Use of Genomics to Improve Healthfulness And Quality of Meat

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Introduction

Beef is a nutritious source of protein and micronutrients for consumers. While beef is a good source of these nutrients on average, there is considerable variation from animal to animal in the concentration of many of these nutrients. Furthermore, beef is perceived as having a high fat content with undesirable composition, i.e., high percentage of saturated fatty acids (SFA). We are interested in developing molecular tools for beef producers to be able to select replacements with favorable nutrient concentrations, because nutrients are difficult and expensive to measure directly.

Material and methods

Resource Population and Phenotypes. This study utilized 2,285 Angus bulls (n = 540), steers (n = 1,311), and heifers (n = 434), which were finished on concentrate diets in Iowa (n = 1,085), California (n = 360), Colorado (n = 388), and Texas (n = 452). Groups of animals were harvested in commercial facilities when they had reached typical US market endpoint. Carcass data were collected at 24 to 48 h postharvest at the 12^{th} – 13^{th} rib interface of the longissimus dorsi. After carcass data were collected, a sample of longissimus dorsi that was anterior to the data collection interface was transported to a university meat laboratory where a 2.54 cm thick sample was removed and aged at 0°C for 14 d and then frozen at -20°C until Warner-Bratzler shear (WBS) force evaluation. Additionally, a 1.27 cm thick sample from the longissimus dorsi was removed, trimmed of all external fat and connective tissue then frozen at -20°C as a sample for evaluation of mineral and fatty acid concentrations.

Carcass traits collected were: hot carcass weight (HCW); fat thickness (FT) ¾ the lateral distance across the longissimus dorsi; cross sectional area of the longissimus dorsi (LEA); calculated USDA yield grade (YG) [YG = 2.5 + (0.984 × FT, cm) + (0.2 × kidney, pelvic, and heart fat, %) + (0.0084 × HCW, kg) - (0.05 × LEA, cm^2)]; dressing percent (DP) [DP = (HCW / preharvest live weight) × 100]; marbling score (MARB) [4.00 = $Slight^{00}$, 5.00 = $Small^{00}$, 6.00 = $Modest^{00}$, 7.00 = $Moderate^{00}$, 8.00 = $Slightly\ Abundant^{00}$]. Samples for WBS evaluation were cooked on an electric broiler to an internal temperature of 68C. Samples were allowed to cool to room temperature, then six 1.27 cm cores were collected from each

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sample. Each core was sheared with a WBS attachment and the peak force recorded. The average peak force of all cores was then used for analysis.

The longissimus dorsi sample for mineral and fatty acid concentration evaluation was freeze ground in liquid nitrogen to produce a powder that was used for all subsequent analyses. Nine minerals (Calcium; Copper; Iron; Magnesium; Manganese; Phosphorus; Potassium; Sodium; and Zinc) were quantified according to AOAC official methods 999.10 by closed-quartz-vessel microwave digestion (CEM, MDS-2000) and Inductively-Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, SPECTRO Analytical Instruments). Total fatty acids were extracted with a chloroform and methanol (2:1, vol:vol) mixture and quantified (Folch et al., 1957). Fatty acids were derivatized to methyl esters and quantified by a gas chromatograph (model 3400, Varian, Palo Alto, CA) equipped with a Supelco SP-2380 column (100 m × 0.25 mm i.d. × 0.20 μm film thickness) and a flame ionization detector. Atherogenic index was calculated as described by Ulbricht and Southgate (1991).

The ground beef sample designated for mineral and fatty acid concentration was the source of genomic DNA that was genotyped with the Illumina BovineSNP50 beadchip for collection of 54,001 SNP genotypes.

Statistical analyses. Trait means and standard deviations were calculated in SAS. Contemporary groups were defined as gender at harvest nested within harvest date with harvest date nested within each finishing location. There were 33 contemporary groups fit as fixed effects in genomic analyses. Effects of SNPs on each trait were estimated using the Bayes C module of the GenSel program (Kizilkaya et al., 2010), except that Pi (π) was jointly estimated in the analyses. Analytical settings were: a Markov-Chain Monte Carlo of 50,000 iterations, with 10,000 chain burnin; starting value for probability of a SNP not influencing the trait (π) = 0.90; genetic variance = 50% of observed phenotypic variance; residual variance = 50% of observed phenotypic variance.

