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Extensive Analysis of Long-Chain Polyunsaturated Fatty Acids, CLA, *trans*-18:1 Isomers, and Plasmalogenic Lipids in Different Retail Beef types

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The objective of this investigation was to provide a comprehensive analysis of the total lipid composition of present-day retail beef meat available at the consumer level and to evaluate the total lipid composition with special emphasis on the nutritional value. For this purpose, 40 beef cuts were obtained from four cattle farms based on either a natural grazing system (NGS) or an intensive production system (IPS). The total lipid composition was analyzed using complementary chemical and chromatographic procedures. The content of *n*-3 LC-PUFA, CLA, total *trans*-18:1, and branched-chain fatty acids was significantly higher in NGS beef than in IPS beef. The *trans*-18:1 and CLA profiles were affected by the different production systems, whereby they can be utilized empirically to differentiate between feeding regimen and production management. Fatty acid ratios that have health implications (*n*-6/*n*-3, LA/αLNA, and AA/EPA) were remarkably beneficial for NGS beef compared with IPS beef. In conclusion, from the human health perspective, beef raised on NGS is clearly superior with regard to a more favorable fatty acid profile in comparison to IPS beef.

KEYWORDS: Retail beef meat; total lipid composition; production system; breed

INTRODUCTION

In recent times, consumers' awareness of the relationships between diet and health has increasingly influenced the demand for foods reported to promote the maintenance of health and prevent disease. Beef consumption in the European Union (EU) and United States has significantly decreased over the past decades, not least because of consumers' uncertainty based on negative media attention. In general, lipids from animal sources are perceived as detrimental to health in comparison to plant-derived lipids. Health-related concerns about beef consumption are largely due to statements that beef meat may contain high proportion of fat, cholesterol, saturated fatty acids (SFA), and *trans* fatty acids (TFA), which potentially contribute to a number of diseases (e.g., cardiovascular disease, colorectal cancer, and metabolic syndrome). However, the fat content and fatty acid (FA) composition of beef meat varies mainly depending on farm management systems, which include breed, sex, age, husbandry, and feeding strategy (*1–3*). In the past few years, extensive research has been undertaken aimed at improving the FA composition of beef meat by increasing the content of conjugated linoleic acids (CLA), *n*-3 long-chain polyunsaturated FA (*n*-3 LC-PUFA), and vaccenic acid (VA), as well as reducing the *n*-6/*n*-3 ratio and the polyunsaturated to saturated fatty acid (P/S) ratio (reviewed in refs *4–7*). The purpose of these feeding strategies is to promote and maintain human health and prevent diseases. However, there are questions regarding the commercial adaptability and viability of such experimental results to produce products with these improved lipid profiles in present-day realities of commercial beef production. The objective of this study was to evaluate the FA content and composition of present-day beef produced under typical commercial production practices found in Germany and to see how they compare with current health recommendations. For this purpose beef cuts of two different production systems were sampled [intensive production system (IPS) vs natural grazing system (NGS)] using typical breeds currently used in this country.

Improved methods were used to analyze the total lipid composition of beef meat including the free FA, *O*-acyl- (glycosides, ester), *O*-alkenyl ether- (plasmalogenic lipids), and *N*-acyl-linked lipids (sphingolipids) (*8, 9*). Meat lipids are a complex mixture of molecular species each containing FA in different combinations. Typically in meat, the common FA vary in chain length (C10−C26), chain structure (straight- and branched-chain), degree of saturation (0−6 double bonds),

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double bond type (conjugated and nonconjugated double bonds), configuration (cis, trans), and position of double bonds. Considering the lipid complexity of meat samples and the chemical nature of all lipid structures, it is not possible to obtain a FA composition by a single analytical method (*8, 10*). To evaluate the content of the various individual FA and other alkyl groups in meat, it was necessary to apply accurate and reliable methods for the total determination. The latter provided an additional objective of this investigation to describe comprehensive methods for the total lipid composition of beef meat using complementary chemical and chromatographic techniques.

MATERIALS AND METHODS

Materials. A total of 40 meat samples were obtained from beef raised by four privately owned farming operations that employed the two main production systems currently practiced in agricultural beef production in Germany (combination of typical breed and husbandry system). Meat samples were collected from four beef cattle breeds: Angus, Scottish Highland Cattle (Scott), Limousins (Limo), and Simmentals, that were reared in a NGS or IPS. The Limo and Simmental cattle were raised in an IPS, kept indoors and fattened intensively on corn silage ad libitum supplemented with restricted portions of soy and cereal meal, whereas the Angus and Scott were reared under NGS production conditions grazing only on pasture throughout the entire vegetative season.

The animals were slaughtered at the typical commercial production weights with carcasses ranging between 250 and 300 kg. The beef cuts (~250 g) were obtained from musculus longissimus dorsi between the 12th and 13th ribs of the left side of beef carcasses from each head of cattle tested after 14 days of aging at 4 °C. Upon collection, samples were trimmed of visible subcutaneous and intermuscular fat, coarsely chopped, minced in a food processor (Moulinette, Germany), freeze-dried, and stored at −80 °C for subsequent lipid analysis.

