GENETIC VARIATIONS AND ASSOCIATIONS FOR IMPROVING MEAT PRODUCTION AND MEAT QUALITIES IN SHEEP AND GOATS

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SUMMARY

Characteristics associated with a naturally occurring mutation of the callipyge gene in sheep are presented. Assignment of the callipyge locus to the telomeric region of ovine chromosome 18 was confirmed and an unusual mode of expression was verified. Heterozygotes (CN) receiving the mutant C allele from the sire and the wildtype N allele from the dam expressed extreme muscularity, whereas NC heterozygotes were of normal phenotype, that is, reciprocal heterozygotes produced different phenotypes. Furthermore, both homozygous genotypes (CC and NN) produced normal phenotypes. Callipyge lambs had greater dressing percentage, deposited lean tissue more rapidly and fat tissue less rapidly, and produced compact 25.67 kg carcasses with 71.9% lean compared to 64.4% carcass lean for normal lambs. Callipyge loin chops had less marbling and greatly increased shear force values, associated with evaluated levels of postmortem calpastatin activity. Despite the significant beneficial effects of the mutant callipyge allele, the detrimental effects on various meat quality traits must be circumvented before the allele can be exploited by the sheep industry.

Keywords: callipyge, imprinting, composition, QTL, carcass.

INTRODUCTION

Excellent general reviews pertaining to genetic aspects of developmental growth and meat quality of sheep and goats were published recently (*e.g.,* Bennett 1990; Dikeman 1990; Simm 1994; Koohmaraie 1995, 1996; Clarke *et al.* 1996). These authors discussed a broad range of genetic influences on the efficient production of lean meat and its association with pleasant eating experiences. Rather than retrace their collective, thorough efforts, we will focus on developments concerning the callipyge gene which have stimulated considerable interest in the U.S. and abroad due to significant effects on carcass and meat quality traits and the unusual mode of expression. Our research with a resource population segregating at the callipyge locus was recently summarized by the junior author (Freking 1997).

HISTORICAL PERSPECTIVE

In 1983, a Dorset ram was born in the Moffat flock of Oklahoma and expressed extreme muscular development, encouraging his use as a sire for the production of "club lambs", a showring segment of the U.S. sheep industry. The ram, named Solid Gold, passed on the muscular phenotype to only part of his progeny and further descendants. The extreme muscling was not evident at birth, but generally developed to a distinct degree by two months of age. The muscular phenotype was characterized by large longissimus muscles that often protruded above the spinous process, creating a trough appearance over the dorsal midline. Rear legs were very thickly muscled, particularly high

on the inner sides, and set wide apart; animals often appeared to walk with stiff, shuffling movement of their hindquarters. The unusual phenotype was not favored in the showring and its existence went largely unknown to sheep scientists for several years.

A graduate student at Texas Tech University, Sam Jackson, was the first researcher to study the genetic peculiarity. Matings were made in the fall of 1991, exposing upgraded rams (15/16 Rambouillet, 1/16 Dorset) that expressed the muscular phenotype to normal ewes. Progress reports of Jackson *et al.* (1992a,b,c) provided evidence consistent with a single, non recessive, autosomal gene with large effects on muscle and fat development. News of the Texas Tech findings spread quickly and several institutions secured well-muscled male descendants of Solid Gold from industry flocks to initiate research with matings in the fall of 1992. Researchers from at least 14 institutions published more than 30 abstracts from 1993 through 1996. The locus was assigned to the telomeric region of ovine chromosome 18 and given the descriptive name of *callipyge*, Greek for beautiful buttocks, in 1993 (Cockett *et al.* 1993, 1994). An unusual type of parental imprinting, initially termed dominant negative imprinting (Cockett *et al.* 1995) and subsequently polar overdominance (Cockett *et al.* 1996), was associated with the callipyge genotypes and phenotypes. As foreign producers and scientists keenly followed rapid developments, the mutant callipyge allele (C *vs* wildtype N) was exported into several countries, including Canada, UK, and Hungary.