Results and discussion

Carcass traits. The observed values for hot carcass weight (332 ± 32.4 kg), dressing percentage (60.7 ± 1.69), fat thickness (1.24 ± 0.47 cm), loin eye area (81.2 ± 7.98 cm^2), kidney, heart and pelvic fat (2.08 ± 0.40%), calculated yield grade (2.90 ± 0.67), and Warner-Bratzler shear force (3.53 ± 0.77 kg) were similar to U.S. industry averages. However, marbling score (5.96 ± 1.03) was higher than industry average, which may be due using Angus cattle for this study. In almost all cases, year, sex, location, and slaughter group significantly ($P<0.0001$) influenced all carcass traits with the exception that sex was not significant ($P>0.10$) for fat thickness or yield grade. Contemporary group was there after defined as year, sex, location and slaughter group for all subsequent statistical analyses.

The BayesCπ analyses indicated that between 28.1% and 50.4% of the observed variance could be explained by an animal's 54K genotype (**Table 1**). However, as all of the animals in the study were used in this analysis, the estimation of the variance accounted for by genotype will be inflated compared to validating the results on unrelated individuals. Unfortunately, BayesCπ also indicated that to achieve these levels of variance accounted for requires a large proportion of all available markers (**Table 1**). Thus in all cases, the number of markers used in these analyses greatly exceeded the number of individual animals. For example, 7,128 markers could account for 49.7% of the variance observed in yield grade, but

the number of animals with phenotypic data was only 2,284. Thus, if the number of markers was limited to no more than the equivalent of one SNP per animal, less variance would have been explained by the markers.

Table 1. Summary of BayesCπ analyses of carcass traits.

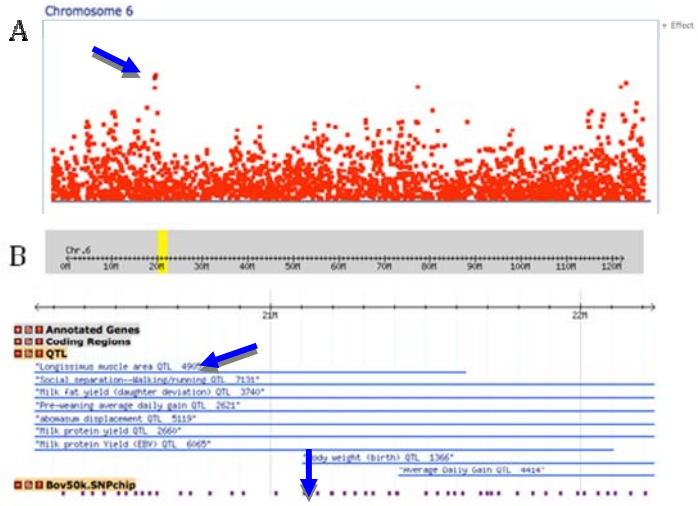
Trait	n	Posterior Mean of Residual Variance[a]	Posterior Mean of Genetic Variance[a]	Proportion of Variance accounted for by Markers	Posterior Mean of π
Dressing Percentage, %	726	1.724	0.685	0.284	0.313
Hot Carcass Weight, kg	2,284	0.031	0.031	0.504	0.521
Fat Thickness, cm	2,283	0.071	0.045	0.396	0.583
Loin Eye Area, cm^2	2,284	35.38	14.73	0.294	0.638
Calculated Yield Grade[b]	2,284	0.153	0.152	0.497	0.844
Marbling Score[c]	2,284	0.464	0.382	0.451	0.551
Warner Bratzler Shear, kg	2,251	0.266	0.104	0.281	0.868

[a] units for variance are the square of the units of the trait

[b] Yield Grade = 2.5 + (0.984 × FT, cm) + (0.2 × kidney, pelvic, and heart fat, %) + (0.0084 × HCW, kg) - (0.05 × LEA, cm^2)

[c] 4.00 = $Slight^{00}$, 5.00 = $Small^{00}$, 6.00 = $Modest^{00}$, 7.00 = $Moderate^{00}$, 8.00 = Slightly $Abundant^{00}$

Figure 1. Visualization of Loin Eye Area SNP effects.



[A] Absolute effect associated with a SNP.

[B] Location of SNP on genome and proximity to previously identified QTL.

