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Association of myostatin on early calf mortality, growth, and carcass composition traits in crossbred cattle[1,2]

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ABSTRACT: The objective of this study was to investigate a potential association of an inactive myostatin allele with early calf mortality, and evaluate its effect on growth and carcass traits in a crossbred population. Animals were obtained by mating F_1 cows to F_1 (Belgian Blue × British Breed) or Charolais sires. Cows were obtained from mating Hereford, Angus, and MARC III (¼ Hereford, ¼ Angus, ¼ Pinzgauer, and ¼ Red Poll) dams to Hereford, Angus, Tuli, Boran, Brahman, or Belgian Blue sires. Belgian Blue was the source of the inactive myostatin allele. Myostatin genotypes were determined for all animals including those that died before weaning. Early calf mortality was examined in the F_2 subpopulation (n = 154), derived from the F_1 sires mated to F_1 cows from Belgian Blue sires, to evaluate animals with zero, one, or two copies of inactive myostatin allele. An overall 1:2:1 ratio (homozygous active myostatin allele:heterozygous:homozygous inactive myostatin allele) was observed in the population; however, a comparison between calves dying before weaning and those alive at slaughter showed an unequal distribution across genotypes ($P < 0.01$). Calves with two copies of the inactive allele were more likely ($P < 0.01$) to die before weaning. Postweaning growth traits were evaluated in the surviving animals (n = 1,370), including birth, weaning, and live weight at slaughter, and postweaning ADG. Carcass composition traits analyzed were hot carcass weight, fat thickness, LM area, marbling score, USDA yield grade, estimated kidney, pelvic, and heart fat, retail product yield and weight, fat yield and weight, bone yield and weight, and percentage of carcasses classified as Choice. Charolais lack the inactive myostatin allele segregating in Belgian Blue; thus, in the population sired by Charolais (n = 645), only animals with zero or one copy of the inactive myostatin allele were evaluated. Animals carrying one copy were heavier at birth and at weaning, and their carcasses were leaner and more muscled. In the population sired by Belgian Blue × British Breed (n = 725), animals with two copies of inactive myostatin allele were heavier at birth, leaner, and had a higher proportion of muscle mass than animals with zero or one copies. Heterozygous animals were heaviest at weaning and had the highest live weight, whereas animals with zero copies had the highest fat content. The use of the inactive myostatin allele is an option to increase retail product yield, but considerations of conditions at calving are important to prevent mortality.

Key Words: Carcasses, Crossbred Cattle, Growth, Mortality, Myostatin

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Introduction

The Germplasm Evaluation program at the U.S. Meat Animal Research Center characterizes breeds representing several biological types of cattle. The fifth cycle of this program (Cycle V), includes two tropically adapted breeds (Tuli and Boran) compared with Brahman and the Belgian Blue breed, which has a high frequency of double-muscling. Growth, carcass composition, and meat quality traits have been evaluated in two generations from this cycle (Wheeler et al., 2001; Casas and Cundiff, 2003). Evaluation of these traits is important in establishing the potential value of alternative germplasm resources in the beef industry; however, the effect of the inactive myostatin allele that causes double muscling in Belgian Blue remained unevaluated on numerous traits in this population.

Myostatin was first discovered in mice (McPherron et al., 1997), and was then identified in cattle as the gene responsible for double muscling (Kambadur et al., 1997; McPherron and Lee, 1997; Grobet et al., 1998).

[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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Several distinct mutations have been identified that explain the increased muscling in cattle (Grobet et al., 1998; Dunner et al., 2003). One of those mutations is an 11-base pair deletion (Kambadur et al., 1997; Grobet et al., 1998; Dunner et al., 2003) in the third exon of the gene that results in the production of a truncated inactive protein. This allele has been associated with double muscling or muscle hypertrophy in several breeds (Dunner et al., 1997; Kambadur et al., 1997; Wiener et al., 2002) and is fixed in the Belgian Blue breed (Kambadur et al., 1997; Grobet et al., 1998; Dunner et al., 2003). The objective of this study was to examine the potential association of the inactive myostatin allele with early calf mortality, and to determine the effect of this allele on growth and carcass composition traits in crossbred cattle from the Cycle V of the Germplasm Evaluation program.

