Comparison of the Fatty Acid Composition of Subcutaneous Adipose Tissue from Mature Brahman and Hereford Cows[1]

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ABSTRACT: The fatty acid composition of adipose tissue was measured in 37 mature Brahman and 32 mature Hereford cows to determine breed effect. Diet was held constant among all cows. When biopsied, cows were on oats and native cool-season annual pastures of good quality. Real-time ultrasound measurements of subcutaneous fat were taken at three locations (between the 12th and 13th rib, at the rump, and at the perianal region) to determine overall fatness. Overall fat thickness from these measurements was 1.3 cm for Brahman cows and 1.7 cm for Hereford cows ($P < .01$). Subcutaneous adipose tissue biopsy samples were collected from the perianal region, and fatty acid composition was determined using a gas chromatograph. Fatty acids were expressed in both normalized (area percentage) and gravimetric (grams/100 grams of fresh tissue) formats. In addition to greater overall subcutaneous fat thickness, Hereford cows contained 5 g more of fatty acids per 100 g of fresh adipose tissue than Brahman cows ($P < .05$). Subcutaneous adipose tissue from Hereford cows was higher ($P < .01$) in total saturated fatty acids and lower in mono- and polyunsaturated fatty acids than subcutaneous adipose tissue from Brahman cows. Compositional differences remained when breeds were compared by analysis of covariance at a common body fatness. The data suggest a genetic basis for the differences in fatty acid composition of Brahman and Hereford cows.

Key Words: Fatty Acids, Cows, Beef, Adipose Tissue, Breeds

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Introduction

The modern, genetic approach to manipulating fatty acid profiles in cattle should be to select for individuals or breed types capable of transmitting to their descendants the ability to accumulate adipose tissues with less palmitate and(or) more oleate and(or) stearate because the latter have desirable (either neutral or hypocholesterolemic) effects in humans (Grundy et al., 1982; Bonanome and Grundy, 1988). Leat (1977) has shown a linear relationship between fatness measured ultrasonically and a monounsaturated:saturated fatty acid ratio (C16:1 + C18:1/C18:0). Therefore, considering this relationship and the fact that fat thickness is easily measured and has a moderately high heritability, genetic change in fatty acid composition may be feasible. It is also recognized that previous management and physiological status of the cattle may influence fatty acid composition (Terrell et al., 1969; Christie, 1979; Eichorn et al., 1986). To compare breeds in a fair way is difficult because of the confounding of age, fatness, plane of nutrition, and other extrinsic factors (Yoshimura and Namikawa, 1983; Eichorn et al., 1986). Previous examinations of adipose tissue fatty acid composition in cattle have been largely confined to *Bos taurus* types. The primary objective of this study was to evaluate the fatty acid composition of subcutaneous adipose tissues of mature cows of two differing species of cattle, *Bos taurus* (Hereford) and *Bos indicus* (Brahman), managed under controlled conditions. It was anticipated that the investigation would demonstrate the feasibility of predicting differences in fatty acid composition between progeny groups in a follow-up study.

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Materials and Methods

Animals and Management. This research was approved by the Texas A&M University Animal Care Committee. Brahman (n = 37) and Hereford (n = 32) cows were sampled from the purebred herds at the Texas A&M University Agricultural Research Center at McGregor. The cows used for this study were all multiparous, lactating, and between 15 and 60 d postpartum when fat biopsies were collected on April 29 and 30, 1987. Average age of the cows was 4.5 yr. The cows had been managed together through the winter under controlled conditions and breed groups were similar in age and stage of lactation. Cows were fed hay and a salt-limited grain diet in the early part of winter. By February 1, all Brahman and Hereford cows were feeding on oats planted for grazing. Cows grazed oats until March 1, when wet and cold weather forced the removal of the cattle from oat pasture and hay was fed for approximately 1 wk. After that, cattle were on oat pasture and native cool-season annual pastures of good quality.

