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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.

Keywords: Beef, genome wide association, nutrient profile

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 et al., 2004; Mateescu et al., 2013a; Mateescu et al., 2013b). 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 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 *i*th 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)

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.

and between cuts within each trait. Additionally, the cattle genome was separated into 1 Megabase (Mb) windows and SNP variance within a window was summed to give an estimate of the total SNP variance for each window (n = 2,677). The percentage of top 5% (n = 134) windows in common across traits and cuts were then compared with GEBV correlations among traits and between cuts. The top 0.5% 1-Mb windows (n = 13) for each trait were extended by 1-Mb in both directions and a positional candidate gene approach was conducted using *Bos taurus* build UMD_3.1 assembly (Zimin et al., 2009). Due to the limited functional annotation of the *Bos taurus* genome, human orthologs of beef cattle positional candidate genes were obtained and used for functional characterization by using Ensembl Genes 69 database and the BioMart data mining tool (http://www.ensembl.org/biomart/martview/dd0c118c99ed15210cc6e97131d873fb). Overrepresented gene ontology terms, and pathway analysis were identified using DAVID (http://david.abcc.ncifcrf.gov). The proportion of SNP variance explained by the top 0.5% windows was calculated by removing all SNP within the top 0.5% windows and rerunning the analysis (subset), with the same parameters as the initial run (full). The percent of variance explained was calculated as 1 − (subset posterior mean genetic variance / full posterior mean genetic variance).

To determine if associations on BTA2 were due to the myostatin *C313Y* mutation, which is not included on the BovineSNP50_v2 BeadChip, linkage-disequilibrium (LD) between *C313Y* and all the SNP within the top 0.5% windows on BTA2 was estimated with the Haploview software (Barrett, Fry, Maller, & Daly, 2005). Significant SNP on BTA2 in high LD with *C313Y* would suggest the importance of the myostatin mutation in explaining a portion of the additive variation.

3. Results

3.1. Genomic heritabilities

The posterior mean (standard deviation; SD) genomic heritability estimates (proportion of phenotypic variation explained by the markers) for sodium, iron, potassium and protein from the ST were 0.05 (0.05), 0.35 (0.09), 0.65 (0.09), and 0.75 (0.06) respectively. The posterior mean (SD) genomic heritability estimates for sodium, iron, potassium and protein from the LTL were 0.15 (0.08), 0.35 (0.13), 0.75 (0.08), and 0.70 (0.08), respectively. The posterior mean (SD) genomic heritability estimates for CH, PUFA and MUFA as a percentage of total lipid content for the ST were 0.45 (0.10), 0.65 (0.06) and 0.60 (0.07), respectively. When analyzed as mg/100 g of total wet tissue, the posterior mean (SD) genomic heritability estimates for CH, PUFA and MUFA for the ST were 0.45 (0.11), 0.45 (0.04) and 0.60 (0.10), respectively. The posterior mean (SD) genomic heritability estimates for CH, PUFA and MUFA for the LTL as a percentage of total lipid content were 0.50 (0.09), 0.70 (0.06) and 0.40 (0.10), respectively. When analyzed as mg/100 g of total wet tissue, the posterior mean (SD) genomic heritability estimates for CH, PUFA and MUFA for the LTL were 0.50 (0.06), 0.70 (0.08) and 0.85 (0.04), respectively.

3.2. Genomic estimated breeding value and phenotypic correlations

The mean prediction error variances (PEV), across all animals, of GEBV for ST sodium, LTL sodium, ST protein, LTL protein, ST potassium, LTL potassium, ST iron and LTL iron were 32.08, 70.2, 0.27, 0.54, 7,727.57, and 10,596.26, respectively. The mean PEV of GEBV for fatty acid traits as a percentage of total lipid content were 1.25, 0.04, 4.96, 2.98, 4.31, and 0.79 for ST cholesterol, LTL cholesterol, ST MUFA, LTL MUFA, ST PUFA, and LTL PUFA, respectively. The mean PEV of GEBV for fatty acid traits measured on a gravimetric scale were 3.23, 4.08, 667,883.93, 867,919.28, 3,222.09, and 5,632.32 for ST cholesterol, LTL cholesterol, ST MUFA, LTL MUFA, ST PUFA, and LTL PUFA, respectively. Correlations between GEBV are presented in Table 3 and Table 4. In the ST, significant correlations were estimated between protein and iron (− 0.58), sodium (− 0.41), and potassium (0.75). In the LTL, significant correlations were estimated between protein and sodium (− 0.26) and potassium (0.74). The correlation between ST and LTL protein was 0.73 ($P < 0.01$). Significant correlations between potassium and iron differed in the direction depending on the cut (0.20 and − 0.45 for LTL and ST, respectively). For minerals, correlations between LTL and ST for the same trait were significant and ranged from 0.31 to 0.99.

When lipid traits were analyzed as a percentage of total lipids, correlations between CH and PUFA (0.84 for ST and 0.89 for LTL) and CH and MUFA (− 0.77 for ST and − 0.78 for LTL) were significant. The same was true for the estimated correlations between MUFA and PUFA (− 0.91 and − 0.80 for ST and LTL, respectively). When lipid traits were analyzed as mg/100 g, the strength of associations were still moderate to high, but in some cases the direction of the correlations changed. Significant correlations existed between CH and MUFA (− 0.54 and − 0.62 for ST and LTL, respectively), and PUFA (− 0.47 and − 0.51 for ST and LTL, respectively). The correlations between PUFA and MUFA were 0.89 ($P < 0.01$) and 0.87 ($P < 0.01$) for ST and LTL, respectively.

When lipid traits were analyzed as mg/100 g of wet tissue, significant negative correlations existed between protein and both MUFA

Table 2. Summary statistics for nutrient traits.