Materials and Methods

Animals

Casas and Cundiff (2003) provide a detailed explanation of the population and its management. Briefly, animals for this study were produced by F_1 cows. These F_1 cows were produced by mating Hereford, Angus, and MARC III (¼ Hereford, ¼ Angus, ¼ Pinzgauer, and ¼ Red Poll) mature dams to Angus, Hereford, Tuli, Boran, Brahman, and Belgian Blue sires by AI. To avoid confounding sire breed effects with heterosis effects, no purebred Hereford or Angus matings were made. Hereford and Angus were treated as one group (British Breeds). The F_1 cows obtained from these crosses were mated to Charolais, Belgian Blue × MARC III, Belgian Blue × Angus, or Belgian Blue × Hereford sires during two consecutive years. Matings were made by multisire natural service mounting within sire breed. Individual sires of progeny were not identified. All sires with Belgian Blue inheritance were treated as the same group (Belgian Blue × British Breed). Offspring were born during spring of 1998 and 1999. Ear notches were collected for calves that died between birth and weaning within 24 to 48 h after death. Male calves were castrated within 24 h of birth. Offspring were weaned in mid-October at an average age of 214 d (SD = 14 d). After a 30-d adjustment period, animals were randomly assigned to pens and fed separately by sire breed for 247 d (SD = 14 d). A growing diet was fed until animals reached approximately 320 kg, and then a finishing diet until slaughter. The growing diet included corn silage, corn, and a urea-based liquid supplement containing approximately 2.7 Mcal of ME/kg of DM and 12.5% CP. The finishing diet, fed from approximtely 320 kg to slaughter, contained approximately 3.05 Mcal of ME/kg of DM and 13.1% CP (Casas and Cundiff, 2003). Animals were slaughtered during the summers of 1999 (n = 695) and 2000 (n = 675).

Traits Analyzed

Preweaning mortality was included in the study. To obtain an unbiased estimate for the effect of myostatin, mortality was only evaluated in the Belgian Blue F_2 subpopulation (n = 154), consisting of offspring obtained from the Belgian Blue × British Breed sires mated to F_1 cows from Belgian Blue grandsires. This Belgian Blue F_2 subpopulation is the only one in which offspring can inherit two copies of the inactive myostatin allele. In all other crosses, it is impossible for the offspring to inherit two copies of this allele.

Traits analyzed for growth included birth weight, weaning weight, postweaning ADG, and live weight at slaughter. Carcass traits analyzed were hot carcass weight, fat thickness, LM area, estimated kidney, pelvic, and heart fat (percent), percentage of carcasses classified as USDA choice, marbling score, USDA yield grade (indicates the amount of usable meat from the carcass; a yield grade of 1 yields the greatest percentage of retail product, 5 the least), retail product percent and weight, fat percent and weight, and bone percent and weight. Retail product, fat, and bone yields were estimated using prediction equations that included carcass yield grade traits (LM area, adjusted fat thickness, and estimated kidney, pelvic, and heart fat) and marbling score (Shackelford et al., 1995).

Myostatin Genotyping

A saturated salt procedure (Miller et al., 1988) was used to obtain DNA from ear notches (dead animals) and blood (live animals). Blood samples were collected in 60-mL syringes with 7 to 10 mL of 4% (vol/vol) EDTA. Blood was spun at 1,300 × *g* for 25 min, and buffy coats were aspirated. Both tissues were frozen until DNA was extracted. Fahrenkrug et al. (1999) described the primers and amplification conditions. Samples were electrophoresed in 3% agarose gels for 2,000 volt-hours. Gels were stained with ethidium bromide and results recorded photographically. Animals were scored as having zero, one, or two copies of the inactive myostatin allele (11-base pair deletion), which corresponds to non-double-muscled, heterozygous and double-muscled animals, respectively.

