Pleiotropic effects in Hereford, Limousin, and Piedmontese F_2 crossbred calves of genes controlling muscularity including the Piedmontese myostatin allele[1]

R. E. Short*[2], M. D. MacNeil*, M. D. Grosz*[3], D. E. Gerrard†, and E. E. Grings*[4,5]

*Fort Keogh Livestock and Range Research Laboratory, ARS, USDA, Miles City, MT 59301 and
†Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

ABSTRACT: Objectives were to determine 1) effects on traits measured from birth to slaughter in F_2 cross calves from sire breeds that differ in potential for lean tissue growth but have similar mature BW and 2) the gene action of the mutant Piedmontese myostatin allele. Hereford (normal muscling, H), Limousin (moderate increase in muscling, L), and Piedmontese (muscular hypertrophy, P) sires (20 to 25 per breed) were bred at random to crossbred cows to produce F_1 calves that were *inter se*-mated within sire breed to produce F_2 calves that were grown out, finished, and slaughtered. Piedmontese-cross calves were genotyped for the G-A transition mutation at the myostatin locus characteristic of P (msP). Genotypes were classified on the basis of having zero (P_0), one (P_1), or two (P_2) copies of msP (H, n = 227; L, n = 207; P_0, n = 40; P_1, n = 107; and P_2, n = 37). Limousin-cross F_2 calves had heavier birth (but dystocia was not affected) and weaning weights, gained faster, had more muscle, less fat, larger pelvic area, and more efficient feed conversion than Hereford-cross F_2 calves. Normal-muscled Piedmontese-cross F_2 calves (P_0) were similar to Hereford-cross F_2 calves except that they required less assistance at birth in heifer dams, had less fat, gained slower, were less efficient, and had larger pelvic area. Addition of msP alleles (P_1 and P_2) consistently increased muscle through hyperplasia, decreased fat, and increased adjusted efficiency, but many of those changes were not linear. Residual variances for breed were heterogeneous for most traits related to muscularity. This heterogeneity was caused by increased variances for L and P and(or) lower variances for H. Accounting for the msP alleles decreased the variance for P in most traits, but heterogeneity remained for most traits among the five genotypes because L remained high, H was low, and(or) P_2 was low. We conclude that differences in muscularity affect most traits, and when differences in muscularity include the msP allele, there is an incremental, but not equal, change in most traits with the addition of each copy of the msP allele. Advantages of L could be captured through normal crossbreeding and selection schemes but with some caution because of potential problems from increased variability. Advantages of P could be best captured through more complex breeding and selection programs that would lessen potential negative impacts and through marketing systems that do not penalize for very low fat.

Key Words: Animal Production, Cattle, Muscular Hypertrophy, Pleiotropy

 J. Anim. Sci. 2002. 80:1–11

Introduction

Decreasing fat may be an approach to increase efficiency and consumer acceptability of beef (Byers et al.,

[1]This research was conducted under a cooperative agreement between USDA, ARS and the Montana Agric. Exp. Sta. USDA-ARS, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

[2]Correspondence: Rt. 1, Box 2021 (phone: 406/232-8235; fax: 406/232-8209; E-mail: bshort@larrl.ars.usda.gov).

[3]Current address: GenomicFX, 12024 Visa Parke Dr., Austin, TX 78645.

[4]We give special thanks to those who meticulously and diligently provided input into this research and who really made all of this possible: Butch Arnoldt, Benny Bryan, Rick Harris, Dave Phelps, and Mike Woods for animal care and feeding; Brooke Shipp for feed and slaughter data collection, WB shear tests, ultrasound scans and tracings, and data summaries; Sue Reil for feed analyses; Norman Bellows for ultrasound scans and tracings; Byron Hould for slaughter arrangements; Sandi Lockie for animal records; Larry French for genotyping; and Mary Ellen French for feed data summaries and manuscript preparation.

[5]We gratefully acknowledge the assistance of the North American Limousin Foundation and the Piedmontese Association of the United States in soliciting semen from their respective memberships. Donations of Limousin semen by Charlie Hunt, Shane Lindsay, Terry O'Neill, Bob Yackley, Mark Smith, Bullis Creek, JCL Cattle Co., Randy Winter, Walters and Gordon, Karl Dean, and Jerry Bayli are appreciated. Donations of Piedmontese semen by Laurence Soukup, Kalista Farms, Ritter Piedmontese, Harry Armstrong, Norway Farms, Georgetown Farms, Wilmeth Cattle Co., and MyAnn Farm are likewise appreciated. Finally, we acknowledge the donation of additional semen from Hereford, Limousin, and Piedmontese bulls by American Breeders Service.

Received December 4, 2000.

Accepted August 1, 2001.

1988). However, breeding programs that focus only on fat/lean traits must ensure that total production efficiency and product consistency are not compromised because of concurrent responses in other traits (pleiotropism). Moderate changes in muscularity and composition can be achieved using breeds such as Limousin (Koch et al., 1976), but effects that are more dramatic can be achieved with breeds such as Piedmontese that are double-muscled (Wheeler et al., 1997; Cundiff et al., 1998a,b).

Control of skeletal muscle growth is at least partly accomplished through the putative hormone myostatin, which limits or regulates formation of muscle cells (Kambadur et al., 1997). There are several known mutations of the myostatin gene that result in an interruption of the production or activity of myostatin (Grobet et al., 1998), and those mutations cause a condition known as double muscling (dramatic increase in skeletal muscle development). A trait that was most likely double muscling was first identified by Culley (1807) and Youatt (1834). In spite of extensive subsequent research, the mechanism(s) that control expression of the gene and its various alleles that cause double muscling are not completely understood (Oliver and Cartwright, 1968; Arthur, 1995). Recent advances in molecular genetics have resulted in assays that accurately identify occurrences of myostatin mutations, which allow exact determinations of genotypes (Fahrenkrug et al., 1999) and, in appropriately designed experiments, gene action.

The objectives of this research were to determine 1) effects on means and variances of traits measured from birth to slaughter in F_2 cross calves from sire breeds that differ in potential for lean tissue growth but have similar mature weights and 2) the gene action of the inactive mutant Piedmontese myostatin allele.

