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Diet Alters the Fatty Acid Composition of Individual Phospholipid Classes in Beef Muscle

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The objective of this study was to investigate the effect of diet on the distribution of phospholipid classes and fatty acid profiles of individual phospholipid classes in longissimus muscle of beef. An experiment was established to examine the effect of pasture-based versus concentrate diet offered to two different breeds (German Holstein and German Simmental bulls) to enhance the content of beneficial fatty acids in beef and improve the meat quality for the consumer. High-performance thin-layer chromatography was utilized to separate the phospholipid classes. The fatty acid composition of the individual phospholipid classes was determined by gas chromatography. The main phospholipid classes in the muscle were phosphatidylethanolamine and phosphatidylcholine, representing approximately 60% of the total phospholipids, followed by phosphatidylinositol ranging between 11.8 and 14.8%. The results have shown that the fatty acid profiles in the detected seven phospholipid classes can be affected by different feeding systems. Pasture-based feeding resulted in an enrichment of total and individual *n*-3 fatty acids in all phospholipid classes of muscle lipids of bulls compared with those fed on concentrate. In contrast, pasture-based diet significantly decreased the proportion of total and individual *n*-6 fatty acids in phospholipid classes, except in the sphingomyelin fraction. The total saturated fatty acid proportions in the phospholipid classes were different and ranged between 4.5% in the cardiolipin fraction and 50.5% in the sphingomyelin fraction of muscle lipids of bulls. Furthermore, the diet effects on the saturated fatty acid proportion in the different phospholipid classes differ widely. The results have shown that the C18:1 *trans* and CLA profiles in the detected seven phospholipid classes can be affected by different feeding systems.

KEYWORDS: Phospholipid classes; beef muscle; pasture; thin-layer chromatography; fatty acids; CLA

INTRODUCTION

The relationship between balanced nutrition and health has increased consumer interest in the nutritional value of food. Fat, especially animal fat, has been the subject of much interest and debate because of the implications for human health and association with risks for some diseases (i.e., coronary heard disease; cancer) when consumed in excess. Animal experiments and clinical intervention studies indicate that a lower ratio of *n*-6/*n*-3 fatty acids in dietary fat is desirable in reducing the risk of many of the chronic diseases (*1*−*3*). Animal production practices, particularly the nutrient composition of the diet, can change carcass and meat quality and the fatty acid profile of beef (*4*−*8*). Feeding animals grass relative to concentrate-rich diets has been reported to affect several meat quality characteristics of beef, in particular, color, flavor, and fatty acid composition (*8*−*11*). A pasture-based feeding system, including fresh and conserved forages and also occasional dietary supplements, leads to improvements in the nutritional quality of meat from cattle for the consumers. Pasture feeding and diets containing proportionally high concentration of linolenic acid (C18:3 *n*-3) in the fat, such as fresh grass and grass silage, resulted in increased deposition of *n*-3 fatty acids in the muscles (*5*, *7*, *8*, *10*).

Intramuscular fat mainly consists of triacylglycerols (TAGs) and phospholipids (PLs). TAGs serve as energy stores and are deposited in adipocytes. PLs are amphiphilic molecules with lipophilic acyl chains and a hydrophilic head and components of biological membranes. The PL content in beef muscle is nearly constant and typically ranged between 0.5 and 0.6 g/100 g of fresh muscle (*11*−*13*). In beef muscle nine different phospholipid classes, phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), cardiolipin (CL), sphingomyelin (SM), phosphatidylserine (PS), lysophosphati-

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dylethanolamine (LPE), lysophosphatidylcholine (LPC), and phosphatidic acid (PA), have been described (*13*−*16*). PE is usually the most abundant PL and PC the second most abundant PL in animal tissues, and as such they are obviously the key building blocks of membrane bilayers. Cardiolipin, first found in animal hearts, is a specific lipid component of mitochondria, and in animal tissue it is believed to be an important cofactor for cholesterol translocation from the outer to the inner mitochondrial membrane and may also have a specific role in the import of proteins into mitochondria (*17*). Phosphatidylinositol is the primary source of the arachidonic acid (C20:4 *n*-6) required for the biosynthesis of eicosanoids, including prostaglandins, via the action of the enzyme phospholipase A_2, which releases the fatty acids from the position *sn*-2 and seems to have crucial roles in the interfacial binding of proteins (*18*). Sphingomyelin is known to have various important roles in animals, besides serving as a substitute for PC as a building block of membranes by forming a stable and chemically resistant outer leaflet of the plasma membrane lipid layer. Evidence has accumulated to propose that SM and cholesterol metabolism is integrated in cells (*19*, *20*). Also, SM serves as a precursor for ceramides, long-chain bases, and sphingosine-1-phosphate (*19*).