Methods. Neutral and polar lipids were extracted from the pulverized samples (3 g) using chloroform/methanol/water (2:2:1.8, v/v/v) according to the method of Bligh and Dyer (*11*). Total lipids were derivatized using two separate methylation procedures. To accurately analyze the total lipid contents of these meat samples, the results of both acid-catalyzed and base-catalyzed methylation procedures were utilized in combination to compensate for shortcomings in the individual derivatization methods. Two individual samples of beef lipid extracts were prepared for each meat sample. Each sample consisted of an aliquot of 2.5 mg of beef sample lipid extract dissolved in 3 drops of benzene, and then one of the test solutions was methylated under basic conditions using 0.5 mL of 0.5 N $NaOCH_3$/methanol (Supelco Inc., Bellefonte, PA) for 30 min at 50 °C, and the other test solution was methylated under acidic conditions using 0.5 mL of 5% HCl gas in anhydrous methanol (w/v) for 1 h at 80 °C. The base-catalyzed methylation method facilitated proper CLA isomer analysis. This methylation method produced fatty acid methyl esters (FAME) from *O*-acyl lipids and prevented double-bond isomerization (e.g., cis/trans conjugated bonds to trans,trans isomers) and the formation of methoxy artifacts, but was limited in its application to the transesterfication of alk-1-enyl ether lipids, free FA, and *N*-acyl lipids (*12, 13*). In contrast, the acid-catalyzed methylation method was employed to convert all common lipid structures efficiently to methyl esters including plasmalogenic lipids to dimethylacetals (DMA) (*8, 9, 14*). Taking into account that muscle lipids consist of 10−15% long-chain alkyl groups (*15*), the acid-catalyzed methylation is a mandatory procedure. Prior to gas chromatographic (GC) analysis the derivatization products were purified by thin-layer chromatography (TLC) on silica gel G plates (Fisher Scientific, Ottawa, ON, Canada) using *n*-hexane/diethyl ether/acetic acid (85:15:1, v/v/v) as developing solvent. Using this TLC developing solvent mixture the FAME and DMA eluted together (*8, 9*). The TLC bands containing the FAME and DMA were identified using a FAME standard consisting of FAME, triacylglycerol (TAG), free FA, cholesteryl ester, and free cholesterol (18-4A Nu-Chek-Prep Inc., Elysian, MN) after spraying the plates with a 0.01% solution of 2,7′-dichlorofluorescin (w/v) in ethanol and visualization under UV light at 254 nm. This band was scraped off the TLC plate and eluted from the silica gel using 5 mL of chloroform. The eluate containing FAME/DMA was dried with a stream of N_2 and dissolved in *n*-hexane at appropriate concentrations (1−2 μg/μL) for GC analysis.

The resulting methylated product was analyzed using two different GC temperature programs, which permitted the analysis of all the *trans*-18:1 isomers instead of using the silver-ion TLC (Ag^+-TLC) technique (*18*). Silver-ion HPLC (Ag^+-HPLC) was used as a complementary method to resolve all the different CLA isomers; details of the Ag^+-HPLC methodology for identification of CLA isomers have been published previously (*16*). The combined FAME and DMA were analyzed using GC. Under the temperature condition of the GC injector, the DMA were not stable and partially lost one methanol group from the DMA to produce alk-1-enyl methyl ethers (AME) (*14*). The DMA and the AME peaks in the GC chromatogram were identified by first separating the FAME and DMA by TLC using the solvent 1,2-dichloromethane and then analyzing the DMA separately under the same GC condition (*8–10*). The GC used was a Hewlett-Packard model 5890 series II gas chromatograph (Palo Alto, CA) equipped with a splitless injector, a flame ionization detector (FID), an autosampler (model 7673; Hewlett-Packard), and a 100 m CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Varian Inc., Mississauga, ON, Canada). The injector and the detector were both maintained at 250 °C. Hydrogen was used as a carrier gas at a flow rate of 1 mL/min. Two different GC programs were used to identify and resolve the moieties of the meat lipids. The following temperature program was used to separate the methylated products of total meat lipids (FAME and DMA) using two GC temperature ramps. GC analysis A was as follows: 45 °C (held for 4 min), 13 °C/min to 175 °C (held for 27 min), 4 °C/min to 215 °C (held for 35 min). A second method (GC analysis B) was utilized to resolve effectively the positional and geometric isomers of 18:1 using a GC program similar to GC analysis A except that the first ramp temperature was lowered to 150 °C and held for 47 min (*17, 18*).

FAME were identified by comparison of their relative retention times with those of known FA in a mixture of commercial standards (GC reference FAME standard spiked with a four-positional CLA isomer mixture and the long-chain saturated FAME 21:0, 23:0, and 26:0; Nu-Chek-Prep Inc.). Because commercial standards of DMA are not available, plasmalogens were isolated from beef heart lipids (phosphatidylethanolamine) known to contain substantial amounts of this lipid class as described in detail by Cruz-Hernandez et al. (*8*).

The final FA composition was obtained by combining the results of both methylation procedures and chromatographic methods (GC analysis A and B; Ag^+-HPLC). The total alkenyl-ether content was determined as the sum of all DMA/AME peaks in the acid-catalyzed chromatogram belonging to DMA and AME. The relative elution pattern was as follows: AME < DMA < FAME (see ref *8* for further details). The results of the isomeric 18:1 region (acid-catalyzed methylation based on GC analysis B), total CLA (base-catalyzed methylation based on the GC analysis A), and CLA distribution (Ag^+-HPLC) were merged together with the general overall FA distribution achieved by acid-catalyzed methylation (GC analysis A) using several FA in the sample as an internal reference. The results are expressed as grams per 100 g of FA and milligrams per 100 g of fresh muscle. Unidentified peaks were included in the calculation of total FA and, thus, the sum of the identified FA is <100%.