Materials and Methods

Hereford (**H**), Limousin (**L**), and Piedmontese (**P**) bulls (20 to 25 bulls per breed selected from bulls with semen available and as unrelated as possible) were bred at random to crossbred cows to produce F_1 calves over a 3-yr period (1994 to 1996). These sire breeds were selected to represent genotypes that are similar in mature body size but differ in degree of muscularity (Koch et al., 1976; Wheeler et al., 1997; Cundiff et al., 1998a,b). Hereford was considered a control genotype for degree of muscularity, Limousin represented a moderate increase in muscularity, and Piedmontese represented a dramatic increase in muscularity through the effects of a mutated myostatin gene. The majority of the Hereford bulls were from Line 1, an inbred line (average inbreeding coefficient = 0.33) that has been closed since 1934 and have primarily been selected for increased yearling weight (MacNeil et al., 1992), although six were Hereford reference sires that were mostly unrelated to the Line 1 bulls. The F_1 progeny of the Hereford bulls contributed to a simultaneous investigation of response to selection for either high yearling weight or for below-average birth weight and high yearling weight (MacNeil et al., 1999). The crossbred cows that produced the F_1 calves were mainly a composite breed (**CGC**) consisting of ½ Red Angus, ¼ Charolais, and ¼ Tarentaise germplasm, but some dams also included Angus, Hereford, Simmental, and other Continental breeds in their genetic background.

From F_1 calves born in the 1st yr, 8 to 10 bulls from within each sire breed (total of 27) were selected at random (but restricted to those with CGC dams and only one per original sire) to be used throughout the study. These bulls were *inter se*-mated (by natural service or AI for 45 d starting about June 15) at random to F_1 females of the same sire breed (except half-sib matings were not allowed) to produce F_2 calves. The remaining F_1 males from the 1st yr and all males from the 2nd and 3rd yr were fed out and the data were used to characterize management and breed effects (Grings et al., 2001; MacNeil et al., 2001). The *inter se* mating system to produce the F_2 generation was designed to allow alleles of major genes to segregate independently so that the genotypic and phenotypic effects of these alleles on means and variances could be identified.

Spring-born, F_2 calves were produced over a 3-yr period (1996 to 1998), and a set of fall-born calves was produced during the 2nd yr of the study. The management of the fall-born calves included a 90-d grazing period during the growing phase, and none of these calves was individually fed during the finishing phase. Otherwise, management was the same as for the spring-born calves. The group of fall-born calves was included in the analyses as an additional contemporary group (year). Only two F_1 bulls chosen at random from each sire breed were used to produce the calves in the fall-calving group of the 2nd yr and the calves for the 3rd yr.

Data collected at calving (F_1 dams giving birth to F_2 calves) included birth weight, dystocia score (Bellows et al., 1971a,b), and percentage of dams with assisted deliveries. All F_2 calves were weaned at approximately 6 mo of age and were group-fed a growing diet (Table 1) until weekly weights for heifers were ≥ 341 kg and for steers were ≥ 386 kg. Animals were then individually switched to the finishing diet (Table 1).

To obtain individual animal feed intake measurements during the finishing period, animals were moved during the growing phase into an individual animal feeding barn that had a 150-animal capacity with six animals per pen. Feed intake was monitored with a Calan-Broadbent feeding system (American Calan, Northwood, NH) in which feed was weighed into individual bunks and access to each bunk was restricted by an individual animal electronic identification system. Animals were stratified by sex, sire breed, and weight for assignment to pens and, within pen, assignments to feeding gates were at random with two animals from each sire breed within each pen. If there

Table 1. Content of diets (% on a DM basis) fed during the growing and finishing phases

	Diet	
Component	Growing	Finishing
Corn silage	49.70	42.80
Barley	31.00	53.40
Alfalfa hay	14.00	—
Soybean meal	3.70	2.60
Urea	0.80	0.50
Calcium carbonate	0.40	0.30
Salt	0.20	0.16
Vitamin A, D, E[a]	0.10	0.06
Trace mineral mix[b]	0.10	0.05
Vitamin E[c]	—	0.10

[a]Contains 4,400 kIU/kg vitamin A, 440 kIU/kg vitamin D, and 122 kIU/kg vitamin E.
[b]Contains 20% Mg, 2.7% S, 6% Zn, 5% Fe, 4% Mn, 1.5% Cu, 0.11% I, 0.01% Co, and 0.01% Se with wheat mids and mineral oil as carriers.
[c]Contains 44.05 kIU/kg vitamin E.

were more than 150 animals in any given year, the excess were pen-fed during both the growing and finishing periods. Animals were weighed weekly, and the weight on the week that the target weight was attained was recorded as the on-test weight. During the week of the on-test weight, each animal was measured for hip height and ultrasound scans were taken to estimate semitendinosus area (scan taken from the posterior surface of the right round at the apex of the curvature, approximately halfway down the round), longissimus muscle area, and backfat (scans for longissimus muscle area and backfat were taken between the 12th and 13th rib of the right side).

As each individual animal attained the target weight it was switched to the finishing diet that was fed for either 90 or 132 d. Cattle were slaughtered once per week at the end of the finishing period, and because of limitations on weekly slaughter capacity, days on the finishing diet were allowed to vary ± 1 wk. Therefore, days-on-test for the finishing diet was included as a continuous linear variable rather than as a discrete 90 or 132 days-on-test. Samples of the as-fed diet were obtained weekly during the finishing period. Composite samples were analyzed for nutrient composition (AOAC, 1990). These data were used to convert feed intake data to ME and CP based on analyses of samples taken during the corresponding feed intake period.

Before slaughter, each animal was weighed and assigned a visual muscle score (1 = least to 9 = most muscling, where a score of 5 = average muscling for a Hereford-cross steer). On the day of slaughter, liver weight, hot carcass weight, and pelvic area (only for fall-born yr-2 and yr-3 calves, estimated by multiplying pelvic height by pelvic width) were recorded. The split carcass was allowed to hang in a cooler (4°C) for 2 d. Then the left half was processed to obtain cold carcass weight; longissimus muscle area between the 12th and 13th ribs; marbling score (based on BIF [1996] standards, where degree of marbling scores of 3.0 to 3.9 denote trace amounts [USDA Standard], 4.0 to 4.9 denote slight amounts [USDA Select], 5.0 to 5.9 denote small amounts [USDA Low Choice], etc.); and fat depth over the 12th and 13th rib. The left half was further fabricated into standard, untrimmed cuts that included weights for the boneless two-piece chuck, 107 rib, 2 × 3 short loin, sirloin, 160 round, boneless brisket, plate, flank steak, butt tender, and kidney-pelvic fat. The first five of these cuts were added together to estimate the weight of primal cuts. The data from the left half were multiplied by 2 to estimate total carcass data. Yield grade (where lower values are less fat trim and more meat yield) was calculated based on the following formula (BIF, 1996): yield grade = 2.5 + (2.5 × fat depth) + (0.0038 × hot carcass weight) + (0.2 × kidney-pelvic-heart fat) – (0.32 × ribeye area).

A 2.5-cm-thick rib steak was cut from the posterior end of the 107 rib, weighed, aged for 14 d at 0°C, frozen at –10°C, and later evaluated for tenderness using a Warner-Bratzler shear apparatus using procedures recommended by Wheeler et al. (1996). Steaks were thawed for 48 h (4°C) and cooked over an electric grill to an internal temperature of 70°C. Cooked steaks were cooled to room temperature for 2 h before core samples were taken (1.25 cm diameter). Five or six cores were tested and averaged to determine shear force.

