Evaluation of associations between prion haplotypes and growth, carcass, and meat quality traits in a Dorset × Romanov sheep population[1,2]

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ABSTRACT: There is concern about potential antagonistic correlated responses due to intensive selection for scrapie-resistant haplotypes of the prion (*PRNP*) gene in sheep. The objective of the present research was to test for associations of *PRNP* haplotypes for codons 136, 154, and 171 with growth, carcass, and meat quality traits in an F_2 Dorset × Romanov population (n = 415) segregating the 2 callipyge alleles. Haplotypes of the 3 *PRNP* codons were determined for each sheep, and breed of origin of each gamete was predicted by genotyping 6 microsatellite markers flanking the *PRNP* locus. Twenty-five growth, carcass, and meat quality traits were evaluated. Data were analyzed using a basic model consisting of fixed effects of year, sex, and callipyge genotype, the random effect of sire, and 7 covariates corresponding to the probability that a lamb inherited a specific *PRNP* haplotype of either Dorset or Romanov origin. A fixed effect of litter size was added to the model for growth traits. The model for carcass traits contained the linear and quadratic effects of chilled carcass weight and the interactions among callipyge genotype and linear and quadratic terms. For meat quality traits, the model contained chilled carcass weight as a covariate and the interaction between callipyge genotype and chilled carcass weight. A contrast between the resistant ARR haplotype and the average effect of other *PRNP* haplotypes was tested to investigate the effects of potential selection for ARR within each breed of origin (Dorset, ARR vs. ARQ, VRQ, and AHQ; Romanov, ARR vs. ARQ and VRQ). There was limited evidence that selecting for scrapie resistance would cause correlated responses due to linkage disequilibrium. Associations of only 3 traits with PRNP haplotypes were detected in either breed of origin. In Romanov, the ARR haplotype was associated with longer carcasses ($P < 0.013$), narrower rumps ($P = 0.038$), and less marbling ($P = 0.022$) than the average of ARQ and VRQ haplotypes. No significant contrasts were detected for Dorset. This study is the first to account for breed of origin while investigating haplotype associations in an F_2 population. This study provided limited evidence of associations between *PRNP* haplotypes and growth, carcass, and meat quality traits.

Key words: carcass trait, growth trait, meat quality trait, prion gene, scrapie resistance, sheep

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INTRODUCTION

The existence of transmissible spongiform encephalopathies, such as bovine spongiform encephalopathy in cattle and scrapie in sheep, is a significant hazard to livestock industries. Traditionally, culling of affected animals (and animals sharing the same production area) has been the standard method of scrapie control in the sheep industry. Unfortunately, this method is not effective with prion diseases, due to their long incubation time and unusual method of transmission.

A genetic component to scrapie susceptibility has been known for many years. The 3 most important genetic markers associated with scrapie susceptibility are located at codons 136, 154, and 171 of the prion (**PRNP**) gene (for review see Baylis and Goldmann, 2004). The *PRNP* haplotype encoding alanine, arginine, and arginine (ARR) at the respective 136, 154, and 171 positions is associated with increased resistance to scrapie, whereas the valine, arginine, and glutamine haplotype (VRQ) is associated with increased susceptibility to scrapie. The scrapie susceptibility of 3 other common *PRNP* haplotypes (ARQ, AHQ, and ARH) is intermediate or unknown.

[1]Mention of a trade name, proprietary product, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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Many countries currently have scrapie eradication plans utilizing these genetic markers (Byrne, 2003). Several researchers have investigated possible antagonistic associations between the *PRNP* locus and milk (de Vries et al., 2005), performance (Alexander et al., 2005), and growth (Brandsma et al., 2004) traits. However, consistent evidence supporting antagonistic associations has not been found. The objective of this study was to test for associations between *PRNP* haplotypes and a comprehensive set of growth, carcass, and meat quality traits in an F_2 population of Dorset × Romanov sheep.

