Association of Genetic Variation to Healthfulness of Beef

A.S. Leaflet R2009

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Summary and Implications

The objective was to determine the natural variability in beef fatty acid composition and to identify single nucleotide polymorphisms (SNPs) in the stearoyl-CoA desaturase (SCD) gene to test the association of SNPs with fatty acid composition. We have used common gas chromatographic techniques to determine the fatty acid composition of phospholipids and triacylglycerols (TAGs) extracted from beef muscle (*longissimus dorsi*) from 800 sire-identified cattle originating from Iowa State University beef cattle breeding selection projects. Heritability of individual fatty acids and indexes of fatty acid desaturase and elongase systems were calculated by evaluating specific ratios of fatty acids (product/precursor). In general, we found that TAG composition is heritable, but phospholipid composition is not. The atherogenic index of TAGs as proposed by Ulbright and Southgate had a heritability estimate of 0.55 and 0.45 for the TAG and total lipids, respectively. Individual fatty acids of TAGs also had high heritability estimates. For example, the heritability estimates of 14:0 and 16:0 in TAG were 0.49 and 0.40, respectively. Monounsaturates 16:1 and 18:1 in TAGs both had heritability estimates greater than 0.5. Future research will focus on DNA sequencing of candidate genes from sires that are phenotypically divergent for a trait of interest. Ultimately, we plan to develop DNA markers for use in selecting breeding stock to improve healthfulness of fatty acids in beef. Three SNP candidates in the SCD gene were tested by using Iowa State University beef cattle. Two of the potential SNPs were homozygous in this population (n=123). We have classified 172 cattle into two genotypes, VA and VV, based on the third SNP. The ratio of palmitoleic acid (C16:1) concentration vs palmitic acid (C16:0) concentration of TAG is significantly associated with the SNP (P=0.02). There is no significant association of the SNP with the fatty acid composition of phospholipids.

Introduction

The consumption of beef has decreased, especially in comparison to poultry meat products, over the last 20 years. According to the Economic Research Service (USDA), during the years 1970-1999, red meat consumption decreased 11% per person, whereas poultry intake increased 102%. Similarly, the American Meat Institute reports a 15% decrease in beef consumption during the years 1980-2000. One of the major factors affecting beef intake has been the recommendations of dieticians and health professionals. The general consumer has been trained that it is desirable to decrease consumption of foods rich in saturated fatty acid (SFA). Often it is recommended that beef be excluded from the diet because it has been identified as a food rich in SFA. Atherosclerosis and other vascular diseases are correlated positively with SFA intake.

Much of the beef that Americans consume (43.2%) is eaten as ground beef, which is often rich in fat, ranging from 5 to 30% fat by weight. Therefore, to prevent beef, an important source of iron, essential amino acids, and B vitamins, from being further excluded from the American diet, we propose to improve the fatty acid composition of beef by capitalizing on the natural genetic differences between animals. It is our thought that improvements in the healthfulness of the fatty acid composition of beef can be made while maintaining other positive physical and chemical attributes of the product (e.g., tenderness, juiciness, flavor, texture).

Being able to improve the fatty acid composition of beef while maintaining or improving other carcass characteristics is supported in the literature. In a 2002 Australian study by Pitchford and others, Hereford cows were mated to 97 sires from seven breeds and fatty acid composition differed based on breed of sire, but the fatty acid composition data did not correlate with other carcass characteristics, such as hot carcass weight and intramuscular fat content. The team of Australian researchers also report similar trends compared with what we have observed in our data base of fatty acid composition in that desaturase indexes are highly heritable, whereas elongase indexes are not heritable. In another study from Australia, Ch'ang et al. (1980) analyzed perirenal fat from 255 sheep to determine the genetic effects of sire on the five major fatty acids. In this study, 30 sires of the same breed were used and a strong sire effect on the concentration of palmitic, palmitoleic, and oleic acids was observed. There was also a strong sire effect on the delta-9 desaturase index (16:1/16:0). The heritabilities that Ch'ang et al. (1980) calculated for individual fatty acids and delta-9 desaturase indexes are similar to the heritabilities that we have calculated from our

data base, giving us further confidence in our data base of fatty acid composition.