Statistical Analyses

Preweaning mortality (1 = dead, 0 = alive) was calculated from animals that died before weaning. The total number of animals that survived to slaughter and the total number of animals included in the analysis are shown in Table 1. A likelihood ratio test (Lynch and Walsh, 1997) was used to establish whether preweaning mortality and survival to slaughter deviated from Mendelian inheritance for myostatin in the F_2 subpopulation derived from Belgian Blue. The likelihood ratio test has a sampling distribution of "G," which approximates a χ^2 distribution (Lynch and Walsh, 1997).

Table 1. Frequency of offspring with zero, one, or two copies of the inactive myostatin allele that were dead before weaning or that survived to slaughter, in the F_2 subpopulation of Belgian Blue × British Breed

No. of inactive myostatin alleles	Offspring dead before weaning		Offspring that survived to slaughter		Total No. of offspring	
	Observed	Expected	Observed	Expected	Observed	Expected
0	7	4	40	39	47	42
1	7	13	61	62	68	77
2	14	11	25	25	39	35
Total	28	28	126	126	154	154

Growth and carcass composition data were analyzed with the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model included the fixed effects of maternal grandsire breed (British Breed, Tuli, Boran, Brahman, and Belgian Blue), maternal granddam breed (Hereford, Angus, and MARC III), sire breed (Charolais and Belgian Blue × British Breed), sex class (steers and heifers), year of birth (1998 and 1999), all possible two-way interactions among these fixed effects, and number of copies of the inactive (11-base pair deletion) myostatin allele within sire breed (zero and one for Charolais, and zero, one, and two for Belgian Blue × British Breed). Random effects were maternal grandsire within breed and residual. Age at weaning and days on feed were included in the model as covariates for carcass composition traits. Levels of significance associated with the effect of grandsire breed were tested with the random effect. Least squares differences and probability values were estimated for the number of copies of the inactive myostatin allele. Probability values are nominal and do not correct for multiple testing.

Results

Mortality

A portion of the total population is an F_2 because the dams and the sires were Belgian Blue × British Breed crosses. In the calves from this portion of the population, Mendelian expectations were that the ratio of animals with zero, one, or two copies of the inactive myostatin allele would be 1:2:1. The total number of offspring did not differ from the expected ratio; however, a comparison between calves dying before weaning and those alive at slaughter showed an unequal distribution across genotypes ($G = 5.9$; $P < 0.015$). Calves with two copies of the inactive allele were more likely to die before weaning. The number of individuals in each group is shown in Table 1.

Growth and Carcass Composition Traits

The effects of maternal grandsire breed, maternal granddam breed, sire breed, and sex class have been previously reported (Casas and Cundiff, 2003); however, the effect of myostatin was excluded in the analysis. Nevertheless, it was possible to show that differences in growth and carcass traits exist among maternal grandsire breeds. No single maternal grandsire breed excels in every trait. Sire and maternal granddam breed differences allow for the optimization of postweaning growth and carcass traits by incorporating these breeds in selection and crossbreeding schemes.

Levels of significance, least squares means, and standard errors are reported in Table 2 for the effect of number of inactive myostatin alleles on growth and carcass composition traits. The effect of myostatin was significant for all traits ($P < 0.05$).

Offspring obtained from the Charolais sire breed segregated a maximum of one copy of the inactive myostatin allele. In spite of the absence of animals with two copies of the inactive myostatin allele, the effect of a single copy was detectable in most traits. Animals that inherited one copy of this allele were heavier at birth and at weaning, and had a heavier hot carcass weight ($P < 0.05$). These animals had larger LM area, lower USDA yield grade, larger retail product yield and retail product weight, and larger bone yield and bone weight ($P < 0.05$). Animals that inherited zero copies of the inactive myostatin allele were fatter than those inheriting one copy, and displayed more fat thickness, more fat yield, more fat weight, as well as greater marbling scores and more estimated kidney, pelvic, and heart fat ($P < 0.05$). These animals also had a larger proportion of carcasses classified as USDA Choice ($P < 0.05$). There was no significant difference between animals with zero vs. one copy of the inactive myostatin allele for postweaning ADG and live weight.