Ultrasound Measurements. Real-time ultrasound measurements of s.c. fat were taken at three locations as follows: at the rib-lumbar area (over the longissimus muscle, between the 12th and 13th rib), at the rump area (over the gluteobicep muscle, halfway between the ischiatic tuber and tuber coxae), and at the perianal region, 5 cm below the ischiatic tuber (biopsy location). The three ultrasound measurements were performed to estimate body fatness and to ensure that overall fatness (average of the three fat measurements) or s.c. fat thickness at the specific biopsy location was held the same for breed comparisons, which were made by means of covariate analyses. Ultrasound readings were taken from each animal using a Johnson and Johnson Ultrasound 210DX linear split-screen array scanner and a 3.5-MHz transducer (Johnson and Johnson Ultrasound, Englewood, CO). Each animal had mineral oil placed on the hair coat at the selected sites to ensure adequate acoustical contact. To obtain s.c. fat measurements, the ultrasound image was recorded on VHS videotape. The tape was played back on a four-head VCR (Panasonic AG-2400 Portable VCR, Panasonic, Secaucus, NJ) and s.c. fat thickness at specific body locations was estimated.

Sample Collection. A single live animal s.c. adipose tissue biopsy sample was collected from all animals at the perianal anatomical site described above. To cause minimum discomfort, the cows were gently but appropriately restrained in a squeeze chute and thoroughly disinfected with a betadine scrub solution, and epidural anesthesia with a 2% HCl solution of lidocaine was administered. After verifying anaesthesia of the surgical field, a sterile surgical scalpel was used to make a 3.75 cm long × 2.5 cm deep incision through the hide to collect approximately 1 g of s.c. adipose tissue. The incision site was closed using metal staple sutures. A furacin spray was applied as a wound dressing to prevent infection. After surgery, animals were checked daily and normal healing occurred in approximately 1 wk. Duplicate biopsy samples were frozen in liquid nitrogen and shipped to the Texas A&M University Department of Animal Science. Upon arrival, the samples were stored (3 mo at −20°C) before preparation and fatty acid analyses.

Fatty Acid Analysis. Total lipids were extracted (in duplicate) using chloroform-methanol (2:1, vol/vol) as described by Folch et al. (1957). Fatty acids were determined by gas chromatography following the same principles described by Slover and Lanza (1979). The extract was mixed with 5 mg of the internal standard (C12:0 methyl ester) and methylated with boron trifluoride-methanol following the procedure described by Morrison and Smith (1964). Fatty acid methyl esters (**FAME**) were analyzed using a flame ionization gas chromatograph (Chrompack, Model 437A, Packard, Raritan, NJ) equipped with a 2-m × 3.175-mm stainless steel column packed with 15% cyanopropyl:phenylpolysiloxane (9:1, wt/wt) on a 100 to 120 mesh solid support. The column was run isothermally at a temperature of 185°C. The FAME in 1 μL of hexane were delivered into the column using a microsyringe. The injection port and detector were maintained at 250 and 275°C, respectively. The flow rates were 25 mL/min for the carrier gas (nitrogen), 25 mL/min for the hydrogen, and 250 mL/min for the breathing air (oxygen). Chromatograms were recorded with a computing integrator (Spectraphysics Model SP 4290, San Jose, CA). The gas chromatograph system was calibrated with standard FAME mixtures (reference standard GLC-68-B, Nu-Check-Prep, Elysian, MN). Identification of sample fatty acids was made by comparing the relative retention times of FAME peaks from samples with those of standards. For quantification of sample FAME, reference standards of pure C14:0, C16:0, C18:0, and C20:0 fatty acid triglycerides were saponified, mixed with the internal standard and methylated, and the methyl esters were chromatographed to obtain response factors of each external standard. The fatty acid response factors were entered in the equation used for quantification of sample FAME as grams of individual fatty acid triclycerides per 100 g of wet tissue (Slover and Lanza, 1979). Data also were calculated as normalized area percentages of fatty acid.

Data Management and Statistical Analysis. Statistical analyses were conducted using the GLM procedure of SAS (1985). Normalized and gravimetric averages from duplicate samples were calculated. After consolidation of individual fatty acid data in both normalized and gravimetric formats, fatty acid data were grouped in categories and their ratios calculated, following criteria for unsaturation (i.e., total unsaturates, total monounsaturates, total polyunsaturates, total saturates) and desirability (in terms of physiological effects in humans). Analyses of

Table 1. Ultrasound measurements of subcutaneous fat thickness at three body locations for mature Brahman and Hereford cows

Body location	Breed		Pooled SE	Significance level
	Brahman	Hereford		
	cm			
Loin area[a]	.76	.70	.25	.29
Rump area[b]	1.49	1.68	.81	.35
Biopsy location[c]	1.71	2.71	.85	.0001
Average fat thickness	1.32	1.69	.58	.0098