MATERIALS AND METHODS

Animal Populations and Phenotypic Data

Sheep were produced to investigate the chromosomal location, gene action, and phenotypic effects of the callipyge locus (Freking et al., 1998a). Nine Dorset rams were mated with 255 Romanov ewes to produce Dorset × Romanov F_1 lambs. Eight F_1 sires and 138 F_1 dams (from 9 Dorset grandsires and 114 Romanov granddams) produced 432 F_2 offspring in 1994 and 1995. Traits included in the current study are listed in Table 1. Weights were recorded at birth and at an average of 8 (weaning), 12, and 20 wk of age. Preweaning ADG was calculated using the weight gain from birth to weaning and age at weaning. Postweaning ADG from 12 to 20 wk of age was calculated in a similar manner.

Lambs were serially slaughtered at 23, 26, 29, 32, 35, and 38 wk of age, with approximately 30 lambs slaughtered per age group each year. Live weight was recorded on each lamb on the morning of slaughter. Kidney-pelvic fat and HCW were recorded at the time of slaughter. Following a 24-h chill, shoulder and rump widths were recorded. Each carcass was assigned a subjective leg muscling score, normally scaled from 1 (low cull) to 15 (high prime). However, in this population, scores ranged from 9 to 17 because some carcasses displayed degrees of muscling beyond the high prime classification due to effects of the callipyge mutation.

Carcasses were split along the dorsal midline and the length of the carcass was measured from the anterior edge of the first rib to the anterior edge of the aitchbone. Metacarpal bone length was measured as an additional indicator of skeletal development. Fat depths were measured at the midpoint of the longissimus muscle between the 12th and 13th ribs and at the midline of the fourth sacral vertebra. The left side of the carcass was retained for later use in shear force determination. The right side of the carcass was cut between the fifth and sixth ribs and between the last 2 lumbar vertebrae to produce 3 carcass subsections. Subsections from the right side corresponded to anterior (shoulder, neck, foreshank, and breast), middle (loin, rib, and flank), and posterior (sirloin, leg, and hindshank) portions of the carcass. Each subsection was individually weighed. Longissimus muscle depth, width, surface area, and subjective marbling score (on a scale from 0 = devoid to 600 = moderate) were measured between the 12th and 13th ribs.

Table 1. Least squares means (LSM) and residual standard deviations (RSD) for growth, carcass, and meat quality traits in a Dorset × Romanov F_2 population[1]

Trait	n	LSM	RSD
Growth traits			
Birth weight, kg	415	4.12	0.64
Preweaning ADG, kg/d	363	0.25	0.04
Postweaning ADG, kg/d	363	0.47	0.09
Carcass traits			
Slaughter traits			
Weight at slaughter, kg	354	47.8	2.1
Kidney and pelvic fat weight, kg	354	1.20	0.32
HCW, kg	354	26.13	0.06
Conformation traits			
Shoulder width, cm	354	19.82	0.78
Rump width, cm	354	22.47	0.49
Carcass length, cm	354	61.8	1.4
Metacarpal length, cm	354	19.11	0.33
12th rib fat depth, cm	354	0.455	0.17
Fourth sacral vertebra fat depth, cm	354	1.43	0.36
Loin eye area, cm^2	354	17.5	1.6
Loin eye depth, cm	354	3.61	0.27
Loin eye width, cm	354	6.28	0.33
Legscore[2]	354	12.92	0.78
Anterior carcass weight, kg	354	8.56	0.39
Middle carcass weight, kg	354	8.16	0.38
Posterior carcass weight, kg	354	8.84	0.33
Composition traits			
Carcass water, kg	354	13.15	0.58
Carcass fat, kg	354	7.20	0.74
Carcass protein, kg	354	4.07	0.17
Carcass ash, kg	354	1.13	0.10
Meat quality traits			
Warner-Bratzler shear force,[3] kg	325	1.59	0.28
Longissimus marbling[4]	354	345.3	75.3

[1]Least square means and residual standard errors were calculated using statistical models identical to those described in the text, except for the exclusion of the 7 *PRNP* covariates.
[2]Subjective leg muscling score, average choice = 11, average prime = 14.
[3]Warner-Bratzler shear force values were natural log transformed before analysis.
[4]Subjective longissimus marbling, ranging in value from 0 (devoid) to 600 (moderate).

Proximate chemical analysis was performed on each of the carcass subsections. Each subsection was frozen and ground separately to measure ash, water, protein, and ether extract (fat) content (AOAC, 1990).