Among the various methods for the analysis of phospholipids, high-performance thin-layer chromatography (HP-TLC), high-performance liquid chromatography (HPLC), and ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR) are the most applied ones (*21*−*24*). In the past two decades, HP-TLC and HPLC were the preferred separation methods of PLs for qualitative and quantitative analysis because of relatively low cost compared with ^{31}P NMR measurements (*21*). Recently, results of investigations into PL class profiles and fatty acid composition of individual PL classes of dairy products and hen eggs were published (*22*, *25*, *26*). However, until now, studies into PL class composition and fatty acid profiles of the PL classes in different beef tissues are sparsely described in the literature (*13*, *15*). It is known that pasture feeding affected the distribution of fatty acids in the PL and TAG fraction of the beef muscle lipids (*7*, *12*). However, there is a lack of information about the diet effects on PL class profiles and fatty acid composition of individual phospholipid classes in intramuscular fat of beef.

A large study was established to investigate the effect of feeding pasture versus concentrate to two different breeds (German Holstein and German Simmental) on fatty acid composition of beef and effects on other important meat quality attributes including color shelf life and sensory characteristics. The results on aspects of meat quality and fatty acid composition in total lipids, neutral and polar lipid fractions including C18:1 *trans* and conjugated linoleic acid isomers (CLA), and fatty aldehyde composition have been reported by Nuernberg et al. (*8*) and Dannenberger et al. (*7*, *27*, *28*), recently. This paper reports the effects of diet and breed on the fatty acid composition of individual phospholipid classes in longissimus muscle of German Holstein and German Simmental bulls.

MATERIALS AND METHODS

Materials. Sixty-four bulls of two breeds (5−6 months old), German Simmental ($n = 31$) and German Holstein ($n = 33$), were randomly assigned to two dietary treatments, concentrate or pasture. German Simmental ($n = 16$) and German Holstein bulls ($n = 17$) fed the concentrate were kept in a stable and fed using single-fodder workstations. The concentrate ration consisted of winter barley, molasses particles, soybean extraction particles, calcium carbonate, sodium chloride, and a mixture of minerals and vitamins. The forage component of the diet consisted of maize silage (13.8 kg/day), concentrated feed (3.2 kg/day), soybean extraction particles (0.15 kg/day), hay (0.1 kg/day) and straw (0.09 kg/day). German Simmental (*15*) and German Holstein (*16*) bulls were kept on pasture during two summer periods (approximately 160 days) followed by indoor periods (approximately 190 days) when animals received semi ad libitum a high-energy diet. The latter consisted of wilted silage (15 kg/day), hay (0.7 kg/day), a mixture of minerals, and a mixture of vitamins along with a special concentrate diet containing 12% barley, 10% coarsely cracked linseed, and a mixture of minerals and vitamins. Nuernberg et al. (*8*) reported the experiment details and chemical composition including the fatty acid composition of the diets.

All bulls were slaughtered at 620 kg of live weight in the abattoir of the Research Institute for the Biology of Farm Animals Dummerstorf (Germany). Samples of the longissimus muscle for the analysis of phospholipids were taken at the sixth rib of the left carcass side immediately postslaughter and stored frozen immediately at −70 °C until lipid extraction and further chromatographic procedure of the muscle lipids.

Methods. *Extraction of Muscle Lipids and Separation of Lipid Classes.* The intramuscular lipids from 2 g of muscle was extracted with chloroform/methanol (2:1, v/v) according to Folch et al. (*29*) by homogenization (Ultra Turrax, 3 × 15 s, 12000 revolutions per minute) at room temperature. The details of muscle lipid extraction were described previously (*8*). To obtain the PL fraction the isolated total lipids were separated by thin-layer chromatography on precoated silica gel 60 HP-TLC plates using the solvent mixture *n*-hexane/diethyl ether/acetic acid (70:30:2, v/v). The phospholipid band was scraped off and eluted with chloroform/methanol (1:2, v/v) followed by chloroform/methanol (2:1, v/v). The details of separation of the lipid classes were described by Lorenz et al. (*12*).