Trait	Units	n	Mean	Minimum	Maximum	Standard deviation
LTL[a] MUFA	(% of fat)	224	46.25	33.2	55.00	4.31
LTL MUFA	(mg/100 g)	227	6087.70	270.97	13849.38	3233.42
ST[b] MUFA	(% of fat)	223	45.11	26.6	56.70	5.55
ST MUFA	(mg/100 g)	227	2461.14	37.24	10308.06	1977.84
LTL PUFA	(% of fat)	223	5.27	2.66	15.30	2.21
LTL PUFA	(mg/100 g)	224	572.60	149.86	1197.99	180.07
ST PUFA	(% of fat)	222	8.50	1.14	25.60	5.20
ST PUFA	(mg/100 g)	227	378.87	36.24	735.02	132.31
LTL cholesterol	(% of fat)	222	0.50	0.14	2.84	0.45
LTL cholesterol	(mg/100 g)	225	45.76	33.00	59.00	4.48
ST cholesterol	(% of fat)	223	1.94	0.22	17.10	2.48
ST cholesterol	(mg/100 g)	225	46.26	32.00	58.00	4.73
LTL sodium	(ppm)	226	418.69	336.50	491.20	32.14
ST sodium	(ppm)	227	393.92	317.40	478.60	29.02
LTL potassium	(ppm)	227	3015.18	2283.00	3614.00	268.71
ST potassium	(ppm)	226	3484.30	2867.00	4087.00	227.99
LTL iron	(ppm)	224	13.65	8.99	19.56	2.06
ST iron	(ppm)	226	13.92	7.50	25.50	2.62
LTL protein	(%)	225	21.69	17.34	27.44	1.86
ST protein	(%)	227	22.91	18.58	26.17	1.33

a. *M. Longissimus dorsi* (LTL).
b. *M. Semitendinosus* (ST).

Table 3. Genomic estimated breeding value (GEBV) correlations with lipid traits measured as a percent of total fat[abcdef].

Trait[abc]	STPR	STI	STS	STOP	STCH	STMUFA	STPUFA	LTLPR	LTLI	LTLS	LTLPO	LTLCH	LTLMUFA	LTLPUFA
STPR	–	− 0.58 (0.01)	− 0.41 (0.01)	0.75 (0.01)	0.61 (0.01)	− 0.69 (0.01)	0.74 (0.01)	0.73 (0.01)	− 0.02 (0.75)	− 0.32 (0.01)	0.62 (0.01)	0.68 (0.01)	− 0.66 (0.01)	0.64 (0.01)
STI	–	–	0.55 (0.01)	− 0.45 (0.01)	− 0.58 (0.01)	0.54 (0.01)	− 0.63 (0.01)	− 0.57 (0.01)	0.31 (0.01)	0.49 (0.01)	− 0.48 (0.01)	− 0.54 (0.01)	0.51 (0.01)	− 0.60 (0.01)
STS	–	–	–	− 0.13 (0.05)	− 0.29 (0.01)	0.20 (0.01)	− 0.35 (0.01)	− 0.34 (0.01)	0.13 (0.06)	0.99 (0.01)	− 0.21 (0.01)	− 0.30 (0.01)	0.19 (0.01)	− 0.32 (0.01)
STPO	–	–	–	–	0.49 (0.01)	− 0.64 (0.01)	0.61 (0.01)	0.57 (0.01)	− 0.03 (0.64)	− 0.04 (0.55)	0.60 (0.01)	0.51 (0.01)	− 0.58 (0.01)	0.47 (0.01)
STCH	–	–	–	–	–	− 0.77 (0.01)	0.84 (0.01)	0.68 (0.01)	− 0.06 (0.41)	− 0.20 (0.01)	0.49 (0.01)	0.86 (0.01)	− 0.70 (0.01)	0.73 (0.01)
STMUFA	–	–	–	–	–	–	− 0.91 (0.01)	− 0.75 (0.01)	0.05 (0.46)	0.11 (0.09)	− 0.66 (0.01)	− 0.81 (0.01)	0.86 (0.01)	− 0.77 (0.01)
STPUFA	–	–	–	–	–	–	–	0.80 (0.01)	− 0.11 (0.10)	− 0.26 (0.01)	0.61 (0.01)	0.87 (0.01)	− 0.78 (0.01)	0.82 (0.01)
LTLPR	–	–	–	–	–	–	–	–	− 0.03 (0.67)	− 0.26 (0.01)	0.74 (0.01)	0.81 (0.01)	− 0.75 (0.01)	0.77 (0.01)
LTLI	–	–	–	–	–	–	–	–	–	0.12 (0.06)	0.20 (0.01)	− 0.07 (0.30)	0.005 (0.95)	− 0.15 (0.02)
LTLS	–	–	–	–	–	–	–	–	–	–	− 0.16 (0.02)	− 0.22 (0.01)	0.12 (0.07)	− 0.25 (0.01)
LTLPO	–	–	–	–	–	–	–	–	–	–	–	0.63 (0.01)	− 0.70 (0.01)	0.63 (0.01)
LTLCH	–	–	–	–	–	–	–	–	–	–	–	–	− 0.78 (0.01)	0.89 (0.01)
LTLMUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	− 0.80 (0.01)
LTLPUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	–

a. *M. Longissimus dorsi* (LTL).
b. *M. Semitendinosus* (ST).
c. ST protein (STPR), ST iron (STI), ST sodium (STS), ST potassium (STPO), ST cholesterol (STCH), ST monounsaturated fatty acids (STMUFA), ST polyunsaturated fatty acids (STPUFA), LTL protein (LTLPR), LTL iron (LTLI), LTL sodium (LTLS), LTL potassium (LTLPO), LTL cholesterol (LTLCH), LTL monounsaturated fatty acids (LTLMUFA), and LTL polyunsaturated fatty acids (LTLPUFA).
d. STCH, STPUFA, STMUFA, LTLCH, LTLPUFA and LTLMUFA units as a percent of total fat.
e. GEBV correlations (P value).
f. Standard errors for correlations were 0.067.

Table 4. Genomic estimated breeding value (GEBV) correlations with lipids measured as mg/100 g of total (wet) tissue[abcdef].