Statistical Analysis. The data from the four breeds of the animals were subjected to one-way Aanalysis of variance using the GLM procedure of the SAS software (SAS Systems, release 8.2, SAS Institute Inc., Cary, NC). Means for each breed with their standard errors (SEM) are presented in the tables. The Student−Newman−Keuls' test was used to determine significance ($P < 0.05$) of the differences among the means. This procedure allowed comparison of the breeds within each diet group as well as comparison of the diet groups that also differed in the breeds involved.

RESULTS

Intramuscular Fat. The comparison of the production systems yielded no significant differences in the total intramuscular fat (IMF) content of the longissimus muscle samples.

Table 1. Total Lipid Composition and Total Lipid Content of Beef Muscle from Four Breeds Raised under Two Different Production Systems[a]

	natural grazing system (NGS)		intensive production system (IPS)				natural grazing system (NGS)		intensive production system (IPS)		
selected fatty acid	Angus	Scott	Limo	Simmental	SEM	selected fatty acid	Angus	Scott	Limo	Simmental	SEM
						Total Lipid Composition (Percent of Total FID Response)					
10:0	0.02 a	0.02 a	0.02 a	0.02 a	0.002	18:3 *n*-3	0.98 b	1.91 a	0.64 bc	0.39 c	0.15
12:0	0.04 a	0.04 a	0.03 a	0.02 b	0.003	20:0	0.14 a	0.13 a	0.12 ab	0.10 b	0.006
14:0 iso	0.07 a	0.05 a	0.02 b	0.03 a	0.01	11*c*-20:1	0.11 b	0.13 ab	0.15 a	0.14 a	0.01
14:0	1.97 a	1.69 a	1.35 b	1.95 a	0.09	20:3 *n*-6	0.24 b	0.23 b	0.75 a	0.17 b	0.03
9*c*-14:1	0.40 b	0.31 c	0.22 d	0.47 a	0.02	20:4 *n*-6	0.65 c	0.84 b	1.83 a	0.64 c	0.06
15:0 iso	0.25 a	0.20 b	0.09 c	0.11 c	0.02	20:5 *n*-3	0.25 a	0.32 a	0.31 a	0.05 b	0.02
15:0 ai	0.24 a	0.22 a	0.13 b	0.12 b	0.02	22:4 *n*-6	0.05 c	0.08 bc	0.18 a	0.09 b	0.01
15:0	0.49 a	0.54 a	0.31 b	0.27 b	0.03	22:5 *n*-3	0.43 a	0.47 a	0.53 a	0.14 b	0.04
16:0 iso	0.22 b	0.33 a	0.18 b	0.20 b	0.03	22:6 *n*-3	0.03 c	0.06 a	0.05 b	0.01 d	0.003
16:0	22.69 a	21.12 b	19.49 c	23.68 a	0.45	Σ SFA	45.68 a	41.73 b	39.15 c	43.16 b	0.57
9*c*-16:1	2.59 b	2.45 b	2.02 c	3.13 a	0.14	Σ *cis*-MUFA	36.61 b	36.40 b	34.54 c	41.95 a	0.56
17:0 iso	0.48 a	0.53 a	0.38 b	0.33 b	0.03	Σ *cis*-PUFA	5.38 d	9.95 b	13.54 a	6.62 c	0.28
17:0 ai	0.61 ab	0.67 a	0.51 b	0.51 b	0.04	Σ *trans*-MUFA	4.68 a	3.52 b	3.19 bc	2.75 c	0.18
17:0	1.13 a	1.09 a	0.90 b	0.88 b	0.05	Σ *trans*-PUFA	0.95 a	0.75 b	0.67 bc	0.50 c	0.07
18:0 iso	0.17 a	0.16 a	0.13 b	0.15 ab	0.01	Σ *n*-3 PUFA	1.73 b	2.80 a	1.55 b	0.60 c	0.19
18:0	16.69 a	14.86 b	13.72 c	13.60 c	0.30	Σ *n*-6 PUFA	3.52 d	7.02 b	11.86 a	5.89 c	0.16
Σ *trans*-18:1	4.50 a	3.35 b	3.03 bc	2.65 c	0.17	Σ *n*-3 LC-PUFA	0.75 a	0.89 a	0.91 a	0.21 b	0.06
10*t*-18:1	0.29 b	0.29 b	0.74 a	0.36 b	0.08	Σ *n*-6 LC-PUFA	0.99 c	1.22 b	2.89 a	0.94 c	0.07
11*t*-18:1	2.38 a	0.95 b	0.47 c	0.63 c	0.10	Σ BCFA	2.03 a	2.17 a	1.44 b	1.45 b	0.11
Σ *cis*-18:1	32.90 b	31.23 b	31.42 b	37.67 a	0.60	Σ DMA&AME	2.25 bc	2.78 b	5.01 a	1.91 c	0.35
9*c*-18:1	30.87 b	28.78 b	28.97 b	35.21 a	0.61	14:1/14:0	0.21 b	0.19 bc	0.16 c	0.25 a	0.01
11*c*-18:1	1.17 b	1.51 a	1.50 a	1.47 a	0.05	16:1/16:0	0.11 ab	0.127 ab	0.10 b	0.13 a	0.006
18:2 n-6	2.51 d	5.77 b	8.91 a	4.92 c	0.12	18:1/18:0	1.86 c	1.94 c	2.11 b	2.59 a	0.06
Σ CLA	0.82 a	0.56 b	0.41 c	0.34 c	0.04	9*c*,11*t*-18:2/11*t*-18:1	0.26 c	0.43 b	0.76 a	0.36 bc	0.04
9*c*,11*t*-18:2	0.62 a	0.40 b	0.29 c	0.23 c	0.03						
						Total Lipid Content (Milligrams per 100 g of Fresh Muscle)					
16:0	521 b	612 a	186 c	533 b	11.4	Σ *trans*-MUFA	190 a	194 a	58 c	119 b	11.5
18:0	383 b	431 a	131 d	306 c	7.0	Σ *n*-3 PUFA	39.0 b	79.4 a	14.2 c	13.3 c	5.3
9*c*-18:1	708 b	834 a	276 c	792 a	14.9	Σ *n*-6 PUFA	80.8 d	203 a	113 c	132 b	3.8
18:2 *n*-6	57.7 d	167 a	85.0 c	111 b	3.3	Σ *n*-3 LC-PUFA	17.2 b	25.9 a	8.6 d	4.8 c	1.5
18:3 *n*-3	22.5 b	55.3 a	6.10 c	8.7 c	4.2	Σ *n*-6 LC-PUFA	22.6 c	35.2 a	27.5 b	21.3 c	1.2
20:4 *n*-6	14.9 b	24.4 a	17.5 b	14.3 b	1.1	Σ BCFA	46.6 b	62.9 a	13.7 d	32.6 c	2.8
20:5 *n*-3	5.8 b	9.2 a	2.9 c	1.1 d	0.5	Σ DMA&AME	67.5 b	104 a	55.1 b	58.1 b	8.6
22:5 *n*-3	9.9 b	13.4 a	5.0 c	3.2 c	1.1	PUFA/SFA	0.12 d	0.24 b	0.34 a	0.15 c	0.01
22:6 *n*-3	0.7 b	1.8 a	0.5 b	0.2 c	0.1	*n*-6/*n*-3	2.1 c	2.8 c	8.0 b	10.6 a	0.6
Σ CLA	18.9 a	16.0 b	11.9 c	9.8 c	1.0	LA/αLNA	2.6 b	3.3 b	14.1 a	13.8 a	0.9
9*c*,11*t*-CLA	1.6 a	1.3 b	0.3 d	0.6 c	0.1	AA/EPA	2.6 c	2.7 c	6.1 b	13.0 a	0.4
Σ SFA	1042 b	1202 a	372 d	967 c	13.5	IA[b]	0.47 a	0.37 b	0.32 b	0.37 b	0.03
Σ *cis*-MUFA	1453 b	1894 a	622 c	1779 a	69.4	IT[c]	1.02 a	0.96 a	0.47 b	0.57 b	0.05
Σ *cis*-PUFA	120 c	284 a	128 bc	146 b	7.1						