During carcass fabrication, samples were collected from the semitendinosus and biceps femoris muscles at a point midway down the round for histological evaluation. A 0.5- × 1.0-cm sample was frozen in liquid nitrogen and later processed to determine concentration (number/mm^2) and average cross-sectional area of muscle cells (modified procedures from Gerrard and Judge, 1993). Transverse serial sections of 10 mm were cut on a cryostat (Microm 505M, Waldorf, Germany) at –20°C, dried, fixed, and stained with hematoxylin and eosin. Images from three randomly selected fields from each cross section were captured. Images were processed with Adobe Photoshop (Version 3.0, Adobe Systems, Mountain View, CA) and individual muscle fibers were identified. Images were then segmented and quantified using IPLabs software (Version 3.0.6, Scanalytics, Fairfax, VA).

Blood samples from Piedmontese-cross animals were processed to obtain DNA from white blood cells. The DNA was assayed for the presence of the G-to-A transition at position 938 of the myostatin locus characteristic of Piedmontese (Fahrenkrug et al., 1999). Genotypes were classified based on having zero (**P_0**), one (**P_1**), or two (**P_2**) copies of the mutant allele (**msP**). This assay does not detect other possible mutant alleles. Piedmontese alleles that were not classified as msP as well as both myostatin alleles in Hereford-cross and Limousin-cross were assumed to be normal (**ms**+), which resulted in a breed classification of H, L, and P and a genotype classification of H, L, P_0, P_1, and P_2.

Data were analyzed by a mixed-model ANOVA in SAS (PROC MIXED, SAS, Version 7.00 TS Level 00P1; SAS Inst. Inc., Cary, NC). The original model included all main effects and two-way interactions, but the final analyses used a reduced model that included sire nested within breed as a random variable and breed, genotype nested within breed, sex, days-on-test, year, sex × year, and sex × days-on-test as fixed effects. For efficiency variables and variables measured from birth to weaning, days-on-test and sex × days-on-test, were not included in the model, and for the variables measured at birth and weaning, the effect of age of dam (heifer vs cow) was included in the model.

The main objectives of this experiment were to evaluate the effects of breed and genotype. The other variables were included either as an aid in conducting the experiment or to determine whether they would interact with the breed and genotype classifications. Sex, days-on-test, year, sex × year, and sex × days-on-test effects were highly significant ($P < 0.05$ or greater) for most traits, but none of them interacted with breed and genotype. Therefore, the only variables presented in this paper will be breed and genotype. When significant *F*-statistics were obtained, specific comparisons that are relevant to specific objectives were tested with pairwise *t*-tests. The specific objectives tested pertained to the differences among the five breed and genotype classifications (Table 2). Breed effects were evaluated with Comparisons 1 (H – L) and 2 (H – P_0), and the effects of the msP allele were evaluated with Comparisons 3 and 4. The linear response (additive) to msP was obtained in Comparison 3 ($P_0 - P_2$) and the nonlinear (nonadditive) response in Comparison 4 [$P_1 - 0.5(P_0 + P_2)$].

Efficiency of gain during the finishing phase was calculated two ways. The first was Efficiency = live weight gain/energy intake. The second involved an adjustment of live weight gain for yield of primal cuts for each animal: Adjusted live weight gain = live weight gain (primal cut weight/live weight). Adjusted efficiency was calculated as adjusted live weight gain/energy intake.

In the F_2 generation, alleles segregated independently. One potential consequence of this segregation is that the variation in the F_2 population may be increased, and then if the segregated alleles can be identified, as in the case of msP and ms+, it is possible to determine whether the increased variation is associated with specific allelic combinations. To determine whether this situation existed, selected traits that were considered directly related to muscularity were examined. Residual variances (SD) for each breed and genotype were calculated from an ANOVA using the general linear model of SAS (PROC GLM) with either breed or genotype in the model as well sex, year, days-on-test, sire, and sex × year. Heterogeneity among breed and genotype variances (SD) was evaluated with Bartlett's test.

This research was approved by the Fort Keogh LARRL Animal Care and Use Committee.

Results

It is important to emphasize that there were significant effects of sex, year, days-on-test, and some interactions on most variables, but in no case were there any interactions of these variables with breed or genotype. The effects of breed and genotype discussed below were consistent across all other treatments.

Observed frequencies of the P_0, P_1, and P_2 genotypes deviated from the 1:2:1 expected ratio ($\chi^2 = 4.19$, $P = 0.11$). The observed ratio is a sample of live animals at the time of weaning, and the apparent deviation from that expected could have occurred at any time before weaning.

Birth-to-Weaning Data (Table 2)

Birth weight was increased in Limousin-cross calves (Comparison 1, $P < 0.001$). There was no significant difference between Hereford-cross and P_0 calves, but birth weight increased linearly as one and two copies of the msP allele were added in Piedmontese calves (Comparison 3, $P < 0.001$). Dystocia score was increased in F_1, Piedmontese heifers (assumed to be P_1) as one and two copies of the msP allele were added in the calf (Comparison 3, $P < 0.01$), and there were no other significant effects of genotype on dystocia score in either heifers or cows. Effects of genotype on percentage of F_1 females requiring assistance at calving was similar to effects in dystocia score except that the incidence of assistance in heifers was lower when Piedmontese-cross dams were carrying P_0 calves (Comparison 2, $P < 0.1$), and, in Piedmontese-cross cows, there was still a slightly higher incidence when they were carrying P_2 calves (Comparison 3, $P < 0.1$). The only genotype effect on weaning weight was that Limousin-cross calves were heavier (Comparison 1, $P < 0.1$).

On-Test Data (Table 2)

Limousin-cross calves had larger (Comparison 1) longissimus muscle ($P < 0.001$) and semitendinosus ($P < 0.01$) areas and less fat depth ($P < 0.001$) and were younger ($P < 0.001$) at the on-test measurement. Piedmontese-cross (P_0, Comparison 2) calves did not differ in either longissimus muscle area or semitendinosus area, but they had less backfat ($P < 0.001$) and were older ($P < 0.01$) than Hereford-cross calves. Addition of msP alleles increased muscle area (both longissimus muscle area and semitendinosus area, Comparison 3, $P < 0.001$), with that increase being nonlinear (Comparison 4, $P < 0.001$). Fat depth was decreased (Comparison 3, $P < 0.001$) and age increased (Comparison 3, $P < 0.01$) by the addition of msP alleles, and those effects were nonlinear (Comparison 4, $P < 0.01$ for fat depth and $P < 0.05$ for age).