Trait[abc]	STPR	STI	STS	STOP	STCH	STMUFA	STPUFA	LTLPR	LTLI	LTLS	LTLPO	LTLCH	LTLMUFA	LTLPUFA
STPR	–	− 0.58 (0.01)	− 0.41 (0.01)	0.75 (0.01)	0.64 (0.01)	− 0.67 (0.01)	− 0.62 (0.01)	0.73 (0.01)	− 0.02 (0.75)	− 0.32 (0.01)	0.62 (0.01)	0.54 (0.01)	− 0.76 (0.01)	− 0.62 (0.01)
STI	–	–	0.55 (0.01)	− 0.45 (0.01)	− 0.61 (0.01)	0.51 (0.01)	0.48 (0.01)	− 0.57 (0.01)	0.31 (0.01)	0.49 (0.01)	− 0.48 (0.01)	− 0.49 (0.01)	0.62 (0.01)	0.51 (0.01)
STS	–	–	–	− 0.13 (0.05)	− 0.33 (0.01)	0.36 (0.01)	0.34 (0.01)	− 0.34 (0.01)	0.13 (0.05)	0.99 (0.01)	− 0.21 (0.01)	− 0.20 (0.01)	0.33 (0.01)	0.28 (0.01)
STPO	–	–	–	–	0.54 (0.01)	− 0.55 (0.01)	− 0.47 (0.01)	0.57 (0.01)	− 0.03 (0.64)	− 0.04 (0.55)	0.59 (0.01)	0.49 (0.01)	− 0.60 (0.01)	− 0.43 (0.01)
STCH	–	–	–	–	–	− 0.54 (0.01)	− 0.47 (0.01)	0.66 (0.01)	− 0.04 (0.54)	− 0.26 (0.01)	0.57 (0.01)	0.60 (0.01)	− 0.67 (0.01)	− 0.50 (0.01)
STMUFA	–	–	–	–	–	–	0.89 (0.01)	− 0.66 (0.01)	0.04 (0.57)	0.28 (0.01)	− 0.53 (0.01)	− 0.46 (0.01)	0.69 (0.01)	0.62 (0.01)
STPUFA	–	–	–	–	–	–	–	− 0.58 (0.01)	0.15 (0.03)	0.26 (0.01)	− 0.41 (0.01)	0.40 (0.01)	0.59 (0.01)	0.60 (0.01)
LTLPR	–	–	–	–	–	–	–	–	− 0.03 (0.67)	− 0.26 (0.01)	0.74 (0.01)	0.66 (0.01)	− 0.89 (0.01)	− 0.82 (0.01)
LTLI	–	–	–	–	–	–	–	–	–	0.12 (0.06)	0.20 (0.01)	− 0.007 (0.91)	0.05 (0.48)	0.07 (0.29)
LTLS	–	–	–	–	–	–	–	–	–	–	− 0.16 (0.01)	− 0.14 (0.03)	0.25 (0.01)	0.21 (0.01)
LTLPO	–	–	–	–	–	–	–	–	–	–	–	0.56 (0.01)	− 0.77 (0.01)	− 0.70 (0.01)
LTLCH	–	–	–	–	–	–	–	–	–	–	–	–	− 0.62 (0.01)	− 0.51 (0.01)
LTLMUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	–0.87 (0.01)
LTLPUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	–

a. *M. Longissimus dorsi* (LTL).
b. *M. Semitendinosus* (ST).
c. (STPR), ST iron (STI), ST sodium (STS), ST potassium (STPO), ST cholesterol (STCH), ST monounsaturated fatty acids (STMUFA), ST polyunsaturated fatty acids (STPUFA), LTL protein (LTLPR), LTL iron (LTLI), LTL sodium (LTLS), LTL potassium (LTLPO), LTL cholesterol (LTLCH), LTL monounsaturated fatty acids (LTLMUFA), and LTL polyunsaturated fatty acids (LTLPUFA).
d. STCH, STPUFA, STMUFA, LTLCH, LTLPUFA and LTLMUFA units as mg/100 g of total (wet) tissue.
e. GEBV correlations (P value).
f. Standard errors for correlations were 0.067.

and PUFA for both the ST and the LTL. For both cuts, a significant positive correlation existed between protein and CH. Although weak, significant positive correlations did exist between sodium and fatty acids (MUFA and PUFA) for both cuts. Between sodium and CH for both cuts, significant yet weak negative correlations were estimated. Moderate to strong negative correlations were estimated between potassium and both MUFA and PUFA for both cuts. Moderate positive correlations existed between CH and potassium for both cuts. Correlations between iron and fatty acid traits were variable between cuts as only correlations within the ST were significant. When lipid traits were analyzed as a percentage of total fatty acids, correlations were generally of the same magnitude as when lipid traits were analyzed as mg/100 g of wet tissue. However, the direction of some of the correlations did change.

Phenotypic correlations are presented in Table 5 and Table 6 and in general follow the same trends as the correlations between GEBV. However, a weak, significant negative correlation exists between LTL sodium and fatty acids (MUFA and PUFA). Between LTL sodium and both ST and LTL CH, significant yet weak positive correlations were estimated.

3.3. Top chromosomal regions

The chromosomes and positions of the top 0.5% 1-Mb windows for CH, iron, sodium, potassium, protein, PUFA, and MUFA for the LTL and ST are outlined in Table 7 and Table 8, respectively. The top 0.5% 1-Mb windows explained 4.82, 2.72, 1.13, 2.37, 1.07, 0.69, 4.80, 1.09 and 3.48% of the SNP variance for ST CH (% of fat), CH (mg/100 g), iron, potassium, protein, PUFA (% of fat), PUFA (mg/100 g), MUFA (% of fat) and MUFA (mg/100 g), respectively. The top 0.5% 1-Mb windows explained 1.88,1.17, 4.92, 0.18, 0.97, 9.93, 2.22, 0.35 and 1.67% of the SNP variance for LTL CH (% of fat), CH (mg/100 g), iron, potassium, protein, PUFA (% of fat), PUFA (mg/100 g), MUFA (% of fat) and MUFA (mg/100 g), respectively. For sodium, the percentage of variation explained by the top 0.5% windows was variable. The percentage of top 5% 1-Mb windows in common between traits with a low phenotypic correlation ranged from 2.3% to 21.8%, moderate phenotypic correlation ranged from 6.3% to 21.2%, and traits with high phenotypic correlation ranged from 7.6% to 34.7%. The percentage of top 5% 1-Mb windows in common between traits with a low GEBV correlation ranged from 2.3% to 12.1%, moderate GEBV correlation ranged from 3.1% to 16.0%, and traits with high GEBV correlation ranged from 6.8% to 34.7%.

Linkage-disequilibrium between *C313Y* and SNP within the top 0.5% windows on BTA2 between 0 and 15 Mb for each trait was determined. At least one SNP for both ST and LTL protein, PUFA, MUFA and CH and all showed high D′ (Falconer & Mackay, 1996) values equal to or greater than 0.70. A SNP for LTL potassium at position 8.47 Mb (SNP ID: BTB-0079213) had a D′ value of 1.0 with *C313Y*. Of the D′ values at or above 0.70, SNP position 0.21 Mb (SNP ID: ARS-BFGL-NGS-31104) was shared among ST protein, LTL and ST PUFA, ST MUFA and ST CH. A SNP for ST and LTL MUFA and ST and LTL PUFA located at position 4.56 Mb (SNP ID: Hapmap53000-ss46526222) was in high LD with C313Y. A SNP for ST PUFA, MUFA and CH at position 5.46 Mb (SNP ID: Hapmap57611-rs29011345) was is high LD with C313Y while a SNP for LTL protein, LTL and ST PUFA, LTL MUFA and ST CH at position 9.49 Mb (SNP ID: Hapmap38411-BTA-48376) was as well.