Stearoyl-CoA desaturase is responsible for the conversion of 16:0 and 18:0 to 16:1 and 18:1, respectively, the two major monounsaturated fatty acids of bovine lipids. Studies by Yang et al. (1999) showed that SCD activity was greater in bovine adipose tissue that had a greater proportion of monounsaturated fatty acids. Taniguchi et al. in Japan (2004) found a SNP in the bovine SCD gene that causes an amino acid replacement from valine to alanine. After classifying 1,003 Japanese Black cattle into three genotypes, VV, VA, and AA, these authors observed that the SCD genotypes are associated with percentage of monounsaturated fatty acids (MUFA) and melting point in intramuscular fat. This result suggests that the genotyping of the SCD gene is a useful tool for selection of heart healthier beef cattle.

Materials and Methods

Cattle from Iowa State University beef breeding selection and tenderness projects were used in this study. Rib steaks were collected approximately 24 hours post-harvest and returned to Iowa State University for processing. Bone, external fat, and connective tissue were removed from the *longissimus dorsi* muscle, and the sample was ground to homogeneity in a food processor. Dry matter was determined gravimetrically after drying a portion of the sample in an oven at 120º C for 24 hours. Total lipid was extracted by using organic solvents (chloroform and methanol). Total phosphorus was determined in the lipid extract by wet ashing the lipids followed by a colorimetric assay containing ammonium molybdate, an indicator for phosphorus. Phospholipids were separated from TAGs by using thin-layer chromatography developed in hexane and ethyl acetate (4:1; v:v). The individual lipid spots were derivatized to methyl esters by using acetyl chloride in methanol prior to gas chromatography for determination of fatty acid composition. The fatty acids in the entire sample (phospholipids plus TAGs) were estimated on the basis of a weighted average of phospholipid and triacylglycerol fatty acid composition. In addition to fatty acid composition data, several indexes were evaluated. The atherogenic index as described by Ulbright and Southgate is calculated as shown.

$$= \frac{12{:}0 + 4(14{:}0) + 16{:}0}{\Sigma(\text{MUFAs}) + \Sigma(\text{PUFAs})}$$

Indexes also were used to predict the activity of fatty acid desaturase and elongase systems. In both cases, the ratios of the product to precursor were evaluated. Examples of desaturase indexes would be 16:1/16:0 or 18:1/18:0. Likewise, elongase indexes would be represented by 18:0/16:0 or 16:0/14:0. The resulting data were summarized and analyzed using restricted maximum likelihood (REML) with a sire-maternal grandsire relationship matrix. There were 63 contemporary groups (1-65 cattle per group) and 77 sires (1- 40 progeny per sire) represented in the data.

Two of the SNP candidates in 5' untranslated region (5'UTR) and exon 2 of the SCD gene, respectively, were selected based on IBISS (interactive bovine *in silico* SNP database, sponsored by CSIRO, Australia). The potential SNP in 5'UTP, named SCD316 (position 316 of btcn11869), is a C/T polymorphism. The potential SNP in exon 2, SCD536 (position 536 of btcn11869), is an A/T polymorphism, which cause an amino acid change (A allele codes for isoleucine, and T allele codes for phenylalanine). One previously reported SNP in exon 5, SCD1278 (position 1278 of btcn11869), is a T/C polymorphism, which produces an amino acid substitution (T allele codes for valine, and C codes for alanine). The SNPs were detected by PCR-RFLP (restriction fragment length polymorphism). Three pairs of primers for PCR were: SCD316, F-gtgtgtgcagcatccagttc, R-actttctcggggctgagact; SCD536, F-gactcccctatacttggaagaagat, R-tcaggaggacatgggaactt; SCD1278, F-ggataccgccctttatgacaa, R-tagacgtggtcttgctgtgg. The first SNP site was detected by digestion with spe I, and the third SNP was detected using Fnu4HI. There was no suitable restriction enzyme to detect the second SNP, so a mismatch PCR- RFLP was used to introduce an EcoR V recognition site. The mixed model used for statistical analysis was $Y_{ijkl} = \mu + X_i + G_j + B_k + \beta_{lipid} + \varepsilon_{ijkl,}$ where Y_{ijkl} is C16:1/C16:0 of beef lipid fractions, X_i is the fixed effect of year of sample collections, G_j is the fixed effect of genotypes, B_k is the random effect of sires, β_{lipid} is the linear effect of beef lipids content as a covariant, and ε_{ijkl} is error.

