Redesigning Beef Cattle to Have a More Healthful Fatty Acid Composition

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Summary and Implications

The objective was to determine the natural variability in beef 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.

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 recommendation of dieticians and health professionals. The 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 highly 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 concentrations of palmitic, palmitoleic, and oleic acids were 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.

Materials and Methods

Cattle from Iowa State University beef breeding selection projects 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 120° 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 TAG) were estimated on the basis of a weighted average of phospholipids 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.

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

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.

Results and Discussion

The composite (TAG plus phospholipids) fatty acid composition (Table 1) is similar to other published data on fatty acid composition of beef. Heritability of the fatty acids that are synthesized in beef tissue (e.g., 14:0 and 16:0) tend 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 hearthealthy 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 phospholipids 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 phenotypes 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.

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Fatty	n	Fatty Acidh²		SE	
Acid		Weight %			
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)	794	2.14	0.24	0.18	
& 20:4					
22:5	794	0.53	0.14	0.16	
22:6	794	0.10	D	NC	

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

n = number of animals, h^2 = heritability, SE = standard error, DNC = did not converge

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 and 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 TAGs fatty acids.

heritability in triacylglycerols extracted from lean be				
Fatty	n	Fatty Acid h ²		SE
Acid		Weight %		
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	ofoning	ala h ² - hamitahili	try CE - at	and and amon

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

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	n	Fatty Acidh²		SE
Acid		Weight %		
14:0	795	0.89	DNC	
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)	795	10.15	0.20	0.17
& 20:4				
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, DNC = did not converge

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 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 on improving 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 highly heritable in the phospholipids 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	\mathbf{h}^2	SE
AI	Dì	NC	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	DI	NC	0.10	0.14
8/16	0.02	0.12	0.35	0.20	0.34	0.20

n = number of animals, $h^2 =$ heritability, SE = standard error, DNC = did not converge

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 detected because of 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 over time. 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-healthy" beef.

indexes from total lipids extracted from lean beef.					
Trait	Extreme (+)	Extreme (-)			
Index	EPD as % of	EPD as % of			
	Average	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 5. Expected progeny differences of fatty acid

 indexes from total lipids extracted from lean beef.

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