Separation of Phospholipid Classes. Before use for PL class separation, the HP-TLC plates were dipped into a solvent mixture of *n*-hexane/diethyl ether (9:1, v/v) for 1 h to remove all impurities from the silica gel phase. Up to 20 mg of the total PL fraction, obtained from the HP-TLC separation of lipid classes described above, was applied to the plate in a narrow band. For the separation of the individual PL classes the precoated silica gel 60 thin-layer chromatography plates were developed with a solvent mixture of chloroform/isopropanol/triethylamine/methanol/0.25% potassium chloride solution (30:25:18:9:6, v/v) for 3 h following the method of Pratt et al. (*30*) and Salze et al. (*31*) with changes in the composition of the mobile phase. After drying, the plates were sprayed with a 0.01% aqueous solution of Rhodamin 6G and the bands were visualized under UV light. For the identification of the individual PL classes after HP-TLC a spray solution of Molybdenum blue was used. The identification of the individual PL classes was confirmed by comparison with reference to the R_f values of the standards run alongside on HP-TLC plates and developed in the above solvent system. The separated fractions of PL classes were scraped off and then two times extracted with 5 mL of a solvent mixture (chloroform/methanol, 1:2, v/v). After that, the extracts of the individual PL classes were filtered and evaporated to dryness under a gentle nitrogen stream. The extracts were dissolved in 1 mL of the solvent mixture (chloroform/methanol, 2:1, v/v) and stored frozen at −70 °C until derivatization.

Derivatization and Gas Chromatography Analysis. The extracts of the individual PL classes were treated with 2 mL of a 0.5 M methanolic sodium methylate solution for 20 min at room temperature. Methylation of fatty acids was performed with 1 mL of boron trifluoride/methanol (14% w/v) for 20 min at room temperature. The fatty acid methyl esters (FAMEs) formed were extracted two times with 2 mL of *n*-hexane, then evaporated using a rotary evaporator, and dried under a gentle nitrogen stream at room temperature. The FAMEs were dissolved in *n*-heptane for GC analysis. Nonadecanoic acid methyl ester (C19:0 ME) used as an internal standard was added to the individual PL classes prior to derivatization to methyl ester.

The fatty acid composition of muscle lipids was determined by capillary GC using a CP SIL 88 CB column (100 m × 0.25 mm, Chrompack-Varian) installed in a Perkin-Elmer gas chromatograph Autosys XL with a flame ionization detector and split injection (Perkin-Elmer Instruments). The initial oven temperature was 150 °C, which was held for 5 min, subsequently increased to 175 °C at a rate of 2 °C

min^{-1}, held for 15 min, then increased to 200 °C at 7 °C min^{-1}, held for 20 min, then increased to 220 °C at 5 °C min^{-1}, and held for 25 min. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1}. The split ratio was 20:1, the injector was set at 260 °C, and the detector was set at 280 °C. The amounts were calculated using the internal standard method of Turbochrom workstation software.

Reagents. A reference standard "Sigma-FAME mixture" was obtained from Sigma-Aldrich (Deisenhofen, Germany). Additionally, individual methyl esters of C18:2 *cis*-9, *trans*-11, C18:4 *n*-3, C22:4 *n*-6, and C22:5 *n*-3 were purchased from Matreya (Pleasant Gap, PA). Methyl esters of C18:1 *trans*-11 and C18:1 *cis*-11 were purchased from Larodan Fine Chemicals (Malmö, Sweden). All solvents used for HP-TLC and GC were of HPLC grade from Lab-Scan (Dublin, Ireland). The HP-TLC glass plates coated with 0.25 mm silica gel (20 × 20 cm) were obtained from Merck (Darmstadt, Germany). The phospholipid standards DL-α-phosphatidylcholine, dl-α-phosphatidylethanolamine, L-α-phosphatic acid, cardiolipin, L-α-lysophosphatidylcholine, l-α-phosphatidylethanolamine, L-α-phosphatidylinositole, 3-sn-phosphatidyl-L-serine, and sphingomyelin were purchased from Sigma-Aldrich. For the identification of the individual PL classes after TLC, a spray solution of Molybdenum blue (Sigma-Aldrich) and for the quantification procedure an aqueous solution of Rhodamin 6G (Sigma-Aldrich) were used to visualize the bands under UV light (254 nm). The derivatization reagents sodium methylate were obtained from Fluka (Buchs, Switzerland) and boron trifluoride/methanol (14% w/v) was from Sigma-Aldrich.

Statistical Analysis. All data were analyzed by the least-squares method using the GLM procedures of the Statistical Analysis System (SAS Systems, release 8.2, and SAS Institute Inc., Cary, NC) with fixed factor feeding and breed. All tables contain the least-squares mean (LSM) and the standard error (SEM) of the LSM. All statistical tests of LSMs were performed for a significance level of $p = 0.05$.