[a]At the rib-lumbar area, over the longissimus muscle, between the 12th-13th rib.
[b]At the rump area, over the gluteobiceps muscle, halfway between the ischiatic tuber and the tuber coxae.
[c]At the perianal region, approximately 5 cm below the ischiatic tuber.

variance (SAS, 1985) were performed to examine the variation in fat thickness ultrasound measurements and fatty acid variables explained by breed type as the main effect in the model (Model 1). Additionally, relative proportions of fatty acid variables, expressed in both data formats, were subjected to regression analyses. A series of covariate analyses (**ANOCOVA**) were performed to elucidate sources of variation in fatty acid composition. Preliminary tests for parallelism (equal slopes) were conducted to detect possible interactions between breed type groups and potential covariates (namely, overall s.c. fat thickness and s.c. fat thickness at the biopsy site). When equality of slopes were warranted, ANOCOVA were conducted for comparing relative proportions of fatty acids of breed type groups at a common average fat thickness (Model 2) and at a common fat thickness at the biopsy site (Model 3).

Results

Fat Measurements. Breed type significantly affected overall s.c. fat thickness and s.c. fat thickness at the biopsy location. Brahman cows had less s.c. fat thickness at the biopsy location and exhibited a lower overall body fatness as measured by overall s.c. fat thickness. Breed type did not affect ($P > .05$) thickness of s.c. fat between the 12th-13th rib or at the rump area (Table 1).

Fatty Acid Variables. Table 2 presents levels of significance for testing main effects in three models applied to the normalized fatty acid data set. Model 1 showed significant variation with breed type. Models 2 and 3 for two analyses of covariates used either overall s.c. fat thickness or s.c. fat thickness at the biopsy location as a covariate. When ANOCOVA was conducted for comparing fatty acid composition of breed types at a common s.c. fat thickness at the biopsy location or at a common overall s.c. fat thickness, those breed differences in fatty acid variables detected

Table 2. Statistical analyses for normalized fatty acid data set[a]

Dependent variable	Model[b] 1	2		3	
	Fixed effect breed	Fixed effect breed	Covariate, average fat thickness	Fixed effect breed	Covariate, fat thickness at the biopsy location
Fatty acid[c], area %					
C14:0	NS	NS	NS	NS	NS
C14:1	***	***	NS	***	NS
C16:0	***	***	NS	***	NS
C16:1	***	NA	NA	***	NS
C18:0	**	***	**	***	NS
C18:1	**	**	NS	**	NS
C18:2	**	**	NS	**	NS
C18:3	***	***	NS	***	NS
Total UFA	***	***	*	***	NS
Total MUFA	***	***	*	***	NS
Total PUFA	***	***	NS	***	NS
Total SFA	***	***	*	***	NS
C18:0 + C18:1	NS	NS	NS	NS	NS
Ratios					
UFA/SFA	***	***	*	***	NS
MUFA/SFA	***	***	*	***	NS
PUFA/SFA	***	***	NS	***	NS

[a]n = 69 cows, Brahman and Hereford breeds combined.
[b]*$P < .05$; **$P < .01$; ***$P < .001$; NS = nonsignificant ($P > .05$); NA = not applicable. Covariate analysis was not performed due to lack of homogeneity of within-breed regressions ($P < .05$).
[c]UFA = Unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

Table 3. Statistical analyses for gravimetric fatty acid data set[a]

	Model[b]				
	1	2		3	
Dependent variable	Fixed effect breed	Fixed effect breed	Covariate, average fat thickness	Fixed effect breed	Covariate, fat thickness at the biopsy location
Fatty acid[c], g/ 100 g					
C14:0	NS	NS	NS	NS	NS
C14:1	**	**	NS	**	NS
C16:0	***	***	NS	***	NS
C16:1	NS	*	**	*	*
C18:0	***	***	NS	**	NS
C18:1	NS	NS	NS	NA	NA
C18:2	*	*	NS	NS	NS
C18:3	**	***	*	NA	NA
Total FA	*	NS	NS	NA	NA
Total UFA	NS	NS	NS	NA	NA
Total MUFA	NS	NS	NS	NA	NA
Total PUFA	**	**	NS	**	NS
Total SFA	***	***	NS	**	NS
C18:0 + C18:1	NS	NS	NS	NA	NA
Ratios					
UFA/SFA	***	***	**	***	NS
MUFA/SFA	***	***	**	***	NS
PUFA/SFA	***	***	NS	***	NS

[a]n = 69 cows, Brahman and Hereford breeds combined.
[b]*P < .05; **P < .01; ***P < .001; NS = nonsignificant (P > .05); NA = not applicable. Covariate analysis was not performed due to lack of homogeneity of within-breed regressions (P < .05).
[c]Total FA = Sum of the individual fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

by simple analysis of variance still existed. The same trend was observed when covariate analyses were applied to gravimetric data (Table 3). Breed maintained its significant effect on the majority of the fatty acid variables under study, irrespective of adjustment for body fatness.