Animals obtained from the Belgian Blue × British Breed could have inherited zero, one, or two copies of the inactive myostatin allele. Offspring with two copies of the inactive myostatin allele were heavier at birth, were classified as having the lowest yield grade, had the most retail product yield and retail product weight, and had the most bone yield and bone weight compared with the other two groups ($P < 0.05$). The inactive myostatin allele displayed apparent overdominance for some traits. Heterozygous offspring were the heaviest at weaning and for live weight ($P < 0.05$), although they had similar hot carcass weight and LM area similar to those that inherited two copies of the inactive myostatin

Table 2. Effect of the inactive myostatin allele on growth and carcass traits within the Charolais and Belgian Blue × British Breed sire breeds

		Number of inactive myostatin alleles within sire breed				
		Charolais		Belgian Blue × British Breed		
Trait[a]	*P*-value	0	1	0	1	2
BWT, kg	<0.001	40.2 ± 0.3[c]	42.2 ± 0.7[d]	38.0 ± 0.4[e]	40.0 ± 0.4[f]	44.6 ± 1.1[g]
WWT, kg	<0.001	244 ± 1[c]	253 ± 3[d]	228 ± 2[e]	238 ± 2[f]	227 ± 5[e]
PWADG, kg/d	0.049	1.30 ± 0.01[c]	1.31 ± 0.02[c]	1.26 ± 0.01[ef]	1.27 ± 0.01[e]	1.20 ± 0.03[f]
LWT, kg	0.002	565 ± 3[c]	577 ± 7[c]	538 ± 3[e]	552 ± 3[f]	523 ± 10[e]
HCW, kg	<0.001	358 ± 2[c]	371 ± 4[d]	345 ± 2[e]	357 ± 2[f]	358 ± 7[f]
FAT, cm	<0.001	1.10 ± 0.02[c]	0.82 ± 0.05[d]	1.26 ± 0.02[e]	1.06 ± 0.02[f]	0.43 ± 0.08[g]
LMA, cm^2	0.003	89 ± 1[c]	99 ± 4[d]	90 ± 2[e]	96 ± 2[f]	106 ± 6[f]
MAR[b]	<0.001	544 ± 4[c]	482 ± 12[d]	549 ± 5[e]	498 ± 6[f]	380 ± 18[g]
YG	<0.001	2.60 ± 0.03[c]	1.95 ± 0.08[d]	2.69 ± 0.04[e]	2.22 ± 0.04[f]	1.05 ± 0.12[g]
KPH, %	<0.001	1.94 ± 0.01[c]	1.85 ± 0.03[d]	1.95 ± 0.01[e]	1.91 ± 0.01[f]	1.59 ± 0.04[g]
CH, %	<0.001	60 ± 2[c]	30 ± 6[d]	61 ± 3[e]	41 ± 3[f]	1 ± 9[g]
RPYD, %	<0.001	64 ± 0.2[c]	68 ± 0.5[d]	63 ± 0.3[e]	66 ± 0.3[f]	74 ± 0.8[g]
RPWT, kg	<0.001	230 ± 1[c]	253 ± 3[d]	218 ± 1[e]	236 ± 1[f]	264 ± 4[g]
FATYD, %	<0.001	21 ± 0.2[c]	17 ± 0.6[d]	22 ± 0.3[e]	19 ± 0.3[f]	10 ± 0.9[g]
FATWT, kg	<0.001	77 ± 1[c]	62 ± 2[d]	78 ± 1[e]	69 ± 1[f]	37 ± 4[g]
BONEYD, %	<0.001	14.8 ± 0.03[c]	15.3 ± 0.1[d]	14.5 ± 0.04[e]	14.9 ± 0.05[f]	16.1 ± 0.1[g]
BONEWT, kg	<0.001	53 ± 0.3[c]	57 ± 0.7[d]	50 ± 0.3[e]	53 ± 0.3[f]	58 ± 1.0[g]

[a]BWT = birth weight; WWT = weaning weight; PWAD = postweaning ADG; LWT = live weight; HCW = hot carcass weight; FAT = fat thickness; LMA = longissimus area; MAR = marbling score; YG = USDA yield grade; KPH = estimated kidney, pelvic, and heart fat; CH = percentage of carcasses classified as Choice; RPYD = retail product yield; RPWT = retail product weight; FATYD = fat yield; FATWT = fat weight; BONEYD = bone yield; and BONEWT = bone weight.