[a] All values are means of 10 animals per breed. Means with different letters are significant at the 5% level. Abbreviations: αLNA, α-linolenic acid; AME, alkenyl methyl ethers; BCFA, branched-chain fatty acids; CLA, conjugated linoleic acid; DMA, dimethylacetal; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SEM, standard error of the mean; SFA, saturated fatty acid. [b] IA, index of atherogenity; IA = (12:0 + 4 × 14:0 +16:0)/(MUFA + PUFA) calculated according to ref (*19*). [c] IT, index of thrombogenity; IT = (12:0 + 16:0 +18:0)/[0.5 × (MUFA + *n*-6 PUFA) + 3 × *n*-3 PUFA + (*n*−-3 PUFA/*n*-6 PUFA)] calculated according to ref (*19*).

However, in the Limo the IMF content was significantly lower (1.1 ± 0.1% of wet weight, $P < 0.05$) than for the other breeds, which possessed a similar total lipid content (Simmental, 2.5 ± 0.8% of wet weight; Angus, 2.6 ± 0.9%; Scott, 3.2 ± 1.0%).

Saturated, Monounsaturated, and Branched-Chain Fatty Acids. 16:0 was consistently the major SFA in all breeds followed by 18:0 (**Table 1**). Angus meat had the highest relative proportion of total SFA, whereas Limo meat had the lowest. The proportion of odd-chain SFA (15:0, 17:0) was significantly lower in the meat lipids of IPS ($P < 0.05$). The total *cis*-MUFA content was exceptional for Simmental meat that had significantly higher proportions compared with Limo meat due to higher levels of 9*c*-14:1, 9*c*-16:1, and 9*c*-18:1 ($P < 0.05$). Compared with the animals of the IPS, beef of the NGS had significantly greater relative concentrations of individual and total branched-chain FA (BCFA; $P < 0.05$) but not significantly different within the two production systems. Due to the differences in the IMF content Scott meat showed the highest and Limo meat the smallest absolute content of total SFA, *cis*-MUFA, and BCFA.