Table 4 shows peak area percentage (unadjusted) means for fatty acid variables according to breed type. In both breeds, major fatty acids present in order of predominance were oleic (C18:1), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), and myristic (C14:0) acid. With the exception of C14:0 and the combination of C18:1 and C18:0 area percentages, Hereford and Brahman cows differed (P < .01) in their fatty acid components. Fat samples from Brahman cows were less saturated than counterparts from Hereford cows (P < .001). Hereford cows exhibited a higher C18:0 or C16:0 content and a smaller percentage of monounsaturated (**MUFA**) or polyunsaturated (**PUFA**) fatty acids (P < .01). The unsaturated fatty acid (**UFA**)/saturated fatty acid (**SFA**) ratio was higher (P < .0001) in Brahman adipose tissue, as were the MUFA/SFA and PUFA/SFA ratios. Although somewhat less clear than normalized data, gravimetric data (Table 5) showed, in general, similar trends for breed differences. Fat biopsy samples from Hereford cows contained approximately 5 g more of total fatty acids per 100 g of fresh tissue than counterparts from Brahman cows (P < .05). Indices of unsaturation as calculated from the gravimetric format also were higher in Brahman cows (P < .0001).

Discussion

The present trial involved the comparison of mature cows of *Bos indicus* and *Bos taurus* species that have not been considered previously in fatty acid studies. With the exceptions of the reports of Eichorn et al. (1986) and Rumsey et al. (1972) there are no fatty acid data derived from mature cows in the U.S. literature. Hidiroglou et al. (1987) were probably the first to detail lipid composition of s.c. adipose tissues of *Bos indicus* types but they used Brahman crossbred yearling steers. The present study indicated that fatty acid composition of mature cows differed from fatty acid profiles encountered in the literature for younger bovine animals. The primary difference in the present study was in the higher predominance of C16:1 than of C18:0 for both breeds. The C18:0/C16:1 ratios calculated from the present data (.71 in Brahman and .95 in Hereford cows) contrast with ratios > 1.4 calculated from data reported for mature cows by Rumsey et al. (1972) and Eichorn et al. (1986). The reason for the

Table 4. Mean normalized percentages[a] of major fatty acids in subcutaneous adipose tissue biopsy samples from mature Brahman and Hereford beef cows

Fatty acid[b]	Breed: Brahman (n = 37)	Breed: Hereford (n = 32)	SE	Significance level
C14:0	4.30	3.97	1.07	.22
C14:1	3.18	2.40	.60	.0001
C16:0	22.65	26.00	1.73	.0001
C16:1	10.67	9.40	1.45	.0004
C18:0	7.6	8.9	1.70	.002
C18:1	49.58	47.67	2.64	.004
C18:2	4.29	1.72	.26	.001
C18:3	.94	.73	.28	.0001
Total SFA	34.52	38.82	2.74	.0001
Total UFA	65.85	61.30	3.15	.0001
Total MUFA	63.43	59.50	3.02	.0001
Total PUFA	2.43	1.83	.46	.0001
C18:0 + C18:1	56.60	57.17	2.69	.38
Ratios				
UFA/SFA	1.92	1.59	.22	.0001
MUFA/SFA	1.85	1.54	.21	.0001
PUFA/SPA	.07	.05	.35	.0001

[a]Percentage of the total peak area of the fatty acids listed.
[b]SFA = Saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 5. Mean gravimetric content (g/100 g of fresh tissue) of major fatty acids in subcutaneous adipose tissue biopsy samples from mature Brahman and Hereford beef cows