Table 2. Effects of genotype on production traits in F_2 calves (comparison of means)

	Genotype[a] means					Genotype comparison[b]				Pooled
Data	H	L	P_0	P_1	P_2	1	2	3	4	SD
Birth wt, kg	35.9	39.0	35.7	37.0	40.1	***	—	***	—	4.9
Dystocia score										
Heifers	1.60	1.54	1.24	1.85	2.20	—	—	**	—	0.94
Cows	1.04	1.04	1.01	1.00	1.08	—	—	—	—	0.28
% with dystocia										
Heifers	34.0	29.9	12.9	43.1	49.6	—	†	*	—	0.45
Cows	1.1	2.9	0.8	0.1	7.9	—	—	†	—	0.14
Weaning wt, kg	179	185	174	173	166	†	—	—	—	23.4
n	227	207	49	109	37					
On-test data										
Longissimus muscle area, cm^2	59.4	63.7	59.9	67.4	84.0	***	—	***	***	5.66
Semitendinosus area, cm^2	50.1	54.5	51.6	57.5	75.8	**	—	***	***	8.91
Fat depth, mm	6.43	4.97	4.68	4.41	2.84	***	***	***	**	1.44
Age, d	395	378	409	408	423	***	**	**	*	24.7
n	194	191	48	107	36					
Slaughter data										
Live wt, kg	475	480	464	465	458	—	**	—	—	18.6
Gain, kg	109	113	100	100	96	—	*	—	—	17.4
Carcass wt, kg	273	282	269	278	291	***	*	***	—	11.4
Dressing %	57.5	58.8	57.9	59.7	63.2	***	—	***	***	1.67
Longissimus muscle area, cm^2	74.3	81.4	74.3	86.4	109	***	—	***	***	8.50
% primal cuts	50.7	52.3	50.4	52.5	56.5	***	—	***	***	1.62
Muscling score	4.6	5.0	5.0	5.4	6.7	**	**	***	***	0.58
Marbling score	6.1	5.2	6.0	5.4	4.0	***	—	***	**	0.85
Fat depth, mm	9.8	7.4	6.3	5.6	2.6	***	***	***	**	2.67
Yield grade	2.53	1.99	2.13	1.51	0.10	***	***	***	***	0.56
Kidney pelvic fat, kg	9.14	9.27	10.80	8.79	5.21	—	**	***	*	2.04
Shear test, kg	4.2	4.3	4.2	4.1	4.0	—	—	—	—	0.93
Liver wt, kg	4.93	5.04	5.13	5.00	4.42	—	*	***	***	0.43
n	192	194	48	108	35					
Pelvic area, cm^2	170	174	184	174	168	—	***	*	—	14.7
n	70	60	19	30	13					
Histology data[c]										
ST number cells/mm^2	423	400	422	374	431	—	—	—	*	74.7
ST avg cell area, μ^2	1,867	2,009	1,832	2,104	1,873	—	—	—	*	411
n	16	18	21	20	14					
BF number cells/mm^2	451	438	455	466	448	—	—	—	—	85.6
BF avg cell area, μ^2	1,732	1,922	1,551	1,758	1,775	—	—	—	—	439
n	19	19	23	20	18					
Efficiency data										
Protein intake, kg	107	104	110	108	104	—	—	**	—	9.68
Energy intake, Mcal	2,390	2,339	2,457	2,414	2,302	—	—	**	—	219
Gain efficiency, g/Mcal	47.4	50.4	43.7	42.5	42.4	†	†	—	—	7.95
Adj efficiency, g/Mcal	13.7	15.4	12.6	13.2	15.2	***	†	***	—	2.49
n	130	136	29	80	28					

[a]H = Hereford, L = Limousin, P_0 = Piedmontese with no msP alleles, P_2 = Piedmontese with one copy of the msP allele, and P_2 = Piedmontese with two copies of the msP allele.
[b]Comparison 1 = H – L, Comparison 2 = H – P_0, Comparison 3 = P_0 – P_2, Comparison 4 = P_1 – 0.5(P_0 + P_2).
[c]ST = semitendinosus, BF = biceps femoris.
†$P < 0.1$.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Slaughter Data (Table 2)

All cattle were started on the finishing ration at the same weight and were finished for the same length of time; therefore, differences in BW at slaughter represent differences in rate of gain during the finishing period. Limousin-cross calves were marginally greater in weight (Comparison 1, $P = 0.16$) and gain (Comparison 1, $P = 0.23$), and Piedmontese-cross calves were consistently lighter (Comparison 2, $P < 0.01$) and gained less (Comparison 2, $P < 0.05$). Presence of one or two copies of the msP allele (Comparisons 3 and 4) had no effect on weight or gain. Limousin-cross had a higher dressing percentage and carcass weight than

Table 3. Effects of sire breed and genotype on variability (SD) of traits related to muscularity in F_2 calves

	Sire breed/genotype[a] SD						Heterogeneity[b]	
Trait	H	L	P	P_0	P_1	P_2	Breed	Genotype
Birth wt, kg	1.90	2.52	2.15	2.24	1.91	2.15	<0.001	<0.001
Weaning wt, kg	23.7	21.7	23.1	25.4	23.0	19.0	0.443	0.308
On-test data								
Longissimus muscle area, cm^2	5.87	7.20	10.54	7.02	7.21	8.78	<0.001	0.008
Semitendinosus area, cm^2	8.70	8.04	11.62	7.69	9.04	12.21	<0.001	0.013
Slaughter data								
Live wt, kg	17.1	18.8	19.4	22.2	18.0	19.2	0.205	0.208
Carcass wt, kg	9.4	12.7	13.3	11.9	11.1	10.6	<0.001	<0.001
Dressing %	1.61	1.80	2.33	1.36	1.47	1.36	<0.001	0.032
Longissimus muscle area, cm^2	6.3	10.1	13.5	7.8	7.3	9.7	<0.001	<0.001
% primal cuts	1.38	1.83	2.46	1.52	1.30	1.73	<0.001	<0.001
Muscling score	0.54	0.64	1.04	0.60	0.59	0.71	<0.001	0.098
Marbling score	0.95	0.76	1.03	0.87	0.77	0.72	<0.001	0.009
Fat depth, mm	2.77	2.70	2.55	2.17	2.41	1.71	0.522	0.004

[a]H = Hereford, L = Limousin, P = Piedmontese, P_0 = Piedmontese with no msP alleles, P_1 = Piedmontese with one copy of the msP allele, and P_2 = Piedmontese with two copies of the msP allele.
[b]Probability of SD being heterogeneous for sire breed (H, L, and P) and genotype (H, L, P_0, P_1, and P_2).

Hereford-cross (Comparison 1, $P < 0.001$), and the presence of one and two copies of the msP allele progressively increased carcass weight ($P < 0.001$). The increase in dressing percentage caused by addition of msP alleles was not linear (Comparisons 3 and 4, $P < 0.001$).