Results and Discussion

The composite (TAGs plus phospholipids) fatty acid composition (Table 1) is similar to other published data on fatty acid composition of beef. The heritability of the fatty acids that are synthesized in beef tissue (e.g., 14:0 and 16:0) tends to be greater than the heritability of those fatty acids that are strictly from dietary origin (e.g., 18:2 and 22:5). The standard error values are large as a percentage of the heritability estimate because of the relatively small dataset. It should be noted, however, that a trait with heritability greater than 0.2 can be the focus of selection, and rapid changes in a given trait can be expected in just a few generations. If selection programs for fatty acid composition of beef were begun, the first objective would be to decrease the amount of 14:0 and 16:0 or to increase the amount of monounsaturated fatty acids. These traits have heritability estimates that would allow for rapid improvement of the trait, and these fatty acids are the focus of concern for those advising humans how to eat a heart-healthy diet. Other than 16:1, which is not atherogenic, 14:0 and 16:0 have the highest heritability estimates for the composite fatty acids. The differences in heritability are possibly a result of differences in the fatty acid synthase

enzyme system. This multifunctional enzyme synthesizes fatty acids from two carbon building blocks. Typically, the synthesis stops when the fatty acid is 16 carbons long. Palmitic acid (16:0) then is released from the enzyme, and further processing can occur before the fatty acid is incorporated into TAGs for storage or into phospholipid for membrane synthesis. The 16:0 can be elongated further by a separate enzyme system named fatty acid elongase. Double bonds can be introduced into fatty acids by a family of enzymes named fatty acid desaturases. Any points in these synthetic steps (synthesis, elongation, or desaturation) provide points of focus as candidate genes to describe genotypic differences for the observed phenotypic differences. Therefore, in addition to the methods of traditional breeding programs where phenotype are selected for without necessarily knowing the underlying genotype that is responsible for a trait of interest, we have solid candidate genes to focus molecular attempts at describing phenotypic fatty acid composition differences.

Table 1. Fatty acid composition and fatty acid heritability in total lipids extracted from lean beef.

Fatty Acid	n	Fatty Acid Weight %	h^2	SE
14:0	794	2.81	0.39	0.21
14:1	794	0.64	0.11	0.15
16:0	794	26.28	0.40	0.21
16:1	794	3.35	0.54	0.24
18:0	794	12.79	0.27	0.19
18:1	794	41.05	0.33	0.20
18:2	794	7.46	0.23	0.18
20:3(n3) & 20:4	794	2.14	0.24	0.18
22:5	794	0.53	0.14	0.16
22:6	794	0.10	Did not converge	

n = number of animals, h^2 = heritability, SE = standard error

When only TAG composition is considered (Table 2), the patterns of heritability are similar to the composite sample. This similarity is to be expected because roughly 80% of the total lipids, even in lean beef tissue, are from triacylglycerol. When the heritability of triacylglycerol fatty acids and phospholipid fatty acids (Table 3) are considered, the triacylglycerol fatty acid heritabilities are much greater than those for phospholipids. This difference may be because phospholipids are crucial building blocks of cellular membranes and because slight variation in membrane composition could lead to big differences in terms of cellular fitness and survival. Whereas TAG fatty acids, on the other hand, are a way to store energy, differences in fatty acid composition would not affect the fitness of the animal as much as changes in phospholipid fatty acid composition. Furthermore, the fatty acids in TAGs tend to be shorter chain fatty acids that are more saturated than the fatty acids in phospholipids. In other words, a larger percentage of phospholipid fatty acids are essential fatty acids than are TAG fatty acids.

Table 2. Fatty acid composition and fatty acid heritability in triacylglycerols extracted from lean beef.