The vast majority of experiments reported to date share several common characteristics. Experimental animals were produced by mating rams of callipyge phenotype (CN, with the C allele from the sire and the N allele from the dam) to normal ewes (NN), creating offspring representing two (CN, NN) of the four (CC, CN, NC, and NN) possible callipyge genotypes based on a single locus model. Progeny were subjectively classified as callipyge or normal phenotype based on repeated visual and/or tactile evaluation; ambiguous phenotypes were generally discarded. Often 30 or fewer animals contributed to a study, leading to rather imprecise estimates that generally were detected as statistically significant simply because the magnitudes of effects were so great. Animals within a study were typically sacrificed at a target weight, restricting statistical inference to a specific point in a biologically dynamic system. Furthermore, adjustment for unintended variation in carcass weight was based on a common regression rather than fitting regressions specific to each phenotypic class. Despite these design limitations, a fairly consistent pattern of results has emerged.

RESOURCE POPULATION

A resource population was produced to provide genotypic and phenotypic data to support a genomic scan for chromosomal regions containing loci contributing to the genetic variances of carcass and meat quality traits. The population was constructed to achieve allelic segregation at the callipyge locus in male and female parents of F_2 progeny. In this regard, the objectives were to position the callipyge locus using quantitative traits, to establish the type of gene action associated with the callipyge locus, and to estimate effects of the mutant callipyge allele on carcass and meat quality traits. Directly determined genotypic data, for any locus, were not available for use while the animal component of the experiment was being conducted.

Nine Dorset rams, descendants of Solid Gold and exhibiting the callipyge phenotype, were exposed to 255 Romanov ewes during 1992. The Romanov ewes were assumed to be noncarriers (NN) of the mutant callipyge allele, whereas the genotypes of the Dorset rams were unknown at the time of mating. The F_1 lambs were subjectively evaluated for expression of the callipyge phenotype at 4, 8, and 12 weeks of age; results demonstrated that each Dorset sire was heterozygous (CN) at the callipyge locus. A total of 152 F_1 ewe lambs were retained for breeding, preference given to ewes showing the callipyge phenotype. Only F_1 ram lambs evaluated as exhibiting the callipyge phenotype (CN) were selected as replacement sires. F_1 ewes and rams were *inter se* mated in 1993 and 1994 to produce two replicates of the F_2 generation. Six and five F_1 sires produced the first and second replicates, respectively, with three sires used in common, *i.e.*, eight sires over the two years. A total of 432 F_2 lambs were born to 138 F_1 ewes.

F_2 ewe and wether lambs were serially slaughtered at 23, 26, 29, 32, 35, and 38 weeks of age. Routine traits were recorded at the time of slaughter and on chilled carcasses. The right side of each carcass, including bone, was ground separately for proximate analysis to determine water, fat, protein, and ash content. Fat-free lean tissue was defined as the sum of water and protein. Loin chops from the left side of the carcass were scored for marbling, aged for 14 days and measured for Warner-Bratzler shear force value. Postmortem muscle calpastatin activity was measured by a heated calpastatin assay for the first replicate and by an indirect antibody enzyme-linked immunosorbent assay (ELISA) for the second replicate. Data were collected on 355 carcasses of F_2 lambs, with the exception that chemical composition data were only available for the first replicate (182 carcasses).

A total of 40 markers previously mapped to either ovine chromosome 18 (de Gortari *et al.* 1997) or the homologous bovine chromosome 21 (Kappes *et al.* 1997) were tested in the resource population; 30 loci provided some degree of information. CRI-MAP software was used to conduct linkage analyses and 22 loci were included in the final linkage group which spanned 86.3 cM. Output from CRI-MAP was used to determine the grandparental origin (Dorset or Romanov) of alleles for each chromosome of F_2 lambs and the phase of marker alleles associated with the C allele was determined for each Dorset sire. The probabilities of CC, CN, NC, and NN genotypes were calculated for each F_2 lamb at 1 cM intervals based on expected recombination rates with known phase information from flanking informative markers. Two sets of three orthogonal contrasts of the callipyge genotypes were developed to evaluate gene action. The traditional set included additive (CC-NN), dominance (CN+NC-CC-NN), and reciprocal heterozygote (CN-NC) effects. The second set was derived to include the polar overdominance model of Cockett *et al.* (1996) and consisted of the additive, polar overdominance (CN-CC-NC-NN), and maternal dominance (NC-CC-NN) effects. Each contrast was calculated within animal, with values ranging from -1 to 1. The statistical model fit discrete effects of year, sex, and sire, linear and quadratic covariate effects (slaughter age, carcass weight, or carcass fat), linear effects of three genotypic contrasts, and the interactions of the linear and quadratic covariate effects with genotypic contrasts. Analyses of several key traits (indicators of muscle size, carcass shape, and carcass composition) were conducted at each cM of the linkage group through 20 cM beyond the most telomeric marker. An overall genetic F-test, associated with nine degrees of freedom, was plotted for each analysis