Fatty acid[a]	Breed: Brahman (n = 37)	Breed: Hereford (n = 32)	SE	Significance level
C14:0	2.26	2.54	.67	.09
C14:1	1.87	1.57	.45	.007
C16:0	13.03	16.25	2.9	.0001
C16:1	6.09	5.82	1.11	.32
C18:0	4.70	6.16	1.71	.0008
C18:1	21.00	22.00	4.2	.33
C18:2	.41	.33	.15	.04
C18:3	.64	.51	.17	.003
Total SFA	20.00	25.00	4.95	.0001
Total UFA	30.01	30.23	5.56	.87
Total MUFA	29.00	29.40	5.35	.75
Total PUFA	1.05	.84	.29	.005
C18:0 + C18:1	25.71	28.17	5.51	.07
Total fatty acids	50.01	55.19	9.84	.03
Ratios				
UFA/SFA	1.52	1.24	.20	.0001
MUFA/SFA	1.47	1.20	.19	.0001
PUFA/SFA	.05	.03	.01	.0001

[a]SFA = Saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

discrepancy is unknown. However, it should be pointed out that data presented by Hecker et al. (1975), Leat (1975, 1977), and others strongly suggest that the distribution we report would be expected in mature animals because C18:0/C16:1 ratios in young animals decrease substantially (to values lower than 1.0) as they become older, particularly after 1 yr of age. The general indication is that C18:0 decreases with age and this change is accompanied by concomitant increases in percentages of C18:1 and C16:1 (Leat, 1977).

The only published article that addresses fatty acid compositional differences across bovine species is that of Larick et al. (1989). They examined fatty acid profiles of neutral lipid and phospholipid fractions of longissimus muscle from *Bos taurus* (Hereford), *Bos indicus* (Brahman), and *Bison bison* (American bison). Larick et al. (1989) demonstrated no differences in longissimus muscle neutral lipids between Brahman and Hereford ($P > .05$), but neutral lipids from Brahman muscle tended to exhibit a higher UFA/SFA ratio than those from Hereford muscle. Brahman muscle contained more nervonic (24:1) acid and less arachidic acid (C20:0) in the phosphatidylserine fraction than Hereford muscle ($P < .05$) (Larick et al., 1989). In contrast to this, Ramananarivo and coworkers found that adipose tissue of *Bos indicus* from Madagascar contained more saturated acids than that from French *Bos taurus* (Ramananarivo et al., 1981; Gaydou et al., 1984). However, their results were based on a limited number of samples taken nonsystematically from different sources, including tallow and butter from a local market.

According to Eichorn et al. (1986), breed type differences in fatty acid composition of total lipid extracts from adipose tissues of mature cows are probably the result of variation in the triacylglycerol/phospholipid ratios. Yoshimura and Namikawa (1983) have recommended comparing animals at various stages of fattening to obtain exact information about breed differences. In the present study, covariate analyses (ANOCOVA) were performed to assist in the interpretation of results given the inevitable confounding effect of fatness. It is appropriate, however, to indicate that covariance must be used with caution when potential covariates (fat thickness) are affected by treatments (breed). When this type of adjustment is performed, a portion of the effect caused by breed could be removed (Steel and Torrie, 1980). When analyzed by ANOCOVA, body fatness accounted for some variation in fatty acid composition, but adjustment for this variation did not remove the effect of breed on the overwhelming majority of fatty acid variables under study. Therefore, the effect of breed on fatty acid composition of s.c. adipose tissues was clear and robust, regardless of body fatness. The relationship between fatness and unsaturation in *Bos taurus* types has been indicated by several authors (Waldman et al., 1968; Hecker et al., 1975; Leat, 1975,

1977). In general "the fatter the animal of a given breed the more unsaturated its depot fat becomes" and the influence of fatness is independent of diet (Leat, 1977). However, Leat (1977) also has indicated that the slope of regression line differs markedly between beef (Angus) and dual-purpose (Friesian) breeds. Therefore, fatty acid compositional differences between bovine species are stronger and much less influenced by fatness or, alternatively, breed differences are due to differing degrees of concert among the genes that regulate unsaturation of fat depots and those that regulate the accumulation of lipids in such anatomical locations. The mechanisms of genetic regulation of fatty acid composition remain to be elucidated.

Implications

Genetic manipulation of fatty acid composition of beef adipose tissues is possible because variation occurred across the two bovine species. Whether or not these differences constitute a nutritional advantage for lipids from Brahman or other *Bos indicus* types remains to be demonstrated. Fat biopsy composition of the dam may serve as a guide to the fatty acid distribution in a similar depot of its progeny, but confirmatory data are needed. If the fatty acid composition is highly heritable in *Bos indicus* types, it is necessary to investigate expression in the offspring at younger, more marketable ages.

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