Polyunsaturated Fatty Acids. The relative proportion and the absolute amount of linoleic acid (LA, 18:2 *n*-6) were significantly different among the four breeds and showed no consistent association to production systems (**Table 1**). The long-chain metabolites of LA were remarkably similar among the breeds, irrespective of the production system, except Limo, which contained more than double the relative amount of *n*-6 LC-PUFA, specifically dihomo-γ-linoleic acid (20:3 *n*-6) and arachidonic acid (AA, 20:4 *n*-6). In general, the relative proportion and absolute amount total *n*-3 PUFA and *n*-3 LC-PUFA were significantly higher in meat of NGS beef than in meat of IPS beef, with Scott having the highest average concentrations and Simmental the lowest ($P < 0.05$). The Limo breed was an exception on the basis of the relative proportion. Even though raised in an IPS, it contained levels of *n*-3 LC-PUFA similar to those found in the NGS group and had the

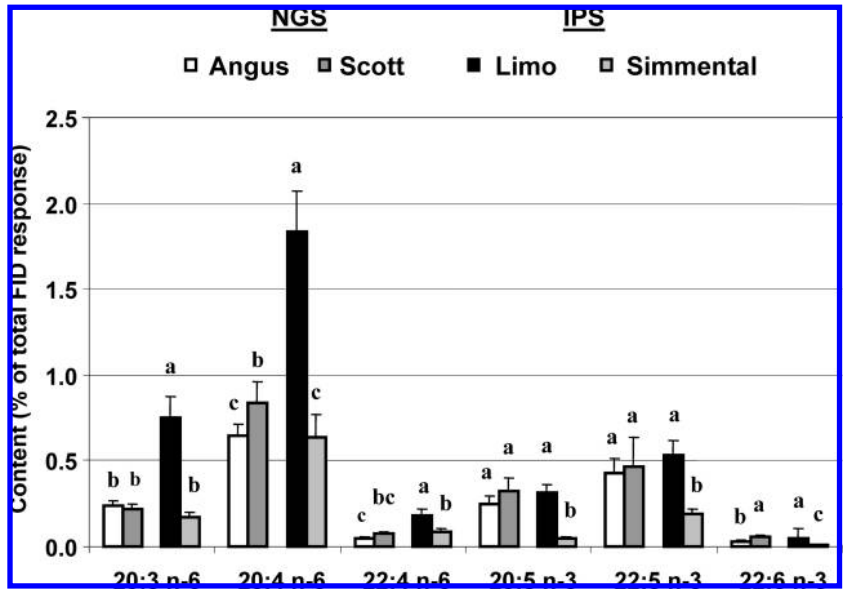

Figure 1. Comparison of LC-PUFA (percent of total FID response) of beef muscle from four breeds raised under two different production systems (all values are means of 10 animals per breed; means with different letters are significant at the 5% level).

Table 2. Relative Distribution (Percent) of *trans*-18:1 Isomers of Beef Muscle from Four Breeds Raised under Two Different Production Systems[a]

trans-18:1 isomer	natural grazing system (NGS)		intensive-production system (IPS)		
	Angus	Scott	Limo	Simmental	SEM
4*t*-	0.24 a	0.31 b	0.29 b	0.31 b	0.03
5*t*-	0.25 b	0.38 a	0.35 ab	0.44 a	0.04
6*t*/7*t*/8*t*-	5.27 d	12.47 a	7.99 c	9.51 b	0.49
9*t*-	9.12 c	11.51 b	10.80 bc	15.89 a	0.57
10*t*-	6.52 b	8.85 b	23.74 a	13.34 b	2.04
11*t*-	52.57 a	28.14 b	15.66 d	23.77 c	1.46
12*t*-	6.85 b	8.68 ab	11.24 a	9.35 ab	0.84
13*t*/14*t*-	11.56 d	20.25 b	22.05 a	16.67 c	0.59
15*t*-	2.15 b	4.42 a	2.58 b	3.94 a	0.24
16*t*-	5.49 b	4.99 b	5.32 b	6.79 a	0.40

[a] All values are means of 10 animals per breed. Means with different letters are significant at the 5% level. Abbreviations: SEM, standard error of the mean.

highest levels of LA and *n*-6 LC-PUFA among all four groups (**Figure 1**). On the other hand, Simmental meat contained approximately half to a fourth the levels found in the other meat types, reflecting a lower content of individual *n*-3 FA (18:3 *n*-3, 20:5 *n*-3, 22:5 *n*-3, and 22:6 *n*-3). The absolute amount of *n*-6 PUFA and *n*-6 LC-PUFA was clearly higher than expected for the Scott beef resulting from the much higher content of LA and AA. Contrarily, Angus beef from the same production system had the lowest *n*-6 PUFA content due to the very low LA levels.

***Trans* Fatty Acids.** Angus meat contained the highest relative proportion of total *trans*-MUFA and *trans*-PUFA and Simmental meat the lowest (**Table 1**). The absolute amount of total *trans*-MUFA, consisting mainly of total *trans*-18:1, was 1.6−3.3 times higher for the NGS-raised beef Angus and Scott compared to Limo and Simmental. The relative proportion and absolute amount of VA (11*t*-18:1) was significantly higher in the beef meat of the NGS. **Table 2** illustrates the relative distribution of the *trans*-18:1 isomers. The meat of the IPS group possessed significantly higher proportions of 9*t*- and 10*t*-18:1, but significantly lower proportions of 11*t*-C18:1 when compared to the NGS group.

Conjugated Linoleic Acids. Despite noticeable variations, the relative proportion and absolute amount of total CLA were significantly higher in the meat of the NGS group compared to that of the IPS group (**Table 1**; $P < 0.05$). The highest total CLA concentrations were found for Angus meat and the lowest for Simmental meat. Comparison of the relative concentrations of the major CLA isomer 9*c*,11*t* showed significantly higher levels in NGS beef meat when compared to IPS beef meat (**Table 1**), but when expressed in relative percent it was only slightly higher (**Table 3**). Distinct differences were observed in the minor isomer distribution of the two production systems. Higher relative proportions of 12*t*,14*t*-, 11*t*,13*t*-, and 11*t*,13*c*-CLA were characteristic in the NGS group, whereas the meat of the IPS group showed significantly higher proportions of 10*t*,12*c*- and 7*t*,9*c*-CLA ($P < 0.05$).