From the left side of the carcass, loin chops (2.5 cm thick) were collected for use in shear force determination. Loin chops were aged at 4°C for 14 d and stored at –20°C until shear force measurement. Storage duration ranged from 2 wk to 5 mo. Before shear force measurement, chops were thawed at 5°C, broiled to an internal temperature of 40°C, and then turned and broiled to an internal temperature of 75°C using an open-hearth electric broiler (Farberware, Bronx, NY). Loin chops were allowed to cool at 4°C for 24 h before 6 cores (1.27 cm diameter each) from 3 chops were obtained, making sure that the cores ran parallel to the muscle fibers.

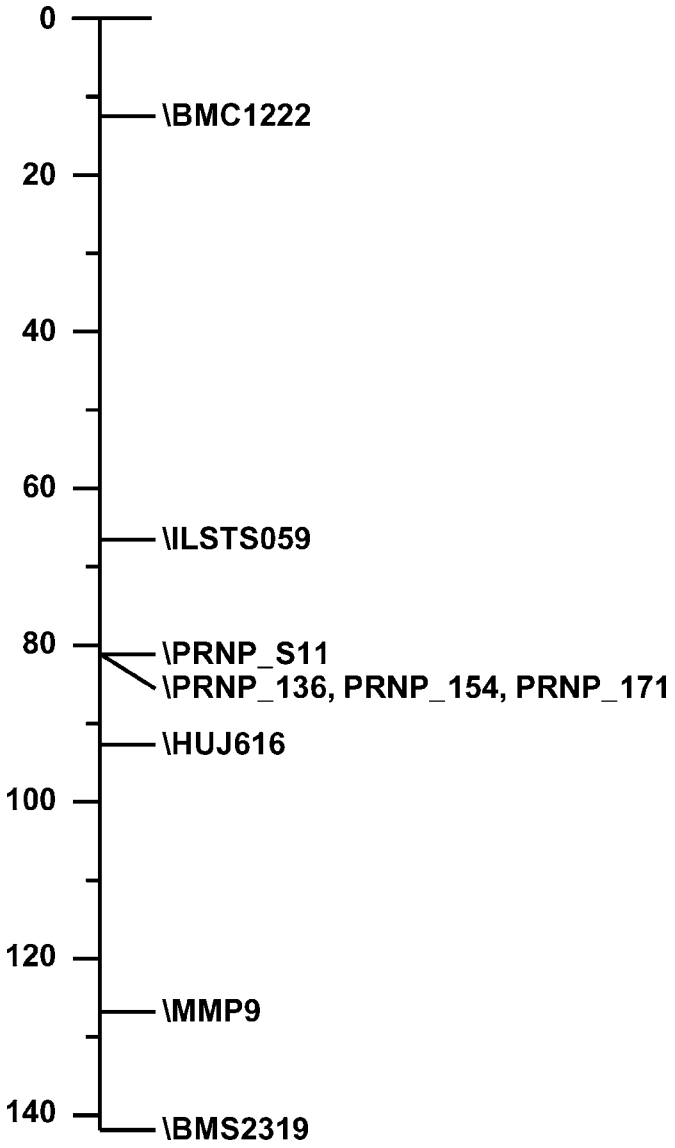

Figure 1. Linkage map of ovine chromosome 13 markers used in the current study. Names of anonymous microsatellite loci are preceded with a backslash symbol, following COGNOSAG nomenclature guidelines (Andresen et al., 1995). Units are in centimorgans.

Each core was sheared once using an Instron Universal Testing Machine (model 1132, Instron, Canton, MA) with a Warner-Bratzler shear attachment, a 50-kg load cell, and a cross-head speed of 50 mm/min. Shear force was calculated as the mean shear force of the 6 cores.

Collection of Genotypic Data

Genomic DNA was extracted from blood, liver, or spleen tissue of 9 Dorset grandsires, 8 Romanov granddams, and all F_1 parents and F_2 lambs using Gentra Generation Capture kits (Gentra Systems Inc., Minneapolis, MN) or a standard saturated salt procedure (Miller et al., 1988). Sheep were genotyped at 6 microsatellite loci (Figure 1) using the standard PCR protocol of de Gortari et al. (1998). Five of these markers were previously linkage-mapped to ovine chromosome 13 (Maddox et al., 2001; http://rubens.its.unimelb.edu.au/~JILLM/jill.htm). The sixth marker, *PRNP_S11*, was not linkage-mapped to chromosome 13, but was identified within the ovine genome sequence (Genbank Accession No. U67922) that contains the *PRNP* gene (Geldermann et al., 2003).