Fatty Acid	n	Fatty Acid Weight %	h^2	SE
14:0	809	3.32	0.49	0.23
14:1	809	0.78	0.12	0.15
16:0	809	28.33	0.40	0.21
16:1	809	3.89	0.50	0.23
18:0	809	13.74	0.30	0.19
18:1	809	45.97	0.54	0.23
18:2	809	2.14	0.09	0.14

n = number of animals, h^2 = heritability, SE = standard error

Table 3. Fatty acid composition and fatty acid heritability in phospholipids extracted from lean beef.

Fatty Acid	n	Fatty Acid Weight %	h^2	SE
14:0	795	0.89	Did not converge	
16:0	795	18.83	0.03	0.12
16:1	795	1.31	0.14	0.15
18:0	795	9.48	0.03	0.12
18:1	795	22.52	0.18	0.17
18:2	795	27.01	0.26	0.18
20:3(n3) & 20:4	795	10.15	0.20	0.17
22:5	795	2.40	0.30	0.19
22:6	795	0.32	0.06	0.13

n = number of animals, h^2 = heritability, SE = standard error

To evaluate the relative activity of candidate enzyme systems, we calculated indexes by placing the product of an enzymatic reaction in the numerator and the precursor for that reaction in the denominator. This calculation then should give an historical account of the activity of a particular enzyme system (e.g., fatty acid elongase or desaturase). In addition, we also calculated the atherogenic index. Table 4 contains the heritability estimates for these indexes in the phospholipids and TAGs and in the composite sample of both lipids from nearly 800 cattle. The atherogenic index, which is dependent on the overall fatty acid composition of a sample, is highly heritable. A selection program based to improve the atherogenic index would be selecting for some combination of (1) fewer short chain saturated fatty acids (14:0 and 16:0), (2) more desaturase activity (conversion of saturated acids into monounsaturated acids), and (3) more elongase activity (the conversion of 14:0 and 16:0 to 18:0, which is neutral with respect to atherogenicity). The indexes were not heritable in the phospholipid fraction but were moderately to highly heritable in the TAG and composite fractions.

Table 4. Heritability estimates and standard errors of atherogenic index, desaturase indexes, and elongase indexes in phospholipid and triacylglycerol fractions as well as total lipids extracted from lean beef.

Index	Phospholipid		Triacylglycerol		Composite	
	h^2	SE	h^2	SE	h^2	SE
AI	Did not converge		0.55	0.24	0.45	0.22
16:1/16:0	0.12	0.15	0.38	0.21	0.44	0.22
18:1/18:0	0.09	0.14	0.26	0.18	0.30	0.19
X:1/x:0	0.12	0.15	0.36	0.20	0.37	0.21
16/14	0.09	0.14	Did not converge		0.10	0.14
18/16	0.02	0.12	0.35	0.20	0.34	0.20

n = number of animals, h^2 = heritability, SE = standard error

Expected progeny differences (EPDs) also were calculated for the traits that had the greatest heritability. Table 5 depicts the EPDs as a percentage of the value of the given trait for the composite lipid samples. In general, these differences that can be attributed to a particular sire are between 5 and 10%. While these changes may not be large enough to make immediate compositional changes in beef fatty acids that are meaningful to the consumer, it does offer hope that improvements could be made. From the data we have collected, traditional breeding selection programs would work to improve the fatty acid composition of beef. We are interested, however, in identifying genetic variance in candidate genes to eventually be able to predict the fatty acid composition of beef tissue based on DNA analysis. If successful, this technology will lead to the development of beef with an improved fatty acid composition or "heart-healthier" beef.

Table 5. Expected progeny differences of fatty acid indexes from total lipids extracted from lean beef.

Trait Index	Extreme (+) EPD as % of Average	Extreme (-) EPD as % of Average
Atherogenic index	8.5	5.8
16:1/16:0	6.9	6.9
18:1/18:0	4.3	5.8
x:1/x:0	4.0	3.9
16/14	4.6	4.4
18/16	6.9	3.9

Table 6 shows the genotype frequency of SCD1278. Nearly 2/3 of the 172 cattle had genotype VV, and 1/3 of the cattle had genotype VA. No animals in this population were homozygous AA. This frequency is different from that of Japanese Black cattle, of which 9% were genotype VV, 63% were genotype VA, and 28% were genotype AA. The frequencies of both the genotypes VV and VA were high. Even though the total number of animals was small, the high frequency resulted in an adequate number of animals in each genotype for accurate assessment of the association of genotype to fatty acid composition traits. SCD316 is a potential regulatory SNP, and SCD536 is a potential coding SNP, which were represented in more than one sequence in IBISS. We have analyzed 123 bovine DNA samples, and no variation was detected in these two sites.