Given the similarities of results between cuts, Manhattan plots for ST cholesterol and potassium are presented in Figure 1 and Figure 2. Functional annotation, enrichment and pathway analysis of the extended top 0.5% 1-Mb windows resulted in enrichments for transcription ($P < 0.01$, $P < 0.01$, $P < 0.01$, and $P < 0.01$) for LTL PUFA (% of fat), MUFA (% of fat), CH (mg/100 g), and sodium, respectively. Enrichments for ST iron were intracellular transport ($P < 0.048$), for LTL iron were innate immune response ($P < 0.01$) and hemostasis ($P < 0.03$), LTL protein were immune response ($P < 0.01$), ST protein were cellular response to stress ($P < 0.01$), and LTL potassium were sodium iron transport ($P < 0.01$). Enrichment for LTL CH (% of fat) was the phosphatidylinositol signaling

Table 5. Phenotypic correlations with lipid traits measured as a percent of total fat[abcdef].

Trait[abc]	STPR	STI	STS	STOP	STCH	STMUFA	STPUFA	LTLPR	LTLI	LTLS	LTLPO	LTLCH	LTLMUFA	LTLPUFA
STPR	–	− 0.32 (0.01)	− 0.09 (0.19)	0.64 (0.01)	0.35 (0.06)	− 0.53 (0.01)	0.59 (0.01)	0.59 (0.01)	− 0.05 (0.46)	0.15 (0.03)	0.49 (0.01)	0.45 (0.01)	− 0.44 (0.01)	0.48 (0.01)
STI	–	–	0.30 (0.01)	− 0.16 (0.02)	− 0.31 (0.01)	0.32 (0.01)	− 0.39 (0.01)	− 0.34 (0.01)	0.35 (0.01)	− 0.06 (0.36)	− 0.30 (0.01)	− 0.27 (0.01)	− 0.32 (0.01)	− 0.36 (0.01)
STS	–	–	–	0.24 (0.01)	0.02 (0.78)	− 0.09 (0.16)	− 0.03 (0.70)	− 0.05 (0.45)	0.02 (0.73)	0.25 (0.01)	0.02 (0.82)	0.03 (0.83)	− 0.07 (0.27)	− 0.008 (0.91)
STPO	–	–	–	–	0.24 (0.01)	− 0.48 (0.01)	0.44 (0.01)	0.39 (0.01)	− 0.06 (0.37)	0.12 (0.07)	0.46 (0.01)	0.28 (0.01)	− 0.37 (0.01)	0.28 (0.01)
STCH	–	–	–	–	–	− 0.59 (0.01)	0.68 (0.01)	0.50 (0.01)	− 0.12 (0.07)	0.17 (0.01)	0.29 (0.01)	0.75 (0.01)	− 0.50 (0.01)	0.56 (0.01)
STMUFA	–	–	–	–	–	–	− 0.85 (0.01)	− 0.61 (0.01)	0.20 (0.01)	− 0.21 (0.01)	− 0.52 (0.01)	− 0.66 (0.01)	0.74 (0.01)	− 0.65 (0.01)
STPUFA	–	–	–	–	–	–	–	0.68 (0.01)	− 0.21 (0.01)	0.19 (0.01)	0.47 (0.01)	0.74 (0.01)	− 0.58 (0.01)	0.71 (0.01)
LTLPR	–	–	–	–	–	–	–	–	− 0.08 (0.22)	0.25 (0.01)	0.64 (0.01)	0.71 (0.01)	− 0.60 (0.01)	0.70 (0.01)
LTLI	–	–	–	–	–	–	–	–	–	0.20 (0.01)	0.18 (0.01)	− 0.11 (0.11)	0.15 (0.02)	− 0.20 (0.01)
LTLS	–	–	–	–	–	–	–	–	–	–	0.50 (0.01)	0.23 (0.01)	− 0.24 (0.01)	0.20 (0.01)
LTLPO	–	–	–	–	–	–	–	–	–	–	–	0.49 (0.01)	− 0.55 (0.01)	0.51 (0.01)
LTLCH	–	–	–	–	–	–	–	–	–	–	–	–	− 0.63 (0.01)	0.82 (0.01)
LTLMUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	− 0.71 (0.01)
LTLPUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	–

a. *M. Longissimus thoracis et lumborum* (LTL).
b. *M. Semitendinosus* (ST).
c. ST protein (STPR), ST iron (STI), ST sodium (STS), ST potassium (STPO), ST cholesterol (STCH), ST monounsaturated fatty acids (STMUFA), ST polyunsaturated fatty acids (STPUFA), LTL protein (LTLPR), LTL iron (LTLI), LTL sodium (LTLS), LTL potassium (LTLPO), LTL cholesterol (LTLCH), LTL monounsaturated fatty acids (LTLMUFA), and LTL polyunsaturated fatty acids (LTLPUFA).
d. STCH, STMUFA, STPUFA, LTLCH, LTLMUFA and LTLPUFA units as percent of total fat.
e. Phenotypic correlations (P value).
f. Standard errors for correlations were 0.067.

pathway ($P < 0.04$). Table 9 lists the potential candidate genes from the functional annotation analysis. GULP1 is a candidate gene for both cuts on a percentage of total fat and wet tissue basis for fatty acids and CH. ITGAV is a candidate gene for both cuts on a percent of total fat and wet tissue basis for fatty acids and for CH when measured as a percentage of total lipids.

4. Discussion

Mateescu et al. (2013a) estimated the heritability based on pedigree information and phenotypic data to be 0.48, 0.00, and 0.15 for LTL iron, potassium, and sodium, respectively. The proportions of phenotypic variation explained by the BovineSNP50 assay were 0.37, 0.03, and 0.09 for iron, potassium and sodium, respectively (Mateescu et al., 2013b). These results are in general agreement with the findings of the current study for the traits of iron and sodium. The vastly different estimates for potassium may be attributed to the admixed population or the small sample size, and the fact that this population was segregating the *C313Y* mutation. One SNP within one of the top 1 Mb windows for potassium was in perfect LD with the myostatin mutation. Lower posterior mean estimates of genomic heritability for ST sodium is likely a function of the lower phenotypic variation of sodium content, which can be explained biologically by the body highly regulating sodium levels (Hollenberg, 1980).