Traits that were considered a more direct measure of muscularity were longissimus muscle area, percentage primal cuts, and muscle score. These were all increased in Limousin-cross over Hereford-cross (Comparison 1, $P < 0.001$). The most dramatic effect was the incremental increase with the addition of one and two copies of the msP allele in Piedmontese (Comparison 3, $P < 0.001$), but that increase was not linear (Comparison 4, $P < 0.001$). Marbling score, fat depth over the loin, yield grade, and kidney-pelvic fat were used as indicators of fat deposition. The responses were the reverse of the muscularity traits, with Limousin crosses having less marbling and fat depth and a lower yield grade than Hereford crosses (Comparison 1, $P < 0.001$); however, Limousin crosses did not differ in amount of kidney-pelvic fat. Piedmontese-cross calves with no msP alleles (P_0) did not differ from Hereford-cross calves (Comparison 2) in marbling score but had a lesser fat depth ($P < 0.001$), a lower yield grade ($P < 0.001$), and less kidney-pelvic fat ($P < 0.01$). The addition of msP alleles within Piedmontese progressively decreased all four estimates of fat (Comparison 3, $P < 0.001$) and the decrease in all cases was nonlinear (Comparison 4, $P < 0.05$ to $P < 0.001$).

Liver weight is an indicator of visceral organ size and metabolic activity. Liver weight was not affected by Limousin, but it was increased in P_0 (Comparison 2, $P < 0.05$), and then it progressively decreased by addition of msP alleles within Piedmontese (Comparison 3, $P < 0.001$), with that decrease being nonlinear (Comparison 4, $P < 0.001$).

Tenderness as estimated by Warner-Bratzler shear test was not affected by breed or genotype.

Pelvic area at slaughter was not affected by Limousin crosses, but it was increased (Comparison 2, $P < 0.001$) in P_0 animals, and adding msP alleles within Piedmontese linearly decreased pelvic area (Comparison 3, $P < 0.05$).

Histology data from both the semitendinosus and biceps femoris muscles were not affected by breed or genotype, except that concentration of cells was less and cell area was larger from the semitendinosus muscles of the P_1 genotype (Comparison 4, $P < 0.05$).

Efficiency Data (Table 2)

Intake of protein and energy was not affected by breed, but as msP alleles were added within Piedmontese there was a linear decrease in intake (Comparison 3, $P < 0.01$). Because effects on intake were similar for protein and energy, only energy intake was used to calculate efficiency of gain (live weight gain/energy intake). Efficiency tended to be increased in Limousin crosses and decreased in P_0 (Comparisons 1 and 2, $P < 0.10$), with no effects of adding msP alleles within Piedmontese. Efficiency of live weight gain is not an accurate estimate of efficiency of edible product; therefore, efficiency of gain was adjusted to account for differences in dressing percentage and percentage primal cuts. This adjusted efficiency was increased dramatically in Limousin-cross animals (Comparison 1, $P < 0.001$), somewhat decreased in P_0 (Comparison 2, $P < 0.10$), and again dramatically increased linearly by adding msP alleles within Piedmontese (Comparison 3, $P < 0.001$); the net effect was that the adjusted efficiency of Limousin crosses and P_2 were the highest.

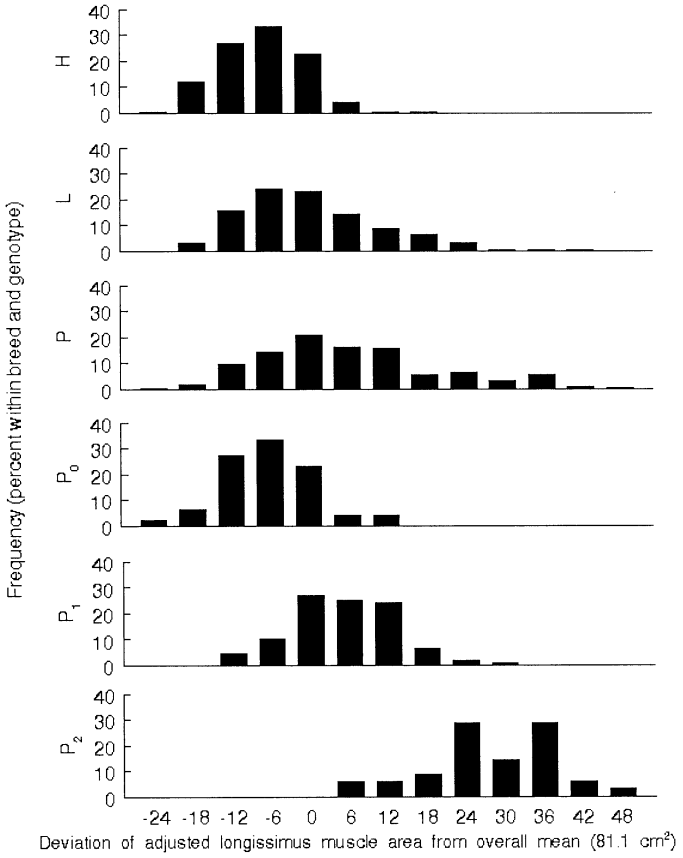

Figure 1. Effect of breed and genotype on distribution of longissimus muscle area adjusted for sex and time-on-feed.

Heterogeneity of Breed and Genotype Variances (Table 3)

Variances of birth weight for both breed and genotype were heterogeneous ($P < 0.001$), and in both cases the cause was a higher variance for Limousin. Partitioning the Piedmontese effect into genotypic components had little effect on the variance. There was no indication of heterogeneity of within-breed or -genotype variances for weaning weight.

At the on-test measurement, both longissimus muscle and semitendinosus area were heterogeneous for breed ($P < 0.001$) and genotype ($P < 0.01$). In the case of longissimus muscle area, the breed heterogeneity resulted from the Herefords being more uniform and the Piedmontese being more variable. When the Piedmontese were partitioned into the three genotypes, the variances went down but the genotypes were still heterogeneous because of the uniformity of the Hereford-cross F_2 progeny. In the case of semitendinosus area, the heterogeneity was caused by a high variance in the Piedmontese, and partitioning the Piedmontese variance into the three genotypes reduced the variances for two of the genotypes, but the P_2 variance remained high. Figure 1 illustrates the overlap of distributions of the breed and genotype classifications for longissimus muscle area.

At the time of slaughter, there was no indication of heterogeneity for variance of live weight in either breed or genotype. For all of the traits directly related to muscularity (carcass weight, dressing percentage, longissimus muscle area, percentage primal cuts, and muscling score) there was a consistent heterogeneity of variance for breed ($P < 0.001$); Piedmontese-cross was most variable, followed by Limousin-cross. Hereford-cross was least variable. Partitioning Piedmontese into the three genotypes reduced the amount of variation for these traits, but genotype variances remained heterogeneous ($P < 0.1$ to $P < 0.001$) because Limousin-cross remained high and Hereford-cross low.

