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Genome wide association study of cholesterol and poly- and monounsaturated fatty acids, protein, and mineral content of beef from crossbred cattle

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ABSTRACT

The objectives were to determine the variation explained by the BovineSNP50v2 BeadChip for cholesterol (CH), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), protein, and minerals in beef cattle, and to identify chromosomal regions that harbor major allelic variants underlying the variation of these traits. Crossbred steers and heifers (n = 236) segregating at the inactive myostatin allele on BTA2 were harvested and steaks were sampled from the *M. semitendinosus* and the *M. longissimus thoracis et lumborum* for nutrient analysis. A Bayes C algorithm was employed in genome-wide association analysis. The resulting posterior heritability (SD) estimates ranged from 0.43 (0.10) to 0.71 (0.08) for lipid traits and 0.05 (0.08) to 0.75 (0.06) for mineral traits. Across cuts, correlations between genomic estimated breeding values (GEBV) were similar for CH, MUFA and PUFA. The top 0.5% 1-Mb windows for all traits explained up to 9.93% of the SNP variance. Slight differences did exist between cuts and between different measurement scales of fatty acids.

1. Introduction

Consumers are becoming increasingly health-conscious and demand healthy and palatable meat, both of which are affected by lipid composition (Dunner et al., 2013). Red meat has relatively high levels of saturated fatty acids and beneficial oleic acid, and low concentrations of beneficial polyunsaturated fatty acids (Dunner et al., 2013). However, fats are not the only nutrients that affect the nutritional value of beef. Beef is an excellent source of iron required in the human diet, yet the consistency of iron content in beef products is highly variable (Duan et al., 2009). Considerable attention has been placed on improving the nutritional value of beef and the development of products that are beneficial to human health and disease prevention (Scollan et al., 2006). It has been illustrated that animal nutritional regime differences can alter the nutrient profile of beef (Realini, Duckett, Brito, Rizza, & De Mattos, 2004) and that genetic factors can also play a role (De Smet, Raes, & Demeyer, 2004; Mateescu et al., 2013a, b). Identification of genetic variants that would allow producers to select for optimum nutritional values with respect to fatty acids, minerals, and vitamins, without sacrificing performance or product quality, could ultimately increase value and consumer satisfaction of beef. Genetic selection aided by genomic predictors may serve as an important and highly applicable tool in improving the nutritional value of beef given the expensive and difficult nature of phenotypic data collection. The objectives of the current study were to determine the proportion of phenotypic variation explained by the Ilumina BovineSNP50v2 BeadChip for cholesterol (CH), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), protein, potassium, iron and sodium, and to identify chromosomal regions that harbor major genetic variants underlying the variation of these traits.

2. Materials and methods

2.1. Experimental design

Crossbred steers and heifers of unknown pedigree and breed fractions (n = 236) with varying percentages of Angus, Simmental and Piedmontese were placed in a Calan gate facility at the Agricultural Research and Development Center (ARDC) feedlot facility near Mead, NE. The project was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Prior to arrival, animals were genotyped for the Piedmontese-derived myostatin mutation (*C313Y*) to determine their myostatin genotype (MG) as either homozygous normal (*313C/313C*, 0 copy, n = 83), heterozygous (*313C/313Y*, 1-copy, n = 96), or homozygous for inactive myostatin (*313Y/313Y*, 2-copy, n = 57). Cattle were fed in four groups over a 2-yr period. Groups 1 and 3 consisted of calf-fed steers and groups 2 and 4 consisted of yearling heifers. Groups 1 and 2 were steers and heifers fed in the first year and groups 3 and 4 were steers and heifers fed in the second year as

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described by Howard, Kachman, Nielsen, Mader, and Spangler (2013). Statistics for carcass traits are summarized in Table 1.

Animals had ad libitum access to water and were fed a diet that met or exceeded National Research Council NRC (1996) requirements. The finishing ration for steers and heifers in year 1 included wet distiller grain with solubles, a 1:1 blend of high moisture and dry rolled corn, grass hay and supplement at 35, 52, 8, and 5% of the diet on a dry matter basis. The finishing ration for steers and heifers in year 2 included modified distiller grain with solubles, sweet bran, a 1:1 blend of high moisture and dry rolled corn, grass hay and supplement at 20, 20, 48, 8, and 4% of the diet on a dry matter basis. Animals were on an all-natural program and were not implanted or fed growth-promoting additives. Cattle were harvested as a group based on average body weight and external fat.