Table 6. Genotype frequency of SCD1278

Genotype	Alleles	Number of animals	Percentage (%)
VV	CC	115	67
VA	TC	57	33
Total		172	100

Table 7 shows the effect of SCD1278 on the desaturase index. The genotypes of SCD were significantly associated with 16:1/16:0 (P=0.02) in TAG. No significant association was detected between SCD1278 with 16:1/16:0 in phospholipid, and with 18:1/18:0 and (18:1+16:1)/(18:0+16:0) in any lipid fractions. Stearoyl-CoA desaturase catalyzes the reaction of introducing a *cis* double bonds at carbon number 9 of 16:0 and 18:0 to synthesize 16:1(n7) and 18:1(n9), respectively. In ruminants, most dietary unsaturated fatty acids are biohydrogenated by the microorganisms in the rumen, and absorbed as saturated fatty acids. The ratio of 16:1 to 16:0 and 18:1 to 18:0 in beef lipid therefore reflect the effect of SCD on substrates. The significant association of SCD1278 with 16:1/16:0 occurred in TAG but not in phospholipids, which is consistent with our results that TAG fatty acid composition is more heritable than phospholipid fatty acid composition. The lack of significant association of SCD1278 with 18:1/18:0 may because we did not quantify the individual 18:1 isomers. Beef fatty acid composition data, which have been collected for other experiments, included several isomers of 18:1 in TAG (data not shown). The major isomer, 18:1(n9), constitutes 70-85% of the total 18:1 concentration. The 18:1 isomers that were not synthesized by SCD interfered with the accurate assessment of the association between SCD genotypes and 18:1/18:0. Taniguchi et al. (2004) observed that Japanese black cattle with genotype AA had the highest MUFA content in intramuscular fat, and cattle with genotype VV had the lowest MUFA content in intramuscular fat. Our study showed a similar trend in that cattle with genotype VA had higher 16:1/16:0 in the TAG fraction of intramuscular fat than the cattle with genotype VV. Casas et al. (2005)

observed that did the allele frequencies of several SNPs are different between *Bos taurus* and *Bos indicus*. They concluded that the genetic markers developed in *Bos taurus* might not suitable in population of *Bos indicus* because of the allele frequency difference. The difference of allele frequency of SCD1278 between Japanese Black cattle and Iowa State University cattle might cause some divergence of the results. The MUFA include several fatty acids that are not synthesized by SCD and may not be a good index for SCD. Our studies showed no significant association of SCD1278 with MUFA concentration in intramuscular fat, indicating that other genetic factors responsible for the variation MUFA should be studied in the future. Our results suggested that SCD1278 is a valuable SNP for regulating the fatty acid composition of beef cattle. More molecular markers should be developed for the selection of heart healthier beef cattle.

Acknowledgements

Financial support for this experiment was provided by the Iowa State University Burroughs Endowment and by the Center for Designing Foods to Improve Nutrition. Animals used in this study originated from the ISU Rhodes Farm at Rhodes, IA, the ISU McNay Farm at Chariton, IA, the ISU Armstrong Farm at Lewis, IA, and the ISU beef tenderness project.

Table 7. Effect of SCD1278 on desaturase indexes

Lipid	16:1/16:0		P-value	18:1/18:0		P-value	(16:1+18:1)/(16:0+18:0)		P-value
	VA	VV		VA	VV		VA	VV	
PL	0.063±0.003	0.068±0.002	0.13	2.30±0.07	2.36±0.05	0.43	0.82±0.03	0.84±0.02	0.50
TAG	0.145±0.003	0.139±0.002	0.02	3.21±0.07	3.34±0.05	0.09	1.19±0.02	1.21±0.01	0.34