and data from the position which maximized the F-test were used to evaluate the significance of each genotypic contrast and its interaction with the linear and quadratic covariate term for lack of fit. Insignificant blocks of effects were deleted from the model. A single position for the callipyge locus was arbitrarily determined from final analyses of key traits and remaining traits were evaluated at the chosen position.

RESULTS

Position and gene action of the callipyge locus. Twenty-two markers were placed in the ovine chromosome 18 linkage group based on 164 to 955 informative meioses per marker. Of note was the limited number of informative markers in the telomeric region of the linkage group where the callipyge locus was previously positioned by Cockett *et al.* (1994). Eight of the nine Dorset rams were informative for 11 to 16 markers each, whereas the remaining ram was informative for a single marker. Comparison of markers in common between this linkage group and the homologous bovine chromosome 21 (Kappes *et al.* 1997) indicated remarkable conservation of locus order and interval distance. The distribution of recombination events per gamete transmitted by F_1 parents to F_2 lambs revealed that 38% of gametes had no crossovers, 47% had a single crossover, and 15% had two or more crossovers. This distribution illustrates the difficulty in fine mapping a QTL locus which partly depends on use of animals with fortuitous recombinant events, yet such occurrences are rare in a single generation.

Preliminary analyses indicated that the reciprocal heterozygote genotypic contrast (CN-NC) was significant for key traits, therefore, the set of contrasts based on the polar overdominance model was investigated. The additive and maternal dominance genotypic contrasts were not statistically significant and were deleted from the final model, leaving the polar overdominance genotypic contrast as the only relevant genetic effect (Figure 1, $P<.0001$); these results confirm the polar overdominance hypothesis of Cockett *et al.* (1996). The approach used by the latter researchers was to assign a specific genotype to each animal and compare the subjective callipygous phenotypic classification with the expected phenotype based on the polar overdominance model. We tested the theory by regressing objectively measured traits on the polar overdominance contrast of genotypic probabilities calculated within animal. Regardless of the method used, qualitative or quantitative, polar overdominance was identified as the operative genetic model. In short, reciprocal heterozygotes expressed different phenotypes (polar), whereas the two homozygous genotypes produced similar, normal phenotypes (overdominance).

Maximum F-tests for the key traits placed the callipyge locus at relative positions 91.0 to 98.0 cM, however, the most telomeric marker was mapped to relative position 86.3 cM. It is not known to what extent chromosome 18 is represented by the existing linkage group, but, based on physical assignment of a common marker to the homologous bovine chromosome, perhaps 15% of the physical length of ovine chromosome 18 is not under marker coverage. The telomeric region lacks highly informative, quality PCR-based markers that exist elsewhere in the linkage group, reducing the ability to resolve the position of QTL. The callipyge locus was arbitrarily set at 91.0 cM for general analyses of traits but the position within this region is not critical as the genotypic probabilities change little because recombination events telomeric to the last marker cannot be

detected (Figure 1). Simulating genotypic and phenotypic characteristics of our data, a 95% confidence interval for QTL resolution covered relative positions 84 to 104 cM. This confidence interval may overlap the estimated positions of the callipyge locus reported by Cockett *et al.* (1994, 1996).