[b]MAR: 400 = $Slight^{00}$, 500 = $Small^{00}$.

[c,d]Within Charolais and traits, means that do not have a common superscript differ, $P < 0.05$.

[e,f,g]Within Belgian Blue × British Breed and traits, means that do not have a common superscript differ, $P < 0.05$.

allele. Heterozygous animals had similar postweaning ADG to offspring inheriting zero copies of the inactive myostatin allele. Offspring inheriting zero copies of the inactive myostatin allele were in general fatter than the other two groups. They had greater fat thickness, more marbling, more estimated kidney, pelvic and heart fat, and more fat yield and fat weight than animals inheriting one or two copies of the inactive myostatin allele ($P < 0.05$). Also, a larger percentage of the carcasses was classified as Choice ($P < 0.05$).

Discussion

Previous studies have suggested that double-muscled animals have higher perinatal mortality (Arthur et al., 1989a; Arthur, 1995); however, the populations used in those studies were not directly tested to determine the myostatin genotype. In addition, other studies of double muscling in crossbred populations using the Piedmontese breed as source of inactive myostatin alleles have reported lower than predicted representation of homozygous individuals (Casas et al., 1999; Short et al., 2002). Because the animals in the current study were genotyped for the Belgian Blue allele of myostatin, including animals that died at birth or before weaning, we were able to directly examine the effect of genotype on mortality even in animals with a single inactive myostatin allele. Furthermore, this study used a broader range of breeds in which the allele was segregating. Although it is known that the Charolais segregates a distinct, inactivating myostatin mutation (mutation Q204X; Antoniou and Grosz, 1999; Dunner et al., 2003), the allele exists at a very low frequency in Charolais in the United States (L. V. Cundiff, personal communication), and our data do not suggest any appearance in the population used. In addition, the calf mortality study was only done in the portion of the population that did not include Charolais germplasm. Although the total number of calves that died was relatively small, the present data indicate that animals with two copies of the inactive myostatin allele are at increased mortality risk, as indicated by the observed 1:1:2 ratio of genotypes among the calves lost. Much of the observed loss is likely related to the increased birth weight of calves. It should be noted that the calves in this study were born in Nebraska in the months of February, March, April, and May, which can present the calves with cold, windy weather conditions with occasional precipitation, such that the relatively low amount of extramuscular fat might contribute to calf loss. The average maximum temperatures for the months of February, March, April, and May are 4.0, 10.0, 16.9, and 22.2°C, respectively, and the average minimum temperatures are –8.4, –3.4, 2.7, and 8.9°C. Precipitation for these months averages 122, 429, 764, and 1,255 mm, respectively (High Plains Regional Climate Center, Univ. of Nebraska).

The number of copies of the inactive myostatin allele resulted in distinct differences in carcass composition. Individuals inheriting two copies of the inactive myostatin allele had a higher proportion of muscle mass and were leaner. Individuals inheriting one copy of the inactive myostatin allele were intermediate between those inheriting two and zero copies of the allele. Those that inherited zero copies of the inactive myostatin allele had the lowest proportion of muscle mass and were fatter. These differences follow the pattern that has been previously reported (Arthur et al., 1989b; Arthur, 1995; Short et al., 2002; Wiener et al., 2002).