Genotypes at *PRNP* codons encoding amino acid residues at positions 136, 154, and 171 were determined using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometer genotyping assays described in Genbank Accession No. AY326330. In our population, alternative amino acids at codons 136 and 154 were defined by a single SNP within each codon, whereas 2 SNP defined 3 amino acids at codon 171. The *PRNP* genotypes were classified using standard single-letter amino acid abbreviations at each locus.

To account for the effects of callipyge genotypes on carcass and meat quality traits, all sheep were genotyped at the callipyge locus using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer assay described by Freking et al. (2002). Animals were genotyped as $C^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, or $N^{Mat}N^{Pat}$ at the callipyge locus, with C representing the mutant allele and N representing the wild-type allele. Superscripts denoted the maternal (Mat) or paternal (Pat) origin of each allele. The 2 classes of heterozygous sheep ($C^{Mat}N^{Pat}$ and $N^{Mat}C^{Pat}$) were differentiated using flanking marker information for ovine chromosome 18 (Freking et al., 1998a).

Linkage distances for chromosome 13 were calculated using CRIMAP version 2.4 (Washington University, St. Louis, MO), and compared with the reported linkage distances (http://rubens.its.unimelb.edu.au/~JILLM/jill.htm). Genotyping errors and deviations from the reported linkage distances were investigated using the CHROMPIC option of CRIMAP. The lack of genotypic information and the inability to resolve pedigree inconsistencies using genotypic data resulted in deletion of data collected on 13 F_2 lambs. No recombinant gametes were detected within the interval containing *PRNP*_136, *PRNP*_154, *PRNP*_171, and *PRNP_S11* loci (Figure 1), and haplotypes were determined based on phase information. The probability of grandparental origin (Dorset or Romanov) of the *PRNP* haplotype for each gamete was estimated using Genoprob (Thallman, 2002). Following Genoprob analysis, the \PRNP_*S11* locus was no longer considered part of the *PRNP* haplotypes, because this locus has no known association with scrapie susceptibility.

Five *PRNP* haplotypes of Dorset origin (ARR, ARQ, VRQ, AHQ, and ARH) and 3 of Romanov origin (ARR, ARQ, and VRQ) were segregating in the F_2 population (Table 2). Because of the low frequency of the Dorset ARH haplotype (n = 4), these data were removed from subsequent analyses. Seven probabilities were calculated for each gamete, each corresponding to the probability of inheriting 1 of the 7 breed-specific *PRNP* haplo-

Table 2. Genotype distribution of haplotypes defined at codons 136, 154, and 171 of the *PRNP* gene in a Dorset × Romanov F_2 population

	ARQ	ARR	ARH	VRQ	AHQ	Frequency[1]
ARQ	63[2]	120	0	63	15	0.387
ARR		36	2	70	15	0.333
ARH			0	2	0	0.005
VRQ				13	20	0.216
AHQ					0	0.060

[1]Frequency of each *PRNP* haplotype within the F_2 population.
[2]Number of F_2 sheep with the corresponding diploid *PRNP* haplotype.

types. Probabilities summed to 2 within each lamb, as each F_2 lamb contained 2 *PRNP* haplotypes. Breed of origin probabilities were distributed in a bimodal manner, with the majority of probabilities greater than 0.95 or less than 0.05. The small number of intermediate probabilities was most likely due to incomplete genotypic information or low marker informativeness for some F_2 chromosomes.