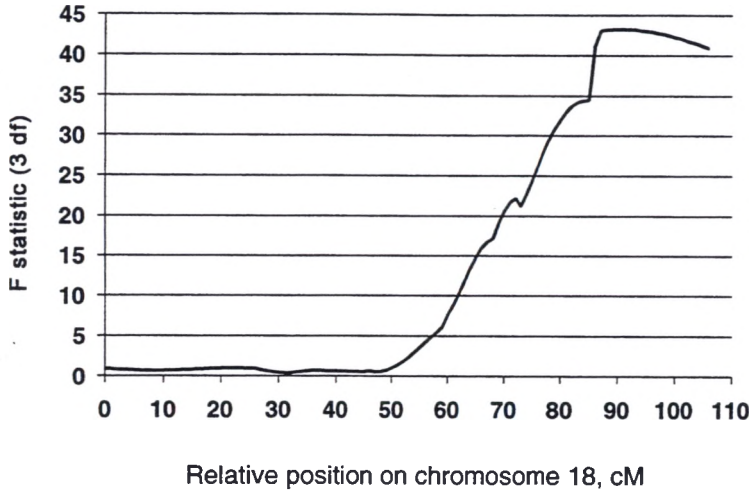

Figure 1. Profile of polar overdominance F-test to position callipyge locus from analysis of carcass fat-free lean regressed on carcass fat.

Effects of polar overdominance. The serial slaughter design allowed effects on response variables to be estimated over a wide range of slaughter ages and carcass weights. To simplify presentation, statistics calculated at the mean slaughter age (215 days) or carcass weight (25.67 kg) are given in Table 1. The callipyge phenotype is associated with the CN genotype, whereas the normal phenotype is associated with CC, NC, and NN genotypes. The callipyge effect is the difference between these phenotypes (genotypes), expressed in actual units of measurement and also residual standard deviation units to allow comparison among traits. Solutions to the equations underestimate true effects if the callipyge locus actually lies distal to the last marker because recombinations go undetected.

Constant slaughter age. Callipyge lambs were slightly lighter at the mean slaughter age while pelt, liver, and kidney-pelvic fat weights also decreased. The latter effects contributed to an increase in carcass weight. Two of the most informative traits are weights of carcass fat and fat-free soft tissue (Figure 2). Callipyge carcasses had more fat-free soft tissue at the initial slaughter group and this advantage was essentially maintained as age increased. Lean growth rate was obviously greater prior to 23 weeks of age. The effect at the mean slaughter age was 1.06 residual standard deviations, accounting for 16.5% of the variance. Callipyge carcasses also had less carcass fat over

Table 1. Least-squares means of normal phenotype (CC, NC, NN), effects of callipyge phenotype (CN) and percent of F_2 variance due to polar overdominance

Trait	Normal	Callipyge effect[A]		Percent variation[B]
		AU	RSDU	
Constant slaughter age (215 d)				
Live weight (kg)	48.97	-1.90	-.35	.5
Pelt weight (kg)	6.174	-.454	-.55	2.8
Liver weight (kg)	.7855	-.0837	-.67	2.5
Kidney-pelvic fat (kg)	1.339	-.323	-.88	6.7
Carcass weight (kg)	26.27	.64	.20	2.3
Carcass fat-free soft tissue (kg)	15.80	2.09	1.06	16.5
Carcass fat (kg)	7.539	-1.829	-1.26	13.3
Constant carcass weight (25.67 kg)				
Live weight (kg)	49.43	-2.80	-1.24	13.7
Dressing percentage (%)	53.13	3.25	1.26	14.4
12th rib fat depth (cm)	.5392	-.1859	-1.04	13.6
4th sacral fat depth (cm)	1.593	-.355	-1.00	10.7
Carcass length (cm)	63.21	-2.31	-1.51	21.6
Femur length (cm)	19.44	-.47	-.75	7.4
Rump width (cm)	22.16	.97	1.77	30.7
Shoulder width (cm)	19.79	.30	.38	2.3
Longissimus area (cm^2)	15.30	4.70	2.31	40.2
Carcass fat-free soft tissue (kg)	16.52	1.94	2.45	44.1
Carcass fat (kg)	7.998	-1.930	-2.41	43.2
Percentage lean (%)	64.36	7.52	2.46	45.1
Marbling score[C]	387.2	-148.7	-1.79	36.2
Shear force (kg)	3.879	3.339	1.83	33.0
Calpastatin activity (U/mg protein)				
Day 0	3.308	1.938	1.94	33.3
Day 7	2.669	2.353	2.13	44.0

[A]AU, actual units; RSDU, residual standard deviation units.