For estimates of fatness at the time of slaughter, the heterogeneity was different from that of muscling. Marbling score was heterogeneous for breed ($P < 0.001$) because of a lower variance for Limousin, and when Piedmontese was partitioned, the variances for the Piedmontese genotypes and level of significance were reduced but still significant ($P < 0.01$) because Herefords remained relatively high. Fat depth was not heterogeneous for breed, but when Piedmontese were partitioned into the three genotypes, there was heterogeneity for genotype ($P < 0.01$). The reduction in variance within the Piedmontese was primarily a result of a decrease in the P_2 genotype. These animals were so lean that there was little opportunity for variation.

Discussion

The lower frequency of P_2 calves than expected would indicate a lower survival for this genotype. This differential survival has also been observed by Casas et al. (1999) and a producer (Jack Dees, personal communication). Most of this differential survival must occur before birth because very few calves were lost at birth or the differential was observed at birth. The cause of this differential survival is not known.

Some increase in muscularity was achieved by using Limousin. A larger increase was achieved by using the myostatin allele associated with Piedmontese. Both observations agree with previous research (Arthur, 1995; Wheeler et al., 1997). This increase in muscularity for both Limousin and Piedmontese crosses was accompanied by an increase in variability and skewness of distribution of most traits. There was no way of further accounting for the cause of these effects in Limousin crosses. This may be an indication that there are major gene effects on muscularity traits in Limousin that we were not able to identify, or the increased variation in Limousin crosses may be caused by the lower relationship among Limousin foundation sires compared to the Hereford foundation sires (MacNeil et al., 2001). In Piedmontese crosses, the effects on variability were reduced in most traits by partitioning into the three Piedmontese genotypes. Experimental designs in past research have not allowed alleles to

segregate independently and have not identified the genotype classes so that these effects on variability could be observed.

Previous research has attempted to determine the effects of muscular hypertrophy and the mode of gene action controlling it (Arthur, 1995). Some research used a crossing system to create heterozygous (ms+/msP), homozygous normal (ms+/ms+), and homozygous muscular hypertrophy (msP/msP) animals that confounded other aspects of the genome with this classification. Other research attempted to classify muscular hypertrophy genotypes based on various visual, carcass, or muscle characteristics that had the same confounding problem as the previous research, and it had the problem of accuracy of classification. The general conclusions (Swatland and Kieffer, 1974; West, 1974; Arthur, 1995) of this research were that muscular hypertrophy is mainly controlled by one gene, but the mode of action could not be exactly determined and varied from a simple dominant-recessive relationship (but there is disagreement as to whether it is dominant or recessive) among alleles at one locus to a more complicated incomplete recessiveness, partial dominance, or incomplete penetrance single-gene relationship or more complicated multiple-gene interactions. The overlap of variances shown in Figure 1 illustrates the problems that past research was confronted with when trying to classify genotypes on the basis of phenotypes.

Past research has shown that 1) the increased muscularity associated with the double-muscled phenotype is the result of increased numbers of muscle fibers (hyperplasia) rather than through increasing size of muscle fibers (hypertrophy), 2) the effect is initiated prenatally, and 3) the effect is caused by blood-borne products (Swatland and Keiffer, 1974; Gerrard et al., 1993). The increased muscularity in both Limousin and Piedmontese crosses that we observed in this study was also through hyperplasia because breed or genotype did not change muscle cell concentration or diameter. The only exception was that the P_1 genotype had a lower concentration and larger diameter of muscle cells in the semitendinosus muscle (hypertrophy). This effect was not observed in the biceps femoris muscle of the P_1 genotype, in either muscle of the Limousin or P_2 genotypes, or in the semitendinosus muscle of the P_1 genotype in the F_1 generation (R. E. Short et al., unpublished data). Because the hypertrophy effect was observed in only the one instance, we conclude that it was a random occurrence.

With the advent of molecular genetics, muscular hypertrophy has been definitively associated with the myostatin gene (Grobet et al., 1997; McPherron and Lee, 1997), various mutant alleles have been identified (Kambadur et al., 1997; McPherron and Lee, 1997; Grobet et al., 1998), and assays have been developed to detect some of the alleles (Antoniou and Grosz, 1999; Fahrenkrug et al., 1999). In our experiment, the F_2 generation should allow the possible alleles for any loci to segregate at random, independent of all other unlinked loci within the genome. Because we were able to identify the presence of the msP allele, the effects of P_0, P_1, and P_2 were evaluated independent of the effects of other genes. These data agree with the findings of Casas et al. (1998, 1999), who used both microsatellite markers and direct genotyping and found similar effects of the msP allele for some of the traits that we report here.

The gene products from the myostatin locus presumably act via autocrine or paracrine mechanisms to control myogenesis. Even though the activity of this gene product is not measured directly, some of the traits that were measured in this experiment, such as carcass weight, marbling, longissimus muscle area, percentage primal cuts, and muscle score are highly related to the effects of this gene and can be used to estimate the genetic control mechanisms. In these traits there is ample evidence that a major portion of the action of the myostatin gene product is additive. However, there is also evidence of some nonadditivity. The nonadditivity was always caused by the addition of the second msP allele having a larger effect than adding the first msP allele. Partial dominance is the term often used to describe this effect. Whether this nonadditivity is real or an artifact due to shape of the dose-response curve of myostatin (and its mutant forms) for the traits measured cannot be determined.

Abbreviations for the allele causing muscular hypertrophy have been m (Nott and Rollins, 1979), M (Bouton et al., 1982), or mh (Hanset and Michaux, 1985), with the normal allele being +. These abbreviations are awkward because there are several mutant versions and the actual gene product has been identified as myostatin, a putative hormone that inhibits myogenesis. Therefore, we have chosen to use ms+ as the abbreviation for the normal allele of this gene that produces active myostatin and msP as the Piedmontese mutant allele that produces inactive myostatin. Other mutations can then be accommodated (ms?) and the gene abbreviation reflects the gene product rather than a phenotypic effect that can be caused by several different mutations of the allele. Recently published assays can detect all known forms of the allele (Karim et al., 2000).

Data from the F_1 phase of this and other experiments (Wheeler et al., 1997; MacNeil et al., 2001) showed that there was a depression in rate of gain in Piedmontese-cross calves. It was hypothesized that this depression was caused by the presence of one copy of the msP allele. The data reported here provide evidence that the gain depression is not caused by the msP allele but is caused by other components of the Piedmontese genotype.