For LTL and ST CH, LTL PUFA and ST MUFA posterior mean estimates of genomic heritability remained the same regardless of the scale of measurement (percentage of total lipids or mg/100 g of whole (wet) tissue). The genomic heritability estimate for LTL MUFA was higher when measured on mg/100 g of whole (wet) tissue than on a percentage of total lipids. ST PUFA genomic heritability was lower when measured on mg/100 g whole (wet) tissue basis. The coefficients of variation for ST PUFA were 0.61 and 0.34 when measured as a percentage of total lips and mg/100 g, respectively. This increase in variation could partially explain the increase in the proportion of variation explained by the markers. Although the ST had lower concentrations of PUFA as measured in mg/100 g of wet tissue, it also had lower values for total lipids. Consequently when PUFA was adjusted for total lipid content, the mean PUFA as a percentage of total lipid content was actually higher than the LTL. The same general trend of the ST containing a higher proportion PUFA and MUFA as a percentage of total fatty acids was also reported by Sexten et al. (2012). Estimates of heritability for fatty acids are sparse in the literature. Pitchford, Deland, Siebert, Malau-Aduli, and Bottema (2002) reported low to moderate estimates of heritability for fatty acid traits in beef cattle. However, Cameron (1990) reported high (0.53–0.71) heritability estimates for palmitic, stearic, oleic, and linoleic fatty acids. This is consistent with the estimate of 0.75 for the heritability of C18:1 in a population of Japanese black cattle (Uemoto et al., 2011), and supports a moderate to high level of genetic control of fatty acids within meat.

Breed differences between Angus, Brahman, and Romosinuano for PUFA percentage but not MUFA percentage were reported by Dinh et al. (2010). When breed comparison was made on a concentration (mg/g) basis, Dinh et al. (2010) reported that significant differences still existed for PUFA and Angus cattle were significantly greater for MUFA concentration than the other two breeds. A rank change between the breeds existed depending on the units of measurement (mg/g or percentage) for PUFA. Huerta-Leidenz et al. (1993) reported significant differences between Hereford and Brahman cows for PUFA and MUFA when normalized, but also reported that these differences began to erode when reported on a gravimetric content scale to the point that the two breeds were not statistically different for MUFA. Sexten et al. (2012) did not observe a significant effect of sire breed (Angus and Charolais) for total SFA, UFA, or MUFA but did report significant interactions between sire breed and weaning date for PUFA.

Table 6. Phenotypic correlations with lipid traits measured as mg/100 g of total (wet) tissue[abcdef].

Trait[abc]	STPR	STI	STS	STOP	STCH	STMUFA	STPUFA	LTLPR	LTLI	LTLS	LTLPO	LTLCH	LTLMUFA	LTLPUFA
STPR	–	− 0.32 (0.01)	− 0.09 (0.19)	0.64 (0.01)	0.40 (0.01)	− 0.48 (0.01)	− 0.43 (0.01)	0.59 (0.01)	− 0.05 (0.46)	0.14 (0.03)	0.49 (0.01)	0.31 (0.01)	− 0.65 (0.01)	− 0.47 (0.01)
STI	–	–	0.20 (0.01)	− 0.16 (0.02)	− 0.33 (0.01)	0.22 (0.01)	0.19 (0.01)	− 0.34 (0.01)	0.35 (0.01)	− 0.06 (0.36)	− 0.30 (0.01)	− 0.29 (0.01)	0.40 (0.01)	0.31 (0.01)
STS	–	–	–	0.24 (0.01)	0.02 (0.78)	0.05 (0.47)	0.02 (0.81)	− 0.05 (0.45)	0.02 (0.73)	0.25 (0.01)	0.02 (0.82)	0.01 (0.84)	0.04 (0.56)	0.03 (0.63)
STPO	–	–	–	–	0.29 (0.01)	− 0.36 (0.01)	− 0.29 (0.01)	0.39 (0.01)	− 0.06 (0.37)	0.12 (0.07)	0.46 (0.01)	0.28 (0.01)	− 0.46 (0.01)	− 0.27 (0.01)
STCH	–	–	–	–	–	− 0.27 (0.01)	− 0.21 (0.01)	0.41 (0.01)	− 0.07 (0.29)	0.15 (0.02)	0.33 (0.01)	0.34 (0.01)	− 0.45 (0.01)	− 0.27 (0.01)
STMUFA	–	–	–	–	–	–	0.82 (0.01)	− 0.47 (0.01)	0.06 (0.35)	− 0.15 (0.02)	− 0.36 (0.01)	− 0.24 (0.01)	0.53 (0.01)	0.46 (0.01)
STPUFA	–	–	–	–	–	–	–	− 0.39 (0.01)	0.16 (0.02)	− 0.11 (0.12)	− 0.24 (0.01)	− 0.19 (0.01)	0.42 (0.01)	0.45 (0.01)
LTLPR	–	–	–	–	–	–	–	–	− 0.08 (0.22)	0.25 (0.01)	0.64 (0.01)	0.46 (0.01)	− 0.82 (0.01)	− 0.76 (0.01)
LTLI	–	–	–	–	–	–	–	–	–	0.20 (0.01)	0.18 (0.01)	− 0.03 (0.67)	0.09 (0.20)	0.08 (0.22)
LTLS	–	–	–	–	–	–	–	–	–	–	0.50 (0.01)	0.23 (0.01)	− 0.24 (0.01)	− 0.28 (0.01)
LTLPO	–	–	–	–	–	–	–	–	–	–	–	0.39 (0.01)	− 0.70 (0.01)	− 0.62 (0.01)
LTLCH	–	–	–	–	–	–	–	–	–	–	–	–	− 0.45 (0.01)	− 0.35 (0.01)
LTLMUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	0.83 (0.01)
LTLPUFA	–	–	–	–	–	–	–	–	–	–	–	–	–	–

a. *M. Longissimus thoracis et lumborum* (LTL).
b. *M. Semitendinosus* (ST).
c. ST protein (STPR), ST iron (STI), ST sodium (STS), ST potassium (STPO), ST cholesterol (STCH), ST monounsaturated fatty acids (STMUFA), ST polyunsaturated fatty acids (STPUFA), LTL protein (LTLPR), LTL iron (LTLI), LTL sodium (LTLS), LTL potassium (LTLPO), LTL cholesterol (LTLCH), LTL monounsaturated fatty acids (LTLMUFA), and LTL polyunsaturated fatty acids (LTLPUFA).
d. STCH, STMUFA, STPUFA, LTLCH, LTLMUFA, LTLPUFA units as mg/100 g of total wet tissue.
e. Phenotypic correlations (P value).
f. Standard errors for correlations were 0.067.

