classes), dam's age group (7 classes) and the fixed effect of PrP genotype group (3 classes). The characteristic of particular fixed effects in models is given in Table 2. Results of the Least Squares Means method were presented using MS Excel software. Differences estimated between variables were tested by the Tukey-Kramer method at the significance level of *P* < 0.05

RESULTS

In addition to the database characteristic, the arithmetic means, standard deviations, minimum and maximum values, and variation coefficients for particular sheep breeds are given in Table 3. In this elementary comparison, Suffolk lambs showed the highest growth performance attributes at 100 days of age, while Texel lambs the lowest. Differences between these two breeds reached 29.53% in LW, 8.07% in MLLT, and 14.25% in BT. As obvious from Table 1, the ARR/ARR allelic group was dominant for all the genotyped lambs during the observed period (43.8% in K, 78.3% in T, 73.2% in CH, and 77.5% in SF). The ARR/ARQ allelic genotype, as the second most abundant variation, represented 42.2% for K, 9.0% for T, 23.3% for CH, and 21% for SF. The population of genotyped lambs contained the lowest distribution of non-ARR allelic genotypes (×××/××× PrP group), representing 0.94% for SF, 2.46% for CH, 11.14% for K, and 0.30% for T. Additionally, $\times \times \times / \times \times \times PrP$ allelic group was presented only in three occurrences in Texel lambs (Table 2); and therefore deleted in the final model. The analysis for growth performance traits of Texel breed included only ARR/ARR vs ARR/××× comparison. The evaluation of PrP genotype groups of the four sheep breeds in relation to growth performance traits

Table 3. Basic statistics for growth performance traits in Suffolk, Kent, Texel, and Charollais lambs (measurements performed at 100 days of age)

Breed	Variable	n	AM	SD	MIN	MAX	CV (%)
Suffolk	LW (kg)	4049	38.00	6.91	17.90	65.50	18.17
	MLLT depth (mm)	4021	29.13	3.62	16.30	41.50	12.42
	BT (mm)	4022	4.07	1.12	1.50	7.70	27.55
Kent	LW (kg)	1790	34.05	5.74	16.80	53.10	16.85
	MLLT depth (mm)	743	27.80	3.56	13.20	37.70	12.80
	BT (mm)	742	3.83	0.95	1.90	9.90	24.96
Texel	LW (kg)	979	32.25	7.28	12.90	59.50	22.58
	MLLT depth (mm)	973	26.78	4.18	13.20	40.40	15.63
	BT (mm)	973	3.49	0.96	1.50	6.90	27.63
Charollais	LW (kg)	447	36.84	6.29	13.50	62.70	17.07
	MLLT depth (mm)	438	28.57	3.47	14.80	37.20	12.16
	BT (mm)	438	3.60	0.96	1.50	6.60	26.71

LW = live weight, MLLT = musculus longissimus lumborum et thoracis, BT = backfat thickness, n = number of observations, AM = arithmetic mean, SD = standard deviation, MIN = minimal value, MAX = maximal value, CV = coefficient of variation

is presented in Figs. 2-4. The variability between *PrP* genotype groups ranged 0.42% (CH)–2.88% (K) in LW, 2.47% (SF)-5.25% (CH) in MLLT, and 0.57% (T)-7.14% (CH) in BT. Most differences were thus negligible or they were burdened with a high standard error. This concerned especially Suffolk and Charollais lambs where no significant differences were noticed, despite of numerically highest values for ARR geterozygotes or ARR allele-absent lambs. However, an ARR allele effect was seen in Kent breed where lambs with two ARR alleles were significantly heavier (+0.99 kg)than those with one ARR copy or those with zero ARR allele carriers. The same effect was monitored for MLLT evaluation in Kent breed. Similarly, Kent lambs of ARR/ARR allelic group reached significantly higher MLLT in comparison to $ARR/\times\times\times$ (+0.84 mm) or $\times \times \times / \times \times \times$ (+1.12 mm) groups. Contrarily, the opposed tendency was detected for MLLT evaluation in Texel lambs. Significantly lower MLLT (-0.69 mm) was demonstrated in Texel lambs of ARR/ARR vs $ARR/\times\times$ allelic groups