Statistical Analyses

Growth and carcass traits were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) and a basic model that included the random effect of sire, fixed effects of birth year, sex, and callipyge genotype, and 7 covariates, each corresponding to the sum of probabilities (essentially 0, 1, or 2) of the breed-specific haplotype in question. To test for associations within breed of origin, specific covariates for Dorset and Romanov haplotypes were included in the model rather than pooled across breeds. That is, haplotype covariates were nested within breed of origin. There is no need to assume that associations, if they exist, are similar in Romanov and Dorset; associations were therefore tested within each breed of origin. To find solutions for the completely confounded covariates, the Romanov VRQ covariate was set to zero. Callipyge genotypes were grouped into 2 classes: $N^{Mat}C^{Pat}$ and NN (which consisted of $C^{Mat}C^{Pat}$, $N^{Mat}N^{Pat}$, and $C^{Mat}N^{Pat}$ sheep). For growth traits only, the additional fixed effect of litter size at birth was included in the model. The statistical model for carcass traits included the linear and quadratic effects of chilled carcass weight as a covariate and the interaction between the linear and quadratic effects of chilled carcass weight and callipyge genotype (Freking et al., 1998b).

Fitting the effect of *PRNP* haplotype in this manner, rather than including the assumed *PRNP* haplotype in the statistical model as a class variable (as in other studies), allows the *PRNP* haplotype to be weighted using the probabilities of breed origin. Probabilities located at the upper and lower extremes of the distribution will be weighted more heavily in the analyses than will intermediate probabilities. The power to detect true effects of the resistant ARR haplotype increases as the contribution of more uncertain genotypes (intermediate probabilities) is reduced.

A single contrast among regression coefficients of *PRNP* haplotype covariates was constructed within each breed of origin. For Dorset, the ARR coefficient was compared with the average of ARQ, VRQ, and AHQ (1, –0.33, –0.33, –0.33), whereas for Romanov, ARR was compared with ARQ and VRQ (1, –0.5, –0.5). Contrasts tested the difference between the resistant ARR haplotype and the average of the other haplotypes, consistent with the use of haplotype information by sheep industries in many countries.

Freking et al. (1999) discussed the nonnormal distribution of Warner-Braztler shear force residuals in these data. Dispersion of residuals increased with greater values of shear force, indicating a nonnormal distribution. Shear force values were transformed by natural logarithm before statistical analysis to account for the proportional relationship between standard deviations and means of callipyge genotypes.

Meat quality traits were analyzed using a model that included the random effect of sire, fixed effects of birth year, sex, and callipyge genotype, and 7 covariates, as described above. For analysis of transformed Warner-Bratzler shear force data, callipyge genotypes were grouped into 3 categories: $N^{Mat}C^{Pat}$, $C^{Mat}C^{Pat}$, and NN (which consisted of $N^{Mat}N^{Pat}$ and $C^{Mat}N^{Pat}$ sheep). For analysis of longissimus marbling data, callipyge genotypes were grouped into 4 categories ($C^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, and $N^{Mat}N^{Pat}$). For shear force, a birth year × callipyge genotype interaction was included to account for the differing distributions of shear force values in sheep born in 1994 and 1995 (Freking et al., 1999). A covariate of chilled carcass weight and the interaction between chilled carcass weight and callipyge genotype were included in the model for both meat quality traits. To test for associations between meat quality traits and *PRNP* haplotypes, breed-specific contrasts were constructed as described above.

RESULTS

The genotypic distribution of *PRNP* haplotypes in the F_2 population is presented in Table 2. Frequencies of the most resistant and most susceptible haplotypes (ARR and VRQ, respectively; Baylis and Goldmann, 2004) were 0.333 and 0.216, respectively. Frequency of the AHQ haplotype was only 0.060.

Table 3. Frequency of *PRNP* haplotypes by breed of origin in a Dorset × Romanov F_2 population

PRNP Haplotype	Breed of origin[1]	
	Dorset	Romanov
ARR	0.559	0.078
ARQ	0.099	0.726
VRQ	0.231	0.196
AHQ	0.111	0.000

[1]Frequency of each *PRNP* haplotype for F_2 gametes of Dorset or Romanov origin.

Dorset and Romanov haplotype frequencies differed greatly (Table 3), supporting the decision to include separate covariates for Romanov- and Dorset-derived haplotypes in statistical models. Because of this situation, the precision of estimates of the contrast of the ARR regression coefficient compared with the average of the other coefficients differed between breeds of origin. Using carcass traits as an example, there were 207 gametes of Dorset origin with the ARR haplotype, and 169 Dorset gametes with ARQ, VRQ, or AHQ haplotypes. For Romanov, there were 27 ARR gametes, compared with 305 ARQ or VQR gametes.