Table 7. Chromosome and position of the top 0.5% 1-Mb windows for LTL traits [ab].

Chromosome	Trait (position[c])									
	LTL CH (% of fat)	LTL CH (mg/100 g)	LTL MUFA (mg/100 g)	LTL PUFA (% of fat)	LTL PUFA (mg/100 g)	LTL potassium (ppm)	LTL MUFA (% of fat)	LTL MUFA (mg/100 g)	LTL PUFA (% of fat)	LTL PUFA (mg/100 g)
1			5–6, 103–104, 117–118	5–6, 21–22	133–134	5–6				5–6
2	1–2, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10	5–6	14–15	1–2, 4–5, 6–7,	8–9, 9–10 7–8, 8–9, 9–10	7–8, 8–9, 9–10,	1–2, 4–5, 7–8, 29–30, 80–81	0–1, 1–2, 3–4, 8–9, 9–10, 13–14	0–1, 1–2, 4–5, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10	7–8, 8–9, 9–10, 5–6, 6–7 7–8, 8–9, 9–10,
13–14										
									13–14, 29–30, 30–31	
3							49–50, 53–54			
4	47–48		87–88, 92–93, 94–95	41–42						
5			8–9							
6					10–11,					
7	21–22		88–89		88–89, 111–112					16–17
8									52–53	
9		97–98			1–2, 60–61		95–96			95–96
10										91–92
11				45–46						
12		68–69					57–58		57–58	
13		79–80	33–34, 45–46			33–34, 34–35	33–34			
15		21–22, 23–24								
16		76–77, 78–79, 79–80		78–79, 79–80	66–67, 67–68, 77–78				42–43	
17						4–5				
18	11–12, 23–24	46–47							11–12	
19	7–8					17–18	7–8			
20		11–12		11–12		50–51				
21			2–3			2–3, 4–5				35–36
25		28–29								
28					34–35					
X	0–1	106–107	120–121		22–23		0–1	0–1	0–1	0–1, 19–20, 24–25, 97–98

a. Cholesterol (CH), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA).

b. Trait refers to a specific nutrient content that was measured from the *M. Longissimus thoracis et lumborum* (LTL).

c. Position refers to location in Megabases (Mb) for a particular chromosome derived from the *Bos taurus* build UMD_3.1 assembly (Zimin et al., 2009).

Table 8. Chromosome and position of the top 0.5% 1-Mb windows for ST traits [ab].

Chromosome	Trait (position[c])									
	ST CH (% of fat)	ST CH (mg/100 g)	ST Sodium (ppm)	ST Protein (%)	ST Iron (ppm)	ST Potassium (ppm)	ST MUFA (% of fat)	ST MUFA (mg/100 g)	ST PUFA (% of fat)	ST PUFA (mg/100 g)
1		6–7	47–48, 65–66		94–95, 144–145	90–91	21–22		41–42, 94–95	
2	1–2, 4–5, 7–8, 8–9, 9–10, 11–12	0–1, 0–2, 3–4, 5–6, 6–7, 8–9, 9–10	55–56	0–1, 1–2, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10	0–1, 1–2, 3–4, 8–9, 29–30, 41–42	1–2, 3–4, 8–9, 65–66	0–1, 1–2, 4–5, 5–6, 7–8, 8–9, 9–10	1–2, 7–8, 8–9	0–1, 1–2, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10	1–2, 7–8, 8–9
3		92–93, 98–99, 100–101, 114–115, 115–116	64–65			9–10,				
4	41–42				26–27					
5	107–108									107–108, 115–116
6	89–90		66–67					12–13		
7				6–7		6–7, 108–109, 110–111				
8						97–98				
9	97–98									
11						26–27		12–13		
12								53–54		53–54
13			46–47, 62–63, 63–64					38–39		
14			5–6, 8–9, 63–64		39–40	7–8				27–28
15	41–42			54–55, 76–77						
16			50–51	24–25	50–51	24–25	73–74, 74–75, 79–80		73–74, 74–75, 79–80	
17	14–15, 40–41									40–41
18								29–30		
19							7–8, 55–56			
21								26–27, 35–36		26–27
25										3–4
27					43–44			35–36		35–36
28								43–44		
29								34–35		1–2
X			48–49		3–4					113–114

a. Cholesterol (CH), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA).

b. Trait refers to a specific nutrient content that was measured from the *M. Semitendinosus* (ST).

c. Position refers to location in megabases (Mb) for a particular chromosome derived from the *Bos taurus* build UMD_3.1 assembly (Zimin et al., 2009).