[B]The difference between the residual variances without and with the genetic effect, expressed as a percentage of the greater variance.

[C]Small = 300, Modest = 400.

the entire range of ages, the effect being -1.26 residual standard deviations at the mean age. Increased lean growth rate and decreased fat growth rate are therefore consequences of the mutant callipyge allele.

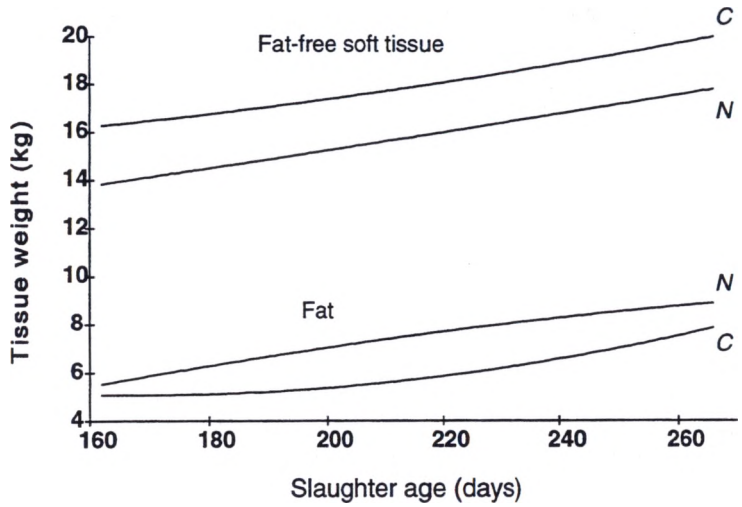

Figure 2. Relationships of carcass fat and fat-free soft tissue with slaughter age for callipyge (C) and normal (N) genotypes.

Constant carcass weight. Callipyge lambs were 2.80 kg lighter than normal lambs, an advantage in dressing percentage of 3.25% (actual units). Fat depths were reduced about 1 residual standard deviation at the 12th rib and 4th sacral vertebra. Differences in carcass shape as determined by objective measures were readily detected as callipyge carcasses were shorter, thicker, and more compact. The callipyge effect accounted for 21.6% of the variance in carcass length and femur length was also affected. Rump width increased by 1.77 residual standard deviations, accounting for 30.7% of the variance, whereas shoulder width was affected to a lesser degree. At the mean carcass weight, longissimus area increased 30%, an effect of 2.31 residual standard deviations. The increase in weight of fat-free soft tissue was similar to the decrease in fat weight, indicating little, if any, effect on bone weight. Percentage lean of normal carcasses was 64.4% compared to 71.9% for callipyge carcasses. Effects on composition traits were about 2.4 residual standard deviations, each accounting for over 40% of the variance. While the callipyge effects on carcass traits were extremely favorable, adverse effects were detected for meat quality. Callipyge loin chops had lower marbling scores, indicating less intramuscular fat. The force required to shear core samples of longissimus muscle from callipyge carcasses was 86% greater than normal carcasses at the mean weight and increased as weight increased. Accordingly, the level of calpastatin activity increased 58 and 88% at day 0 and 7, respectively. These effects on meat quality traits were over 1.7 residual standard deviations, accounting for at least 33% of the variance of each trait. Collectively, other research indicates significantly greater shear force values, particularly for callipyge

longissimus muscle; there was also evidence of greater shear force variability. The increased levels of calpastatin and m-calpain caused reduced rate and extent of postmortem proteolysis and, therefore, increased toughness (Koohmaraie *et al.* 1995). Callipyge meat was less juicy and evaluated as possessing less intense flavor, perhaps due to a lower level of marbling.

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