Animals with two copies of the inactive myostatin allele were heavier at birth than the other two groups, whereas animals with one copy were heavier than animals with zero copies. The difference was 3.5 and 2.0 kg between the groups inheriting two and one copies, and the groups inheriting one and zero copies of the inactive myostatin allele, respectively. This magnitude of effect is similar to that observed in studies of the Piedmontese allele of myostatin (Short et al., 2002), where differences of 3.1 and 1.3 kg were reported for the same contrasts. Nott and Rollins (1979), grouping animals inheriting two, one, and zero copies of the muscular hypertrophy locus, indicated that the difference between these groups was 2.6 and 1.2 kg. In an independent population, Casas et al. (1998), comparing animals inheriting one or zero copies of the inactive myostatin allele from Belgian Blue and Piedmontese, found that the difference between both groups was 4.6 kg, with animals inheriting one copy of the inactive myostatin allele being heavier than animals inheriting zero copies. Casas et al. (1999) found a difference of 5.2 kg between these groups. This difference was observed to be 3.8 kg by Nott and Rollins (1979) and 4.4 kg by Short et al. (2002). The magnitude of the difference among studies is similar, indicating that animals with two copies of the inactive myostatin allele are heavier at birth, whereas animals with zero copies of this allele are the lightest.

Animals with one copy of the inactive myostatin allele were heavier at weaning than both homozygous groups. This trend has been documented before in a population segregating the inactive myostatin allele from the Piedmontese (Casas et al., 1999) and in males of a population segregating the double-muscling locus (Nott and Rollins, 1979). However, Arthur (1995) and Short et al. (2002) indicated that heterozygous animals had weaning weights similar to those with zero copies of the inactive myostatin allele or were slightly lighter. The most likely explanation for these conflicting results is the confounding effects between myostatin differences and breed effects. Maternal effects that could modify performance influence weights expressed early in life.

Animals with one copy of the inactive myostatin allele had a postweaning ADG similar to animals with zero copies of the allele. Animals with two copies of the inactive myostatin allele had a slower growth rate. Nott and Rollins (1979) and Arthur et al. (1989a) found similar performance among these groups. Arthur (1995) indicated that double-muscled animals have a decreased appetite, resulting in lower feed intake during the postweaning period. Furthermore, Arthur (1995) indicated that this decrease in feed intake is due to a reduction in the size of the digestive tract. Thus, double-muscled animals would express their growth potential on a concentrate diet. Animals used in the current study were fed a diet based on corn silage, corn, and a urea-based liquid supplement containing approximately 2.7 Mcal of ME/kg of DM and 12.5% CP (DM basis; Casas and Cundiff, 2003). It is possible that animals with two copies of the inactive myostatin allele used in the current study were unable to express their growth potential because the diet was silage instead of a concentrate. This would be a likely scenario in a production system in the United States.

Animals with two copies of the inactive myostatin allele were lighter than animals with zero or one copy of the inactive myostatin allele when live weight was measured before slaughter. Arthur et al. (1989a) found a similar performance when double-muscled animals, heterozygous, and nondouble-muscled cattle at yearling weight were compared. This weight is associated with the postweaning ADG performance, and it would be expected that animals with two copies of the inactive myostatin allele would have a lighter live weight. Short et al. (2002) found no difference in live weight among animals with zero, one, or two copies of the inactive myostatin allele from the Piedmontese breed. Wiener et al. (2002) also found no statistical difference for weight at 400 d when comparing animals with zero, one, or two copies of the inactive myostatin allele from the South Devon breed. The South Devon breed segregates the same 11-base pair deletion as the Belgian Blue. These conflicting results may indicate that genes other than myostatin may be influencing the expression of weights at a later stage in life.

Implications

The inactive myostatin allele was associated with early life mortality. Animals with two copies of this allele were more susceptible to harsh conditions at birth. Producers would need to consider the conditions at calving in their management decisions before producing calves with two copies of the inactive myostatin allele. The inactive myostatin allele affects growth and carcass traits in crossbred cattle. This allele tends to produce leaner and more muscled carcasses. Production systems that produce calves with one copy of the inactive myostatin allele will benefit from heavier weaning weights and higher yield of lean, compared with calves with zero copies of the inactive myostatin allele.

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