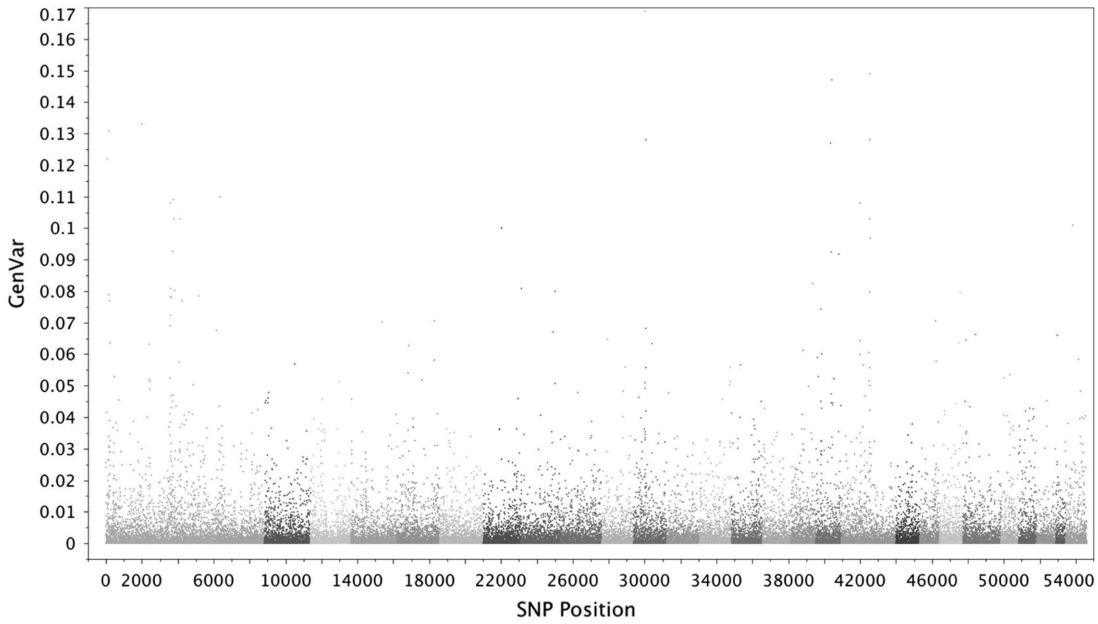

Figure 1. *M. semitendinosus* cholesterol Manhattan Plot. Genome-wide association analysis between 54,609 SNP and *M. semitendinosus* cholesterol (measured mg/100 g of whole wet tissue). The Y-axis GenVar (genetic variance) represents the contribution of a marker to the SNP variance. On the X-axis, alternate gray and black colors represent different chromosomes[a].
a. Chromosome refers BTA1 to BTA29, followed by unknown SNP locations and the X chromosome.

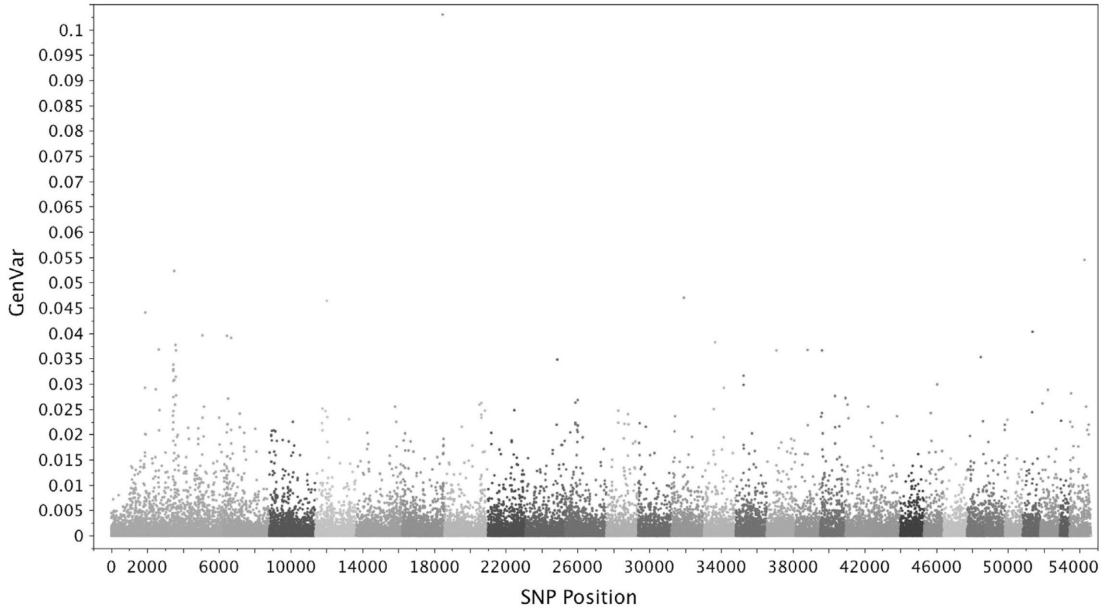

Figure 2. *M. semitendinosus* Potassium Manhattan Plot. Genome-wide association analysis between 54,609 SNP and *M. Semitendinosus* Potassium (measured as ppm). The Y-axis GenVar (genetic variance) represents the contribution of a marker to the SNP variance. On the X-axis, alternate gray and black colors represent different chromosomes[a].
a. Chromosome refers BTA1 to BTA29, followed by unknown SNP locations and the X chromosome.

The current study reports moderate to high proportions of phenotypic variation explained by the BovineSNP50_v2 BeadChip for CH, however, Eichhorn et al. (1986) did not find a significant effect of sire breed for CH leading the authors to conclude that alteration of CH content could not be made genetically. This is consistent with the findings of Elias Calles et al. (2000) who reported that CH content of the LTL muscle was not influenced by sire. The fact that the current study showed genetic control underlying CH content in this population, suggests that selection for decreased CH content in beef may be possible, partially due to the effect of the myostatin mutation.

The interpretation of results relative to fatty acids is conditional on understanding the scale of the phenotypes (percentage of total fatty acids or mg/100 g of wet tissue). When the gravimetric amount of PUFA, for instance, is low the amount of PUFA relative to total fatty acids (percentage of total fatty acids) can simply be high because the amount of total fatty acids was also very low. Similarly, when PUFA content is relatively high as a percentage of total fatty acids (i.e. when the amount of total fatty acids is also low) CH would also be expected to be relatively high when measured as a percentage of total fatty acids. However, the expectation that with an increase in adipose tissue CH increases, PUFA decreases and MUFA increases on a percentage of total lipids basis is challenged in the case of cattle with the double muscling phenotype. Raes, De Smet, and Demeyer (2001) have shown that the double muscling within the Belgian Blue breed has low proportions of MUFA and high proportions of PUFA in muscle lipid compared

with normal animals. This is due to the low concentration of total lipid in the muscle and a high ratio of phospholipid and total lipid. Phospholipids are high in PUFA content in order to perform the function as a constituent of cellular membranes (Wood et al., 2008). However, when PUFA content is high in mg/100 g of whole (wet) tissue, total fatty acid content is also likely high leading to a reduction in the proportionate amount of CH. Moderate to high phenotypic and GEBV correlations between protein and fatty acids can be explained by the fact that both were measured as a percentage of total meat content, and thus a strong relationship would be expected as one changes the other has to change as well.

Significant correlations between GEBV suggest that selection for increased iron concentration in the ST would lead to increased levels of MUFA and decreased levels of both CH and PUFA as a percentage of total lipids. This relationship is expected given that muscles with greater iron content would have more muscle protein and larger muscle fibers, resulting in a dilution of PUFA and cholesterol as the relative contribution of membrane lipids are reduced. In both cuts, selection for increased levels of potassium would have the opposite effects leading to increased PUFA and CH and decreased MUFA as a percentage of total lipids. On a total tissue basis, selection for increased potassium in both cuts would lead towards a correlated decrease in PUFA and MUFA and increase in CH. Selection for increased iron would lead to a correlated decrease in CH and an increase in MUFA and PUFA in the ST on a total wet tissue basis. The relationship between iron and fatty acids expressed on a total tissue basis and between potassium and fatty acids was unexpected, and in part may be due to inherent breed differences that were not accounted for within the admixed population used in the current study.