Table 3. Relative CLA Isomer Distribution (Percent of Total CLA Isomers) of Beef Muscle from Four Breeds Raised under Two Different Production Systems[a]

CLA isomer	natural grazing system (NGS)		intensive production system (IPS)		
	Angus	Scott	Limo	Simmental	SEM
13*t*,15*t*-	0.15 b	0.09 b	0.04 b	0.37 a	0.05
12*t*,14*t*-	1.42 a	1.53 a	0.83 b	0.46 b	0.16
11*t*,13*t*-	3.70 b	4.03 a	2.38 b	1.72 b	0.34
10*t*,12*t*-	0.36 c	0.35 c	0.81 b	1.22 a	0.09
9*t*,11*t*-	1.95 b	2.72 a	2.40 ab	2.17 b	0.15
8*t*,10*t*-	0.16 b	0.72 a	0.37 ab	0.75 a	0.20
7*t*,9*t*-	0.61 b	1.34 a	0.81 b	1.16 a	0.11
6*t*,8*t*-	0.15 b	0.80 a	0.15 b	0.27 b	0.06
12,14-*c*/*t*	0.08 b	0.01 b	0.13 b	0.44 a	0.05
11*t*,13*c*-	8.27 a	6.95 a	3.67 b	1.74 c	0.54
11*c*,13*t*-	0.44 bc	1.08 a	0.68 b	0.28 c	0.11
10*t*,12*c*-	1.07 c	1.36 bc	1.94 b	2.86 a	0.20
9*c*,11*t*-	74.33 ab	76.01 a	72.75 ab	70.75 b	1.11
8*t*,10*c*-	1.91 a	2.22 a	1.94 a	2.07 a	0.16
7*t*,9*c*-	4.27 c	4.51 c	9.93 b	11.38 a	0.51
6*t*,8*c*-	0.28 a	0.29 a	0.37 a	0.25 a	0.04
c,*c*	0.87 b	0.82 b	1.21 b	2.11 a	0.15

[a] All values are means of 10 animals per breed. Means with different letters are significant at the 5% level. Abbreviations: CLA, conjugated linoleic acid; SEM, standard error of the mean.

Plasmalogenic Lipids. Breed affected the total fatty aldehyde content more than the production system. The highest total fatty aldehyde content was found in Limo meat and the lowest in

Simmental meat, but the absolute amount of total fatty aldehydes was up to 2 times higher for Scott meat compared to Angus, Limo, and Simmental meat ($P < 0.05$).

Nutritionally Important Values. Scott meat possessed twice the P/S ratio and Limo meat 3 times the P/S ratio of Angus or Simmental meats. Beef reared in the NGS showed beneficially lower ratios of *n*-6/*n*-3, LA/α-linolenic acid (αLNA), and AA/eicosapentaenoic acid (EPA) than beef raised in the IPS. Differences in the index of atherogenity (IA) were relatively small, although Angus meat had significantly the highest value ($P < 0.05$). The index of thrombogenity (IT) differed markedly between the production systems, being 2 times higher in the NGS.

DISCUSSION

This investigation presents a comprehensive analysis of the total lipid composition of German retail beef meat available at the consumer level. The method proved to be practical for routine determinations. Complementary chemical and chromatographic techniques were applied to analyze all lipid classes including plasmalogens and sphingolipids. Using an improved GC method allowed us to achieve a detailed distribution of the 18:1 positional and geometric isomers without prior silver ion thin-layer chromatography (*18*). Beef reared in the NGS became popular in Germany because it is associated with a health image. Therefore, beef cuts from the two different production systems (NGS and IPS) were chosen to objectively evaluate the total lipid composition with special emphasis on the nutritional value. The selection of the different breeds within each production system was not intentional, but based simply on access to pure breeds from each of the typical production systems. They were the breeds of choice on the selected farms.

Intramuscular Fat. For IMF, there was a fat (Scott), two intermediate (Angus and Simmental), and a lean (Limo) breed. It is recognized that the IMF accretion in beef is influenced by several factors such as muscle type, growth pattern, maturity type, breed, and genetic differences as well as feeding strategy (*20*). The very low IMF content of Limo versus Simmental both on IPS can be traced back to breed differences, as noted by other (*21, 22*). Late-maturing breeds (e.g., Limo) develop more muscle mass and less fat than early-maturing breeds such as Simmental and Angus (*7*). The higher IMF content of the late-maturing Scott beef could be explained by the longer finishing period for this breed that allowed the cattle to obtain higher levels of IMF. Consumers often consider this meat to be unhealthy because of its visible fat content. However, on the basis of the recommended dietary fat allowance of 80 g/day (*23*), the fat intake consumed with 100 g of meat amounts to 1.4% with Limo (lowest) and 4% with Scott (highest) of the daily fat intake. This leads to the conclusion that—on condition that visible subcutaneous fat is removed—beef meat from either production system investigated contributes only marginally to the daily fat intake.