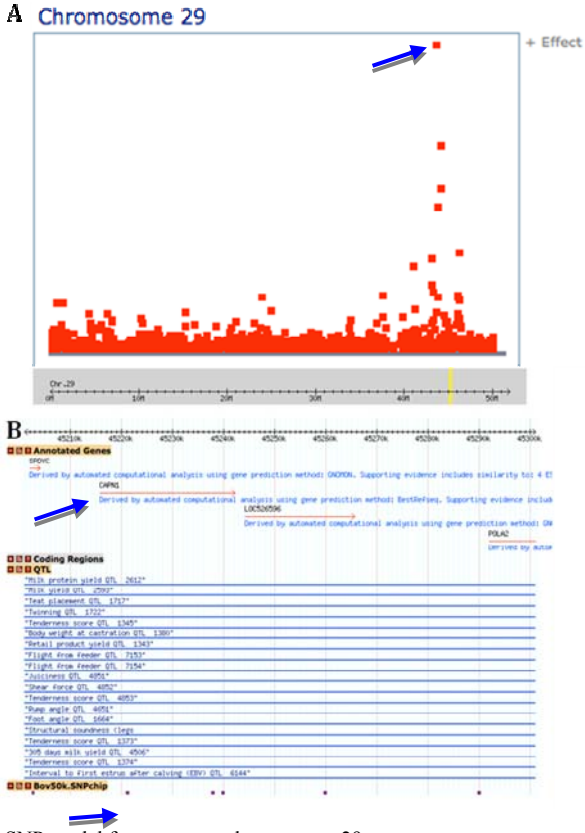
Mapping of either the effects associated with a given SNP or the frequency that a SNP is included in the model indicates that there are numerous regions in the genome that harbor genetic variation that is associated with carcass traits (**Figure 1A**). Examination of QTLdb (Hu et al., 2007) showed the SNP indicated in Figure 1A was located within a previously identified QTL for loin muscle area, (blue arrows). Interestingly, the largest

effects and model frequency for SNP associated with variation in Warner-Bratzler shear appear to be in close proximity with the Calpain 1 locus (**Figure 2**).

Minerals. The observed values for calcium (38.93 ± 20.95 ug/g meat), copper (0.91 ± 2.28 ug/g meat), iron (14.45 ± 3.03 ug/g meat), magnesium (254.4 ± 43.22 ug/g meat), manganese (0.07 ± 0.04 ug/g meat), phosphorus (1968 ± 282 ug/g meat), potassium (3430 ± 495 ug/g meat), sodium (489 ± 93 ug/g meat), and zinc (38.96 ± 7.87 ug/g meat) were similar to previously reported values (USDA, 2009).

In contrast to carcass traits, the amount of variation (0 to 16.1%) that could be accounted for by variation in SNP genotype was much lower, with the exception of iron content (44%) (**Table 2**). These results appear to indicate that genetic variation only accounts for a small proportion of the observed phenotypic variation in mineral content of beef, with the notable exception of iron content. Previously, human whole genome association studies have

Figure 2. Visualization of the Calpain 1 locus.



[A] SNP model frequency on chromosome 29.
[B] Identified QTL and SNP on the SNP chip in close proximity to the Calpain locus.

Table 2. Summary of BayesCΠ analyses of mineral traits.

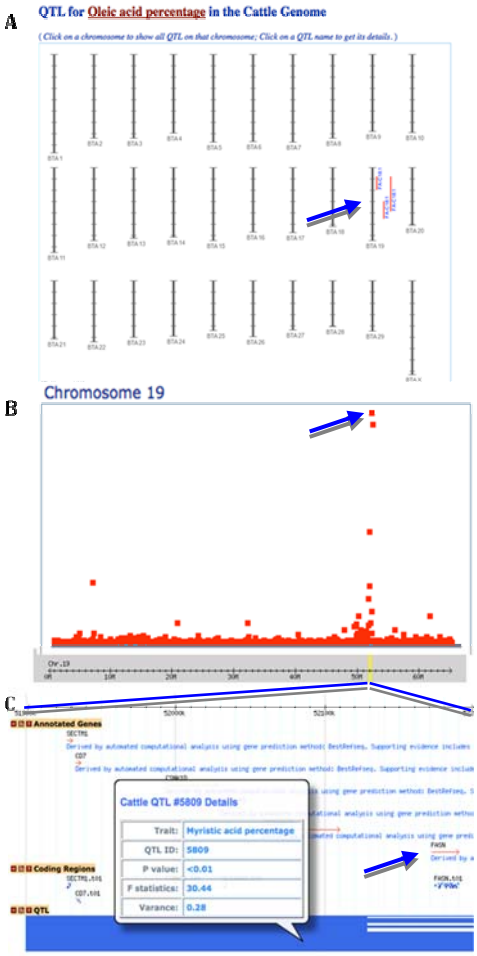
Trait	n	Posterior Mean of Residual Variance[a]	Posterior Mean of Genetic Variance[a]	Proportion of Variance accounted for by Markers	Posterior Mean of π
Calcium[b]	2,238	315.0	0.530	0.001	0.779
Copper[b]	1,990	3.856	0.070	0.017	0.998
Iron[b]	2,228	2.681	3.612	0.440	0.651
Magnesium[b]	2,241	472.1	91.0	0.161	0.215
Manganese[b]	1,978	0.002	0.000	0.093	0.999
Phosphorus[b]	2,239	27,718	3,167.3	0.102	0.355
Potassium[b]	2,192	108,480	0.325	0.000	0.802
Sodium[a]	2,240	3,080	0.423	0.000	0.709
Zinc[b]	2,228	41.1	9.333	0.183	0.215