DISCUSSION

The efficacy of genotyping program resulting in reduced susceptibility of lambs to scrapie disease was confirmed by A r n o l d et al. (2002) in Great Britain. The majority of genotyped lambs were *ARR* homozygotes or *ARR* heterozygotes during the observed period, showing the positive impact of TSE preventive monitoring program in the Czech Republic. Selection for genetic resistant animals against TSE was described also in some previous studies (e.g. G u b b i n s, W e b b, 2005; R o d e n et al., 2006), and therefore their results were involved in evaluating this selection with regard to lambs' growth traits. Generally, the results demonstrated a small variability, showing no consistent evidence for the effect of *PrP* genotype on growth performance traits within observed breeds. No evidence for growth performance traits was demonstrated by Brandsma et al. (2005), Sawalha et al. (2007), Pritchard et al. (2008), while de Vries et al. (2004), Hanrahan et al. (2008) or Moore et al. (2009 a, b) reported occasional influence in several sheep breeds. This was predominantly supported by the results of the present study as well. However, some support for occasional positive effect of ARR/ ARR homozygote allelic group in comparison with no ARR/ARR carriers was demonstrated for LW in Kent lambs. Positive influence of ARR/ARR genotype group on scan weight was demonstrated in Texel (Moore et al., 2009a), while opposed tendency was marked in Welsh Mountain (Moore et al., 2009b) lambs. Others low land (Moore et al., 2009a) or hill land (Moore et al., 2009b) breeds showed no influence of PrP genotype on scan weight. Interestingly, Texel lambs in the present study showed no-evidence for the *PrP* genotype influence on LW, which is thus in oppose with Moore et al. (2009a).

No association was detected between muscle depth and PrP genotype in Poll Dorset, Texel (M o or e et al., 2009a) or Welsh Mountain lambs (Pritchard et al., 2008). Some influence, however, detected as occasional case, was described by H a n r a h a n et al. (2008) for muscle depth in Suffolk and Charollais sheep. The effect of PrP genotype groups in the present study was predominantly low, despite of some significant differences for MLLT in Kent or Texel lambs. Kent lambs with ARR/ARR allelic group showed significantly the greatest MLLT, while Texel lambs the lowest. This opposed tendency between Kent and Texel Fig. 2. Effect of PrP genotype groups (LSM \pm SE) on Suffolk, Kent, Texel, and Charollais lamb live weight

LW = lamb live weight at 100 days of age, ARR/ARR = lambs with two ARR allelic groups in genotype, $ARR/\times\times\times$ = lambs with one ARR allelic group in genotype, $\times\times\times/\times\times\times$ = lambs with no presence of ARR allelic group in genotype





Fig. 3. Effect of PrP genotype groups (LSM \pm SE) on Suffolk, Kent, Texel, and Charollais lamb muscle depth

MLLT = lamb musculus longissimus lumborum et thoracis depth at 100 days of age, ARR/ARR = lambs with two ARRallelic groups in genotype, $ARR/\times\times\times$ = lambs with one ARR allelic group in genotype, $\times\times\times/\times\times$ = lambs with no presence of ARR allelic group in genotype



4.1 4.0 3.9 3.8 3.7 BT (mm) 3.6 3.5 3.4 3.3 3.2 3.1 3.0 Suffolk Kent Texel Charollais $\blacksquare \ ARR/ARR \quad \blacksquare \ ARR/\times\times\times \quad \blacksquare \times\times\times/\times\times\times$

Fig. 4. Effect of PrP genotype groups (LSM \pm SE) on Suffolk, Kent, Texel, and Charollais lamb backfat thickness

BT = lamb backfat thickness at 100 days of age, ARR/ARR = lambs with two ARRalleles in genotype, $ARR/\times\times\times$ = lambs with one ARR allele in genotype, $\times\times\times/\times\times\times$ = lamb with no presence of ARR alleles in genotype breeds could be explained by a lower distribution of PrP allelic groups in Texel breed. Additionally, no influence was detected in Suffolk or Charollais breeds. No significant associations were detected between the PrP genotype and the ultrasonic fat depth in Texel, Suffolk, Charollais or Poll Dorset lambs (Hanrahan et al., 2008; Moore et al., 2009a). The results of this study are in line with those of previous ones. No effect of PrP genotype on BT in all the evaluated breeds was demonstrated. Some previous studies evaluated the influence of other *PrP* alleles or allelic variations on growth performance traits. The evidence for a significant association of VRQ allele with live weight, muscle or fat depth was reported by Brandsma et al. (2004) or Moore et al. (2009a, b). Like in previous discussion, most of these differences were monitored as occasional occurrences and the authors pointed out that the absolute magnitude of VRO allele effect was small. Unfortunately, we were unable to extend the investigation over more specified PrP alleles or PrP allelic groups due to low frequency of VRQ allele. Therefore, the effect of VRQ allele or VRQ allelic combination was expressed as ARR heterozygotes or as no ARR presence group in the present study. As important, selection of genetically resistant animals showed no negative effect on their growth performance traits. Some potential negative associations in the Texel breed should be linked with lower distribution of the other groups and the absence of $\times \times \times / \times \times \times$ allelic group. Furthermore, also the results on Texel breed presented in other available resources did not confirm a direct association between PrP groups and growth performance traits.

CONCLUSION

The results of the current study indicate that breeding on TSE resistance has only minor effect on growth performance traits in sheep breeds management oriented to meat production in the Czech Republic. At least, we were unable to detect a consistent evidence for reduced growth performance traits in relation to genetic resistance against scrapie disease. However, the number of animals in some groups caused as limited factor. This can be a potential reason of opposed trends in Texel and Kent lambs. Potentially lower performance of some animals with a copy of *ARR* allele could be compensated by the different breed or inter-breed effect in crossing.

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