Problems with dystocia and neonatal survival have been consistent problems with double-muscled cattle (Oliver and Cartwright, 1968; Arthur, 1995), and these problems have been the main deterrent to more widespread use of this genetic trait. Bellows et al. (1971a,b)

showed that the main cause of dystocia is an incompatible relationship between birth weight and pelvic area. Addition of one and two msP alleles linearly increased birth weight and linearly decreased pelvic area; that is the first direct evidence (with both pelvic area and birth weight data) of why dystocia is such a problem. Casas et al. (1999) also reported similar effects on birth weight and calving difficulty, although they did not report the calving difficulty effects separately for heifers and cows. In their data, the addition of the second copy of the msP had the largest effect on calving difficulty, whereas in our data set the addition of the first copy had the largest effect. Whether this discrepancy is due to sampling errors or age of dam effects is not known. In both cases, the amount of data is relatively too small to make definitive conclusions for a trait such as calving difficulty and the nonlinear contrasts were not significant. There may have been some adaptation within the Piedmontese breed to compensate for this relationship between pelvic area and birth weight because P_0 calves had larger pelvic areas than Hereford-cross calves and F_1 Piedmontese-cross heifers giving birth to P_0 calves had less calving difficulty. Problems with dystocia in Piedmontese crosses can be kept at an acceptable level by limiting replacement females to having only one copy of the msP allele and breeding replacement heifers to homozygous normal bulls for the first calf crop.

Potential Mating Systems

The use of Hereford genotypes can be through any staightbred or crossbreeding system; their advantages are uniformity, adaptability to a wide range of environments, and ability to perform in production systems in which marketing of carcasses is based on USDA quality grades. Adoption of appropriate selection strategies and crossbreeding systems should stress complementary traits and reflect production objectives.

The use of Limousin genotypes for increasing growth and muscularity traits to a moderate degree could be straightforward. Straightbred and rotational crossbreeding systems could be used with appropriate selection within growth and muscularity traits as well as other complementary traits. Although not measured in this phase of the study, Limousin-cross heifers are older at puberty (Laster et al., 1976; Cundiff et al., 1986; MacNeil et al., 2001). In spite of heavier birth weights, dystocia was not a problem. Depending on the mating system, there could be some increased variability for some traits. The management and marketing of Limousin-based genotypes should take into account this potential for increased variability and the fact that carcasses will be leaner at given ages and weights.

The use of Piedmontese genotypes in breeding systems to capture the benefits of the msP allele are potentially more complicated because of negative effects on birth weight, pelvic area, and calving difficulty. Most researchers (Arthur, 1995; Casas et al., 1999) have concluded that the most appropriate use of double-muscled breeds such as Piedmontese is in a terminal cross system with homozygous normal (ms+/ms+) females bred to homozygous muscular hypertrophy (msP/msP) bulls. This is certainly a viable option to minimize the potential complications of increased dystocia. The limitations of that system are that replacement females must be generated in another system and the benefits of msP are captured only at a moderate level. Now that we know more about genetic control of muscular hypertrophy and have the ability to identify genotypes, a more aggressive approach is possible. We propose a two-step backcross system whereby heterozygous heifers would be bred to a homozygous normal bull for the first calf and then as cows they would be bred to homozygous msP bulls. Half of the calf crop from heifers would be homozygous normal and half would be heterozygous and half of the calf crop from cows would be heterozygous and half would be homozygous msP, which would take greater advantage of the msP allele. Replacement heifers could be either produced in another system such as the first terminal sire system or they could be selected from the heterozygous heifers produced within the system. In larger herds the bulls could be produced from within the system, but in most cases the bulls would be selected from another system. This two-step backcross system would minimize problems with dystocia while more completely capturing the advantages of the msP allele. Selection of replacement breeding stock would necessitate genotyping for the msP allele. The extreme option of breeding homozygous msP cows to homozygous bulls is probably not a viable option except for seedstock producers.

Now that the technology is available to genotype individual animals for msP, there may be instances in which it is desirable to introgress the msP allele into other genetic groups. This introgression can be accomplished efficiently with the aid of genotyping, and it would allow infusion of the msP allele into genomes with other favorable traits such as growth rate. However, care must be exercised to not also lose beneficial traits from the original genome such as younger age at puberty (Cundiff et al., 1996; Grings et al., 1999; MacNeil et al., 2001) for Piedmontese crosses.

In any system that incorporates the msP at some frequency, the management and marketing programs must take into account the decrease in fat at given ages and weights. It would be difficult with homozygous msP animals to achieve a USDA Choice grade at any age or weight. The marketing system must not stress higher proportions of fat, as does any system that relies on USDA quality grades. It is well documented that carcasses from both heterozygous and homozygous msP individuals have the potential to be completely acceptable and even preferred in marketing systems that do not rely on high fat (Arthur, 1995). Also, it is important to emphasize that improvement in

carcass traits gained through myostatin alleles should not rely only on that allele, because as shown in Figure 1, there is ample variation beyond the effects of msP to select for improvement. Casas et al. (2000) have shown that there are QTL within msP genotypes to allow for selection beyond the effects of the msP allele.

Implications

Hereford-based production systems produce high-quality beef with few associated problems and are adaptable to a wide range of conditions. Increased production of lean beef while maintaining tenderness can be accomplished to a moderate degree by using breeds such as Limousin. Effects that are more dramatic can be obtained with Piedmontese through the effects of the mutant myostatin gene that causes double muscling. The effects of this mutant gene for some traits is additive, but many traits also have some form of a nonadditive gene action. Opportunities exist to exploit the positive effects of increased muscularity, but care must be taken to account for all effects. Use of Limousin could be through normal crossbreeding and selection schemes. Advantages of Piedmontese and the msP allele would be best captured through more complex breeding and selection programs that would lessen potential negative effects on some traits and through marketing systems that do not penalize for very low fat.

Literature Cited

Antoniou, E., and M. D. Grosz. 1999. PCR based detection of bovine myostatin Q204X mutation. Anim. Genet. 30:231–232.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.

Arthur, P. F. 1995. Double muscling in cattle: A review. Aust. J. Agric. Res. 1493–1515.

BIF. 1996. Guidelines for uniform beef improvement programs. 7th ed. C. M. Bailey (ed.) Beef Improvement Federation, Colby, KS.

Bellows, R. A., R. B. Gibson, D. C. Anderson, and R. E. Short. 1971a. Precalving body size and pelvic area relationships in Hereford heifers. J. Anim. Sci. 33:455–457.

Bellows, R. A., R. E. Short, D. C. Anderson, B. W. Knapp, and O. F. Pahnish. 1971b. Cause and effect relationships associated with calving difficulty and calf birth weight. J. Anim. Sci. 33:407–415.

Bouton, P. E., P. V. Harris, and W. R. Shorthose. 1982. Comparison of some properties of beef from animals homozygous of heterozygous for muscular hypertrophy. Meat Sci. 6:309–318.