Plasmalogenic Lipids. Meat lipids contain plasmalogens and sphingolipids that may contribute up to 15% of the total fat in the meat (*15*). An acid-catalyzed methylation method is mandatory because both of these lipid classes are stable under alkali conditions, which leads to a significant underestimation of the total lipid content of meat if only a base-catalyzed methylation procedure is used. To date, only a few studies have focused on plasmalogenic lipids in beef meat. Dannenberger et al. (*24*) found that pasture-fed German Holstein and German Simmental bulls had a higher proportion of aldehydes compared with the concentrate-fed bulls. Our high DMA/AME content in the two IPS beef breeds is in agreement with that of Dannenberger et al. (*24*), but the results also suggest an additional breed difference, particularly when expressed on a percent basis (**Table 1**). The plasmologen synthesis is not fully understood, and it is believed that various factors influence this process (*25*). Thus, it is conceivable that breed-typical determinants (e.g., enzymes, phospholipid content) affect the total fatty aldehyde content in the different breeds.

Branched-Chain Fatty Acids. NGS-raised beef accrued 1.5−4.5-fold higher amounts of BCFA than IPS beef. These results support the hypothesis of a highly active and specific rumen microbial ecoflora in grazing ruminants. In NGS, the rations are poorer in energy and richer in fiber (pasture), which leads to a more intensive cellulolytic rumen-bacteria activity that induces an increase in the proportion of BCFA in meat lipids. In contrast, corn silage/concentrate rations, generally used in IPS, are rich in dietary starch and poor in neutral detergent fiber, leading to a reduction in the rumen pH and a subsequent shift in the microbial population from cellulolytic to amylolytic bacteria (*26, 27*). Anticarcinogenic effects have also been attributed to BCFA (*28, 29*), but it is not possible to estimate the associated benefit.

***trans*-18:1 and CLA.** The wide range of *trans*-18:1 and CLA isomers in beef meat originate from bacterial biohydrogenation of dietary PUFA (LA and αLNA) in the rumen (*30, 31*). Ruminal biohydrogenation of these PUFA is a sequential process involving isomerization and hydrogenation of the double bonds producing *trans*-18:1 and CLA isomers as intermediates and stearic acid (18:0) as end product (*32*). The content and composition of *trans*-18:1 and CLA isomers in beef meat are largely determined by dietary components in the animal's diet (*6*).

The content and proportion of total *trans*-18:1 and CLA varied among the production systems and breeds, with significantly higher values in the NGS-raised Angus and Scott beef than Limo and Simmental beef raised in the IPS. Differences in the total CLA and *trans*-18:1 contents of ruminant-derived products between grass- and concentrate-fed animals are well recognized (*16, 33–38*). It would be tempting to compare the *trans*-18:1 and CLA levels in the meat lipids of grass-fed beef to those found in milk fat of grass-fed dairy cows. This would be legitimate if the meat and milk were sampled from the same cow; otherwise, genetic differences might influence the comparison. Furthermore, a comparison of the adipose and milk fat might be more appropriate because both of these matrices consist mainly of TAG. The IMF analyzed in this study consisted of both neutral and polar lipids, and the polar lipids (phospholipids) are known to contain considerably less *trans*-18:1 and CLA isomers than the neutral lipids (*39, 40*).

Meat of NGS-raised beef yielded significantly higher levels of 11*t*-18:1, 12*t*,14*t*-, 11*t*,13*t*-, 11*t*,13*c*-, and 9*c*,11*t*-CLA compared to meat of IPS beef, whereas IPS beef contained significantly higher levels of 10*t*-18:1, 10*t*,12*t*-, 10*t*,12*c*-, and 7*t*,9*c*-CLA. There is clear evidence in the literature that diet rations low in forage and high in starch lead to shifts in the rumen microbial populations due to changes in the ruminal environment that favor the formation of 10*t*-18:1 and a concomitant reduction in the amount of 11*t*-18:1, whereas rumen fermentation in pasture-fed cattle minimizes the formation of the 10*t*-18:1 and maximizes the 11*t*-18:1 formation (*16, 41, 42*). The lower content of 9*c*,11*t*-CLA in the IPS beef in comparison to that in NGS beef also provides evidence for this shift in ruminal biohydrogenation toward 10*t*-18:1 at the expense of 11*t*-18:1 given that the majority of 9*c*,11*t*-CLA (up to 90%) is

endogenously synthesized from 11*t*-18:1 by the Δ9-desaturase enzyme system (*36, 43, 44*). Overall, the nature of the *trans*-18:1 diversity is not yet fully elucidated, and information on the formation of range of the numerous different CLA isomers remains the subject of continuing research. However, it is conceivable that the *trans*-18:1 and CLA isomers originating from the rumen microbes exhibit a multiplicity of enzyme-catalyzed isomerizations and reductions leading to the formation of all possible geometric and positional *trans*-18:1 and CLA isomers.

CLA have received much attention for their potential health-promoting properties (reviewed in ref *44*), but the scientific evidence is diverse and to some extent conflicting, particularly in regard to the 10*t*,12*c* isomer that occurs naturally only in trace amounts. The anticarcinogenic properties of the 9*c*,11*t* isomer show promise in various animal models, although similar results in humans have not demonstrated these beneficial health effects (reviewed in ref *46*). On the basis of the results of this study, 100 g of lean beef provides between 3 mg (∼2 mg of 9*c*,11*t*-CLA) and 22 mg of total CLA (∼16 mg of 9*c*,11*t*-CLA), which represents only a small portion of the daily CLA intake. The intake of TFA is associated with an increased risk of coronary heart disease (*47*). The debate whether naturally occurring TFA from ruminants have the same impact on human health as industrially produced TFA continues because of the lack of definitive scientific studies (*48*). Given that ruminant-derived TFA have been involved in the human diet for centuries compared to industrially produced TFA that were introduced in our diet approximately 100 years ago, it seems unlikely that TFA in ruminant fats constitute a health risk for the average dietary intake of beef, assuming that the TFA profile is not drastically altered.