[a] units for variance are the square of the units of the trait
[b] µg / g beef

identified a number of genomic regions that harbor genetic variation that is associated with hemochromotosis (Andriopoulos et al., 2009; Meynard et al., 2009). It will be very interesting to quantify the value of beef cattle as a genetic model for hemochromotosis.

Fatty acids. The observed means and standard deviations for individual fatty acids were myristic acid (14 carbons with no unsaturated bonds;14:0) (2.70 ± 0.57 vol/wt), palmitic acid (16:0; 26.57 ± 1.84 g/100g), palmitoleic acid (16:1; 3.52 ± 0.70 g/100g), stearic acid (18:0; 13.57 ± 1.88 g/100g), oleic acid (18:1c9; 38.66 ± 2.78 g/100g), linoleic acid (18:2; 3.94 ± 1.29 g/100g), α-linolenic acid (18:3; 0.17 ± 0.16 g/100g), conjugated linoleic acid c9t11 (0.12 ± 0.13 g/100g), and conjugated linoleic acid t10c12 (0.04 ± 0.06 g/100g). The observed means and standard deviations for measures of saturation and desaturation were: atherogenic index (0.69 ± 0.09), n3 desaturation (0.56 ± 0.57 g/100g), n6 desaturation (5.12 ± 1.63 g/100g), total saturated fatty acids (SFA; 45.24 ± 2.40 g/100g), total mono-unsaturated fatty acids (MUFA; 49.08 ± 2.81 g/100g), total poly-unsaturated fatty acids (PUFA; 5.68 ± 1.86 g/100g), and poly-unsaturated to saturated ratio (PUFA/SFA; 12.6 ± 4.30).

Figure 3. Fatty acid synthase locus



BayesCπ analyses indicated that 2.3% to 48.5% of observed variance could be explained by an animal's 54K genotype (**Table 3**). In general, long chain fatty acids appear to be lowly heritable traits with a low proportion of variance accounted by markers. In stark contrast to carcass traits where a large proportion of all available markers were necessary to account phenotypic variance, BayesCπ indicated that relatively few markers were needed to account for proportional levels of observed variance in fatty acids (**Table 3**). For example, 18 SNPs accounted for 32.4% of variation observed in myristic acid (14:0). For seven of the measurements listed in **Table 3** where SNPs accounted for more than 34.2% of the observed variation, less than 864 SNP (<1.6%), which is almost 1/3 the number of animals in this study, were required to account for the proportion of variance explained by markers.

Mapping either the effects associated with a given SNP or the frequency that a SNP is included in the model indicated numerous regions in the genome that harbor genetic

variation associated with fatty acid composition (**Figure 3**). For example, toward the telomeric end of chromosome 19, numerous SNPs appear to be associated with myristic acid concentration (**Figure 3B**). Based on results curated into QTLdb (Hu et al., 2007), previous studies have identified QTLs for myristic, palmitic, oleic and other fatty acids in this region (**Figure 3A**; Morris et al., 2007; Abe et al., 2008). Evaluation of the genes underlying these QTL indicated that fatty acid synthase was within 60 kb of the SNP associated with the largest effect (**Figure 3C**). Previously, we evaluated Fatty acid synthase (FASN) as a candidate gene for its possible association with fatty acid composition (Zhang et al., 2008). A non-synonymous mutation was significantly associated with the concentration of several fatty acids. Morris et al. (2007) observed similar associations with the same mutation. The non-synonymous FASN mutation may be responsible for the effect observed here.