Byers, F. M., H. R. Cross, and G. T. Schelling. 1988. Integrated nutrition, genetics, and growth management programs for lean beef production. In: Designing Foods: Animal Product Options in the Marketplace. pp 283–291. National Academy Press, Washington, DC.

Casas, E., J. W. Keele, S. C. Fahrenkrug, T. P. L. Smith, L. V. Cundiff, and R. T. Stone. 1999. Quantitative analysis of birth, weaning, and yearling weights and calving difficulty in Piedmontese crossbreds segregating an inactive myostatin allele. J. Anim. Sci. 77:1686–1692.

Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, T. S. Sonstegard, T. P. L. Smith, S. M. Kappes, and R. T. Stone. 1998. Association of the muscle hypertrophy locus with carcass traits in beef cattle. J. Anim. Sci. 76:468–473.

Casas, E., S. D. Shackelford, J. W. Keele, R. T. Stone, S. M. Kappes, and M. Koohmaraie. 2000. Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. J. Anim. Sci. 78:560–569.

Culley, G. 1807. Observations on livestock. 4th ed. G. Woodfall, Paternoster-Row, London.

Cundiff, L. V., K. E. Gregory, and R. M. Koch. 1998a. Germplasm evaluation in beef cattle—cycle IV: birth and weaning traits. J. Anim. Sci. 76:2528–2535.

Cundiff, L. V., K. E. Gregory, R. M. Koch, and G. E. Dickerson. 1986. Genetic diversity among cattle breeds and its use to increase beef production efficiency in a temperate environment. In: Proc. 3rd World Congr. Genet. Appl. Livest. Prod., Clay Center, NE. 9:271–282.

Cundiff, L. V., K. E. Gregory, T. L. Wheeler, S. D. Shackelford, and M. Koohmararie. 1998b. Preliminary results from cycle V of the cattle germplasm evaluation program at the U. S. Meat Animal Research Center. In: Proc. 6th World Congr. Genet. Appl. Livest. Prod., Armidale, NSW, Australia. 23:185–188.

Cundiff, L. V., K. E. Gregory, T. L. Wheeler, S. D. Shackelford, M. Koohmararie, H. C. Freetly, and D. D. Lunstra. 1996. Preliminary results from cycle V of the cattle germplasm evaluation program at the Roman L. Hruska U. S. Meat Animal Research Center. Germplasm Evaluation Program Prog. Rep. No. 15. USDA-ARS, Clay Center, NE.

Fahrenkrug, S. C., E. Casas, J. W. Keele, and T. P. L. Smith. 1999. Direct genotyping of the double-muscling locus (*mh*) in Piedmontese and Belgian Blue cattle by fluorescent PCR. J. Anim. Sci. 77:2028–2030.

Gerrard, D. E., and M. D. Judge. 1993. Induction of myoblast proliferation in L6 myoblast cultures by fetal serum of double-muscled and normal cattle. J. Anim. Sci. 71:1464–1470.

Grings, E. E., R. E. Short, M. D. MacNeil, R. A. Roeder, and M. J. Roeder. 2001. Interactions in postweaning production of F_1 cattle from Hereford, Limousin, or Piedmontese sires. J. Anim. Sci. 79:317–324.

Grings E. E., R. B. Staigmiller, R. E. Short, R. A. Bellows, and M. D. MacNeil. 1999. Effects of stair-step nutrition and trace mineral supplementation on attainment of puberty in beef heifers of three sire breeds. J. Anim. Sci. 77:810–815.

Grobet, L., D. Poncelet, L. J. Royo, B. Brouwers, D. Pirottin, C. Michaux, F. Menissier, M. Zanotti, S. Dunner, and M. Georges. 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. Mamm. Genome 9:210–213.

Grobet, L., L. J. Royo-Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset, and M. Georges. 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat. Genet. 17:71–74.

Hanset, R., and C. Michaux. 1985. On the genetic determination of muscular hypertrophy in the Belgian White and Blue cattle breed I. Experimental data. Genet. Sel. Evol. 17:359–368.

Kambadur, R., M. Sharma, T. P. L. Smith, and J. J. Bass. 1997. Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Res. 7:910–915.

Karim, L., W. Coppieters, L. Grobet, A. Valentini, and M. Georges. 2000. Convenient genotyping of six myostatin mutations causing double-muscling in cattle using a multiplex oligonucleotide ligation assay. Anim. Genet. 31:396–399.

Koch, R. M., M. E. Dikeman, D. M. Allen, M. May, J. D. Crouse, and D. R. Campion. 1976. Characterization of biological types of cattle III. Carcass composition, quality and palatability. J. Anim. Sci. 43:48–62.

Laster, D. B., G. M. Smith, and K. E. Gregory. 1976. Characterization of biological types of cattle IV. Postweaning growth and puberty of heifers. J. Anim. Sci. 43:63–70.

MacNeil, M. D., R. E. Short, and E. E. Grings. 2001. Characterization of topcross progenies from Hereford, Limousin, and Piedmontese sires. J. Anim. Sci. 79:1751–1756.

MacNeil, M. D., R. E. Short, and J. J. Urick. 1999. Progeny testing sires selected by independent culling levels for below-average birth weight and high yearling weight or by mass selection for high yearling weight. J. Anim. Sci. 77:2345–2351.

MacNeil, M. D., J. J. Urick, S. Newman, and B. W. Knapp. 1992. Selection for postweaning growth in inbred Hereford cattle: The Fort Keogh, Montana Line 1 example. J. Anim. Sci. 70:723–733.

McPherron, A. C., and S. J. Lee. 1997. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA 94:12457–12461.

Nott, C. F. G., and W. Rollins. 1979. Effect of the m gene for muscular hypertrophy on birth weight and growth to one year of age in beef cattle. Growth 43:221–234.

Oliver, W. M., and T. C. Cartwright. 1968. Double muscling in cattle: A review of expression, genetics and economic implication. Texas A & M Agric. Exp. Station, Anim. Sci. Depart. Techn. Report No. 12, College Station.

Swatland, H. J., and N. M. Kieffer. 1974. Fetal development of the double muscled condition in cattle. J. Anim. Sci. 38:752–757.

West, R. L. 1974. Red to white fiber ratios as an index of double muscling in beef cattle. J. Anim. Sci. 38:1165–1175.

Wheeler, T. L., L. V. Cundiff, R. M. Kock, M. E. Dikeman, and J. D. Crouse. 1997. Characterization of different biological types of steers (cycle IV): Wholesale, subprimal, and retail product yields. J. Anim. Sci. 75:2389–2403.

Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 1996. Sampling, cooking, and coring effects on Warner-Bratzler shear force values in beef. J. Anim. Sci. 74:1553–1562.

Youatt, W. 1834. Cattle: their breeds, management, and diseases. Baldwin and Craddock, Paternoster-Row, London.