Polyunsaturated Fatty Acids. In the recent years, *n*-3 PUFA, in particular *n*-3 LC-PUFA, have received considerable attention in view of their beneficial effects on human health (*49*). Therefore, the content of those FA is of interest when meat quality characteristics are determined. NGS beef had higher total amounts of *n*-3 PUFA and *n*-3 LC-PUFA, primarily as αLNA, EPA, 22:5 *n*-3, and 22:6 *n*-3, than the IPS beef, except for Limo. This difference is consistent with other studies in which pasture- and concentrate-fed cattle were compared (*50–53*). Because PUFA are not synthesized in ruminant tissues (*54*), the amount of LA and αLNA in meat depends on a number of factors: (i) the amount in the diet, (ii) the amount that escapes rumen biohydrogenation and is absorbed, and (iii) the amount that is not oxidized or metabolized in the body. The relatively high proportion of total PUFA in Scott beef was somewhat unique, demonstrating that not only the production system but also breed/genetic factors have an impact on the amount and distribution of PUFA. Also of interest is the Limo breed that contained levels of *n*-3 LC-PUFA similar in content as found in the meat of beef raised on the NGS, despite the lower levels of αLNA. This breed appears to have a greater desaturation and elongation capacity that may be due to its great muscle density (*55*). 22:5 *n*-3 is the most important *n*-3 LC-PUFA, whereas the DHA formation is very low in beef (**Figure 1**). PUFA are preferentially deposited in the phospholipid fraction (*2*), and therefore a higher level of PUFA reflects a higher phospholipid/TAG ratio in the beef meat and, consequently, a lower fat content.

Nutritional Consideration. In general, meat from cattle exhibits a low P/S ratio due to the ruminal biohydrogenation of unsaturated FA. Variations in the P/S ratio result from the IMF content, feeding regimen, and genetic factors (*2, 4*). Our results revealed that the P/S ratio was more affected by the IMF content, which is a characteristic of the breed type, than by the diet. Although the P/S ratio in the beef meat was below the recommended value for the human diet, which is >0.45 (*56*), the P/S ratio was more favorable for Limo, which is a lean breed, than for the fatter breeds. These results are in agreement with previously published data on the Limo breed (*22, 57*).

NGS beef had significantly lower *n*-6/*n*-3, LA/αLNA, and AA/EPA ratios, reflecting the much higher levels of *n*-3 PUFA. In contrast, IPS beef had unfavorable ratios mainly due to the greater proportion of *n*-6 PUFA. The *n*-6/*n*-3 ratio in the Western diet ranges between 10/1 and 17/1 (*58*). According to the German−Austrian−Swiss nutritional guidelines the optimal *n*-6/*n*-3 ratio in the human diet should be 5/1 or below to support optimal regulatory mechanisms and thus prevent chronic diseases (*23*). On the basis of this guideline, the *n*-6/*n*-3 ratios in both NGS beef were within the recommendation, with the Angus having the best ratio of 2. An AA/EPA ratio in the diet of 1.5 is considered to be ideal (*59*). NGS beef approached most closely this recommendation. In contrast, the IPS beef Limo and Simmental had 2- and 5-fold higher ratios, respectively.

Conclusion. Information is widely available on the FA composition of IMF in beef of different production systems and breeds, but comparison of the data is difficult because of differences in experimental designs and analytical methods. This study provides the complete lipid composition of beef meat originating from different German production systems at retail level. On the basis of the results obtained, the tissue levels of FA are the result of not only components leaving the rumen but also subsequent FA metabolic processes in the ruminant animal (uptake, incorporation, degradation, enzyme activities) that are affected by genetic traits.

From the human health perspective beef raised in the NGS is clearly superior with regard to a more favorable FA profile in the IMF compared to beef derived from IPS. Thus, meat from NGS beef possesses distinct benefits for human health, although the contribution of individual FA and FA groups (e.g., 9*c*,11*t*-CLA, *n*-3 PUFA, *n*-3 LC-PUFA) to the recommended daily intake is low. Efforts should be directed toward providing better information for the consumer concerning the fact that beef meat, especially that originating from NGS, is an excellent source of many nutritionally important FA. NGS beef meat can and should be promoted as an important part of a healthy balanced diet.

ABBREVIATIONS USED

αLNA, α-linolenic acid; AA, arachidonic acid; Ag^+-HPLC, silver-ion high-pressure liquid chromatography; Ag^+-TLC, silver-ion thin-layer chromatography; AME, alk-1-enyl methyl esters; BCFA, branched-chain FA; CLA, conjugated linoleic acids; DMA, dimethylacetals; EPA, eicosapentaenoic acid; FA, fatty acids; FAME, fatty acid methyl esters; FID, flame ionization detector; GC, gas chromatography; IA, index of atherogenity; IMF, intramuscular fat; IPS, intensive production system; IT, index of thrombogenity; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acids; Limo, Limousin; MUFA, monounsaturated fatty acids; $NaOCH_3$, sodium methoxide; NGS, natural grazing system; P/S, polyunsaturated to saturated fatty acid ratio; PUFA, polyunsaturated fatty acids; Scott, Scottish Highland Cattle; SFA, saturated fatty acids; TFA, trans fatty acids; TLC, thin-layer chromatography; v, volume; VA, vaccenic acid; w, weight.

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