2.2. Sample collection and analysis

Steaks were sampled from the *M. Longissimus thoracis et lumborum* (LTL) and the *M. Semitendinosus* (ST) three days post-mortem. Steaks were cut to 1.27 cm thick and trimmed to 0.32 cm of subcutaneous fat. Steaks were sent to Midwest Laboratories, Inc. (Omaha, NE) for further analysis. Midwest Laboratories, Inc. followed protocols listed in the Association of Official Agricultural Chemists AOAC (2005). The following methods were used; protein (AOAC 990.03), cholesterol (AOAC 976.26), fatty acid profile (AOAC 996.06), and minerals (AOAC985.01 mod.). Lipid and mineral analysis results were reported for a 113.40 gram serving size. PUFA was defined as the sum of C18:2 trans, C18:2, C18:3 gamma, C18:3 alpha, C20:2, C20:3, C20:4, C20:5, C22:2, C22:5, and C22:6 whereas MUFA was defined as the sum of C14:1 trans, C14:1, C16:1 trans, C16:1, C17:1, C18:1 trans, C18:1, C20:1, C22:1, and C24:1. Fatty acids (MUFA and PUFA) and CH were analyzed as both a percentage of total lipid content and mg/100 g of whole (wet) tissue. The interpretation of these two measurement scales is dramatically different, as a sample with relatively low PUFA content as measured in mg/100 g of whole (wet) tissue would likely have low total lipid content and as a consequence would have relatively high PUFA content when measured as a percentage of total lipids. Potassium, iron and sodium were analyzed as ppm of whole tissue. These values along with protein percentage (whole tissue basis) were obtained using AOAC methods.

2.3. Genotyping

An ear notch sample was collected from each animal. DNA was isolated from 10 to 25 mg of tissue from each animal using the DNeasy blood and tissue kit (Qiagen). The quantity and quality of the DNA sample were assessed by NanoDrop Spectrophotometer (Thermo Scientific) and agarose gel electrophoresis. All animals were genotyped with the Illumina BovineSNP50v2 BeadChip (Illumina Inc., San Diego, CA). Animal genotyping was performed by GeneSeek (Neogen Corporation Lincoln, NE). Myostatin genotyping was performed by Zoetis (Kalamazoo, MI). All samples used had a genotyping call rate above 97.5%. Illumina data analysis software was used to assign quality scores (GenCall) for each genotype. If genotypes were missing or a GenCall score was below 0.20 (Illumina, Inc., 2010), genotypes were replaced with the mean allele frequency. Differences in genotype editing procedures, relative to culling Single Nucleotide Polymorphism (SNP) with low Minor Allele Frequency (MAF), have been shown to have a minimal impact on resulting genomic predictions (Edriss, Guldbrandtsen, Lund, & Su, 2012) and as a result all SNP were utilized for analysis. Myostatin genotype has been shown to have an effect on fatty acid composition. Consequently, outliers, adjusted for group and MG, classified as being >3 SD from the mean of the residual variance (zero), were removed from the analysis. Summary statistics for fatty acid and mineral traits after editing are detailed in Table 2.

2.4. Statistical analysis

A genome wide association study (GWAS) was conducted using Bayesian methods via GenSel platform (Version 0.9.2.045; Fernando & Garrick, 2009). A Bayes C model was employed (Habier, Fernando, Kizilkaya, & Garrick, 2011) with group (concatenation of year (i.e. feeding regime) and sex; 4 classes) fitted as a fixed effect. The proportion of markers having a null effect (π) was set to 0.95. A chain length of 150,000 iterations was run with the first 50,000 discarded as burn-in. The genomic estimated breeding value (GEBV) was estimated by summing posterior mean marker effects by marker genotype across all SNP. Convergence was met for all analyses by starting with high and low a priori heritability estimates until the posterior heritability estimates were trending down and up, respectively and a value in the middle was chosen as the a priori heritability estimate. Phenotypic correlations were estimated using multivariate analysis of variance (MANOVA) procedures with group fitted as a fixed effect. The genomic estimated breeding value (GEBV) of the ith animal was calculated as: $GEBV_i = \sum_{(k=1)}^{K} z_{ik}\hat{a}_k$, where z_{ik} is the genotype call ($-10, 0, 10$) for animal i at marker k and $\hat{a}_k$ is the posterior mean effect at marker k. To estimate potential GEBV re-ranking, correlations between GEBV were estimated across traits within a cut (i.e. ST or LTL) and between cuts within each trait. Additionally, the cattle genome was separated into 1 Megabase (Mb) windows and SNP variance within a window was

Table 1
Summary statistics for carcass traits.

Trait	n	0 copy[a]	1 copy[a]	2 copy[a]	Minimum	Maximum	Mean	Standard deviation
HCW, kg.								
Group 1[c]	59	19	28	12	253.55	372.85	305.88	25.42
Group 2[c]	60	25	26	9	265.80	385.55	319.85	24.96
Group 3[c]	58	20	22	16	268.52	400.98	332.19	26.84
Group 4[c]	59	19	20	20	271.25	434.00	346.24	34.19
Back fat, cm.								
Group 1	59	19	28	12	0.10	1.40	0.73	0.37
Group 2	60	25	26	9	0.10	2.03	0.84	0.41
Group 3	58	20	22	16	0.25	2.29	0.86	0.55
Group 4	59	19	20	20	0.25	3.05	1.02	0.68
Marbling score[b]								
Group 1	59	19	28	12	100	470	294.92	100.75
Group 2	60	25	26	9	100	860	373.00	118.40
Group 3	58	20	22	16	250	880	533.79	166.97
Group 4	59	19	20	20	270	730	426.78	114.75

[a] Refers to the number of copies of the inactive myostatin allele.
[b] Marbling score units: 400 = Sm^{00}, 500 = $Modest^{00}$.
[c] Group 1 refers to year 1 steers, group 2 refers to year 1 heifers, group 3 refers to year 2 steers and Group 4 refers to year 2 heifers.