Sodium was lowly to moderately correlated with all traits measured, in agreement with Mateescu et al. (2013a) who also reported low to moderate correlations between sodium and other mineral traits. However, correlations between GEBV between the different cuts for sodium were high despite the low proportion of variation explained by the markers. This strong GEBV correlation may be due to markers picking up breed/family relationships, which would give rise to a larger positive GEBV correlation.

When measured as a percentage of total lipid content, correlations between GEBV for PUFA and MUFA were strong and negative, while the correlation between CH and MUFA and PUFA were negative and positive, respectively. However, when measured as mg/100 g, there were moderate to strong positive correlations between GEBV for PUFA and MUFA but moderate to strong negative correlations between GEBV for CH and MUFA and PUFA. This trend was observed across both cuts. Consequently, from a selection perspective, the phenotype used (percentage or mg/100 g) would lead to the selection of different animals. This is primarily because increases in fat content dilute fatty acids found in membranes, notably CH and PUFA. Expression of results as mg/100 g of wet tissue thus reflects overall increases in fat content.

Some significant SNP from the top 0.5% 1-Mb windows that were on BTA2 for each trait were in high LD with the myostatin *C313Y* alleles. Consequently, these SNP may simply be an artifact of the importance of the myostatin mutation for some for the traits analyzed. Between all traits and cuts there was a wide range in the number of 1-Mb windows that were on BTA2, ranging from 1 to 9 windows. Traits with few top windows on BTA2 are likely not impacted as much by *C313Y*. Previous work by Aldai et al. (2005) showed significant differences between animals of the Asturiana de los Valles breed of cattle that were homozygous for the myostatin deletion and those that were homozygous normal for protein percentage. The authors also showed that homozygous myostatin animals had lower proportions of MUFA and higher proportions of PUFA illustrating that this mutation has a measureable impact on these traits. This is supported by Wiener et al. (2009) who showed a significant effect of the myostatin mutation in South Devon cattle for both PUFA and MUFA concentrations. Outside of the myostatin mutation, Mateescu et al. (2013b) reported 16 SNP in a single Mb region (103–104 Mb) on BTA2 to explain 1.33% of the phenotypic variation of iron content, although the region reported by Mateescu et al. (2013b) does not overlap with the regions reported in the current study.

Functional annotation analysis resulted in a common gene found among lipid traits was GULP1 (*engulfment adaptor PTB domain containing 1*). GULP1 is an adaptor protein that binds and directs the trafficking of LRP1 (*low density lipoprotein receptor-related protein 1*), which is involved in lipid homeostasis (He & Lin, 2010). ITGAV is associated with metabolic processes and negative regulation of lipid transport and storage (Kim, Shin, Park, & Park, 2013).

Table 9. Predicted functional annotation of candidate genes located in the extended top 0.5% 1-Mb windows.

Ensembl gene ID	Gene	Function	BTA	Position[a]	Trait[b]
ENSG00000026652	AGPAT4	Lipid biosynthetic process	9	98.2	LTLCH (mg), STCH (%)
ENSG00000086848	ALG9	Lipid biosynthetic process	15	22.4	LTLCH (mg)
ENSG00000000419	DPM1	Lipid biosynthetic process	13	79.5	LTLCH (mg)
ENSG00000099377	HSD3B7	Lipid biosynthetic process	25	27.3	LTLCH (mg)
ENSG00000117594	HSD11B1	Lipid biosynthetic process	16	75.4	LTLCH (mg)
ENSG00000145675	PIK3R1	Phospholipid metabolic process	20	11.3	LTLCH (mg)
ENSG00000124212	PTGIS	Lipid biosynthetic process	13	78.3	LTLCH (mg)
ENSG00000105698	USF2	Lipid homeostasis	18	46.1	LTLCH (mg)
ENSG00000144366	GULP1	Lipid transport	2	77.8	STCH (mg), LTLPUFA (mg), STPUFA (mg) LTLMUFA (mg), STMUFA (mg), LTLCH (%), STCH (%), LTLPUFA (%), STPUFA (%), LTLMUFA (%)
ENSG00000157184	CPT2	Fatty acid metabolic process	3	93.6	STCH (mg)
ENSG00000116171	SCP2	Lipid transport	3	93.8	STCH (mg)
ENSG00000187048	CYP4A22	Fatty acid metabolic process	3	99.9	STCH (mg)
ENSG00000138448	ITGAV	Regulation of lipid transport and storage	2	9.6	LTLPUFA (%), STPUFA (%),LTLCH (%), STCH (%), LTLPUFA (mg) STPUFA (mg), LTLMUFA (mg), STMUFA (mg)
ENSG00000198691	ABCA4	Lipid transport	3	49.5	LTLMUFA (%)
ENSG00000117528	ABCD3	Lipid transport	3	49.1	LTLMUFA (%)
ENSG00000153933	DGKE	Phospholipid biosynthetic process	2	7.7	STMUFA (%)
ENSG00000123684	LPGAT1	Phospholipid biosynthetic process	2	9.6	STMUFA (%)
ENSG00000130479	MAP1S	Cytoskeleton organization	7	5.1	STPO

a. Trait refers to a specific nutrient content that was measured as mg/100 g of wet tissue (mg) and as a percent of total fat (%) from the *M. Semitendinosus* (ST) and *M. Longissimus thoracis et lumborum* (LTL).

b. Position refers to location in megabases (Mb) for a particular chromosome derived from the *Bos taurus* build UMD_3.1 assembly (Zimin et al., 2009).

5. Conclusions

In general, the mean estimates of the posterior heritability were moderate to high for fatty acids, suggesting that significant progress could be made through selection with the aid of genomics. The proportion of variation for mineral traits was more variable, although a moderate proportion of variation was explained by the markers for iron and potassium content. Differences did exist for fat traits depending on the scale of measurement (mg/100 g or percentage of total lipid content), in terms of relationships between traits, chromosomal regions underlying genetic variation, and in some cases the proportion of variation explained by the markers. The choice between these two scales would impact the ranking of animals. Potential candidate genes, GULP1 and ITGAV located on BTA2 in close proximity to C313Y were identified and involve regulation of lipids. Further investigation of these traits in other populations as well as analysis of expression of candidate genes identified here will allow for better understanding of lipid transport and regulation in muscle and their subsequent role in determining meat quality of livestock.

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