Table 3. Summary of BayesCΠ analyses of selected fatty acid profile traits.

Trait[a]	n	Posterior Mean of Residual Variance[b]	Posterior Mean of Genetic Variance[b]	Proportion of Variance accounted for by Markers	Posterior Mean of Π
14:0	1,999	0.185	0.089	0.324	0.999
16:0	1,999	1.621	0.883	0.352	0.997
16:1	1,999	0.249	0.172	0.408	0.995
18:0	1,999	1.090	1.030	0.485	0.984
18:1c9	1,999	3.000	2.626	0.466	0.992
18:1t10 + 18:1t11	1,999	0.684	0.502	0.423	0.909
18:2	1,999	0.737	0.409	0.357	0.632
18:3n3	1,999	0.010	0.000	0.025	0.999
CLAc9t11[c]	1,999	0.010	0.000	0.023	0.999
CLAt10c12[c]	1,999	0.003	0.000	0.027	0.999
Atherogenic Index	1,999	0.004	0.002	0.363	0.998
n3	1,999	0.011	0.000	0.019	0.999
n6	1,999	1.262	0.651	0.340	0.662
Saturated	1,999	2.311	2.004	0.464	0.994
Mono-unsaturated	1,999	2.906	2.214	0.432	0.990
Poly-unsaturated	1,999	1.580	0.805	0.337	0.653
Poly-unsaturated / Saturated	1,999	0.001	0.000	0.343	0.333

[a] fatty acids are reported as percentage of extracted lipid
[b] units for variance are the square of the units of the trait
[c] CLA = conjugated linoleic acid

Table 4. Proportion of variance that can be explained by the ten most significant SNPs.

Trait[a]	Proportion of variance accounted for by top 10 Markers	Proportion of 54K variance accounted for by Top 10 Markers [b]
14:0	0.282	0.870
16:0	0.225	0.639
16:1	0.164	0.402
18:0	0.119	0.245
18:1c9	0.185	0.397
18:1t10 + 18:1t11	0.077	0.182
18:2	0.062	0.174
18:3n3	0.047	1.880
CLAc9t11[c]	0.067	2.913
CLAt10c12[c]	0.073	2.704
Atherogenic Index	0.276	0.760
n3	0.001	0.053
n6	0.062	0.182
Saturated	0.188	0.405
Mono-unsaturated	0.153	0.354
Poly-unsaturated	0.060	0.178
Poly-unsaturated / Saturated	0.052	0.152

[a] List of fatty acids

[b] Calculated by dividing the proportion of variance accounted for by top 10 Markers by the proportion of variance accounted for by 54K SNP times 100.

[c] CLA = conjugated linoleic acid

In addition, we evaluated the extent the top ten SNPs associated with each fatty acid could account for variation observed with a given fatty acid measurement (**Table 4**). For example, the top ten SNP associated with myristic acid could account for 87% of the variance accounted for when using 18 SNPs identified by BayesCπ. Upon first glance, it would appear that the top ten SNPs associated with 18:3n3, CLAc9t11 and CLAt10c12 could actually account for more variation than entire list of SNPs identified by BayesCπ. However, this is an artifact as the number of SNPs actually identified by BayesCπ was less than ten. These results indicate that at least for some traits a reduced SNP panel will be able to account for a majority of the variation associated with the SNPs identified by BayesCπ.

Conclusion

Based on these results, it would appear that a relatively large amount of variation in carcass traits is associated with SNPs present on the 54K Illumina SNPchip. However, as the number of SNP needed to account for this variation greatly exceeds the number of animals in this study, it is likely that the amount of variation associated with SNPs is over estimated.

Surprisingly, very little variation in mineral content, with the exception of iron content, is associated with an animal's genotype. In contrast, a large proportion of variation in fatty acid composition is associated with a relatively low number of SNPs. Because all of the animals in this study were used to estimate the effects associated with traits, it is important to remember that the amount of variation that can be accounted for in other populations will most likely be lower than that observed here. Taken together, these results indicate that genetic progress can be achieved by implementation of whole genome selection for carcass traits, meat quality and nutrient composition.

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