No significant contrasts between Dorset-derived PRNP haplotypes were detected. Romanov *PRNP* haplotypes were associated with rump width ($P = 0.013$), carcass length ($P = 0.038$), and longissimus marbling ($P = 0.022$). The Romanov ARR haplotype was associated with longer carcasses, narrower rumps, and less marbling than the average of ARQ and VRQ haplotypes (Table 4).

DISCUSSION

Data collected on 25 growth, carcass, and meat quality traits were analyzed for associations with Dorset and Romanov *PRNP* haplotypes. There is limited evidence herein that selecting for scrapie resistance will cause correlated responses due to linkage disequilibrium. The expected number of false positives ($P < 0.05$) for 50 statistical tests is 2.5 (50×0.05), a value consistent with results of this research. Associations of only 3 traits with *PRNP* haplotypes were detected in either breed of origin (Table 4), all 3 with Romanov. The Romanov breed ranks relatively high in its ability to deposit intramuscular fat (Shackelford et al., 2004), so a slight decrease in marbling is not a major issue. Consistent with within-breed results herein, there is evidence between Romanov and Dorset breeds that a QTL influencing skeletal development (metacarpal length) is located near the *PRNP* gene (B. J. Isler, B. A. Freking, and K. A. Leymaster, US Meat Animal Research Center, unpublished data).

Table 4. Significant contrasts for traits associated with *PRNP* haplotype in the Romanov breed of origin[1]

Trait	Estimate	*P*-value
Rump width, cm	–0.35 ± 0.14	0.013
Carcass length, cm	0.84 ± 0.40	0.038
Longissimus marbling[2]	–48.4 ± 21.0	0.022

[1]Contrast between scrapie resistant (ARR) and the average of ARQ and VRQ *PRNP* haplotypes.
[2]Subjective marbling score in which 300 equals slight and 400 equals small.

A number of studies have investigated associations between *PRNP* genotypes and economically important traits (Prokopova et al., 2002; de Vries et al., 2004, 2005; Alexander et al., 2005). However, only a single study (Brandsma et al., 2004) found multiple associations of *PRNP* genotypes with traits. In the study by Brandsma et al. (2004), associations were detected between the ARR haplotype and breeding values for litter size and 135-d weight in Dutch Texel sheep. Sheep homozygous for the ARR haplotype had greater breeding values for litter size and lesser breeding values for 135-d weight than sheep with zero or one copy of the ARR haplotype. In contrast, the current study did not detect an association between *PRNP* haplotypes and growth traits. However, the differing breed composition of the 2 studies makes direct comparison difficult. The genetic background (breed) of the population under study may influence associations between *PRNP* haplotypes and economically important traits.

In general, it is unlikely that detection of unfavorable associations of specific *PRNP* haplotypes with phenotypic variation of pertinent traits will be a common occurrence in sheep. There are several reasons for this statement. Most traits of economic importance are largely quantitative in nature, although the number of QTL reported in the scientific literature continues to increase. The *PRNP* locus on chromosome 13 segregates independently of loci on the other 26 ovine chromosomes. Within chromosome 13, associations between loci depend on linkage disequilibrium, a temporary phenomenon. If associations are detected, the *PRNP* haplotype resistant to scrapie may in fact be favorably, rather than unfavorably, associated with phenotypic variation. Consistent with goals of the US national scrapie eradication program and experimental results to date, increasing the frequency of resistant *PRNP* haplotypes should be considered a selection goal of breed development programs.

IMPLICATIONS

With the continuing concern about scrapie and other transmissible spongiform encephalopathies, the application of methods to reduce the incidence of these diseases is crucial to the livestock industry. Genetic markers associated with scrapie resistance are used to reduce the incidence of scrapie in sheep populations worldwide. However, before breeding programs focus on intensive selection for scrapie-resistant haplotypes, it is important to understand relationships between

haplotypes and economically important traits. The current study found little evidence of antagonistic relationships between the resistant prion haplotype and growth, carcass, or meat quality traits in crossbred sheep of Dorset and Romanov origin. Within the Dorset and Romanov breeds, advantages of selection for scrapie resistance seem to outweigh any potential adverse correlated responses.

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