RESULTS AND DISCUSSION

Using the TLC procedure, described above, seven PL classes were identified in the longissimus muscle lipids of grass-based and concentrate-fed German Holstein and German Simmental bulls (**Figure 1**). The main PL classes in the muscle were PE and PC, representing approximately 60% of the total PL in the tissue, followed by PI and CL fractions ranging between 11.8 and 14.8% and between 11.6 and 14.0% of the total PL in the muscle lipids, respectively. The proportions of SM, LPE, and LPC varied between 3.7 and 5.3%, between 5.9 and 8.1%, and between 2.2 and 3.1%, respectively (**Figure 1**). No diet effect was observed for the proportion of the main PL classes PE and PC in the muscle lipids of German Holstein and German Simmental bulls. However, the diet affects the proportions of the SM and LPC fraction in the muscle lipids of German Simmental bulls. Pasture diet significantly increased the SM and LPC proportions in the muscle lipids of German Simmental bulls (**Figure 1**). The PS and PA fractions in the muscle lipids of bulls could not be detected by TLC.

Investigations into the composition of PL classes in beef tissues are sparsely described in the literature (*13*−*15*). Due to the different chromatographic techniques used it is difficult to compare the results. Yeo and Horrocks (*16*) reported that in beef top loin steak from steers the main PL classes PC and PE constituted approximately 90% of the total PLs. The proportions of PI, PS, and SM varied between 1.1 and 4.7%. The PL classes CL and the lysophospholipids were not detected. Larick and Turner (*14*) determined the influence of finishing diet on the PL class profiles and fatty acid composition in pectoralis major muscle of Angus and Angus/Hereford steers. Those authors found that the PC plus LPE fraction (coeluted) was the predominant PL class present, accounting for approximately 60% of total PLs. The PE fraction ranged between 7 and 8% of the total PLs. However, the LPC fraction varied between 20 and 21% of the total PL and SM, PI and PS ranged between 4 and 5% and between 2 and 3%, respectively. The CL fraction was not identified in the muscle lipids (*14*). Recently, Kivini et al. (*25*) reported that total PLs in hen egg yolk consisted of predominantly three PL classes, approximately 70% PC, 28% PE, and 2% SM.

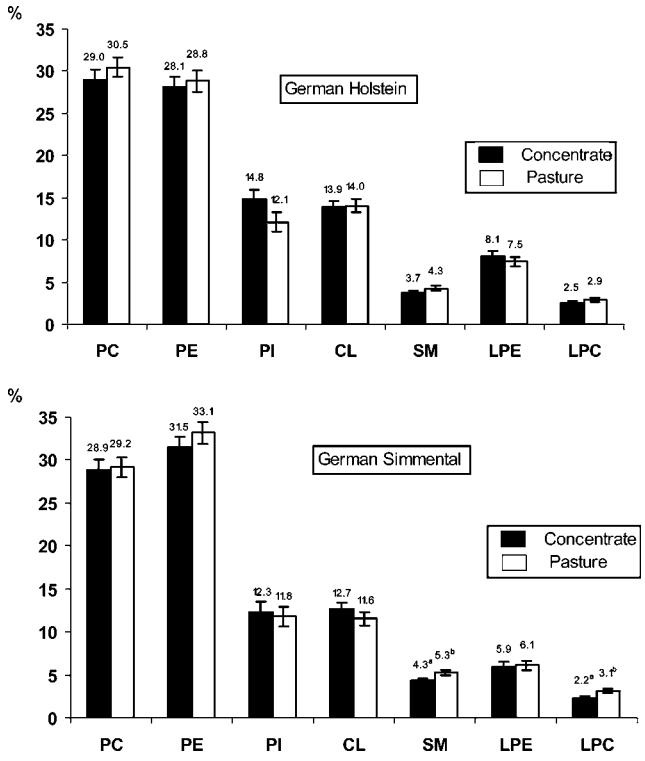

Figure 1. Effect of diet on the distribution of phospholipid classes in longissimus muscle lipids of concentrate- and pasture-fed German Holstein and German Simmental bulls (columns with different letters are significantly different between feeding groups, $P < 0.05$).

Furthermore, there is a lack of information in the literature describing the effect of diet on the fatty acid composition of PL classes in beef muscles besides the PL class composition. It is recognized that feeding diets with high proportions of *n*-3 polyunsaturated fatty acids (PUFA) in the lipids such as grass, although very low in total fat content, and fish oil (rich in C18:3 *n*-3 and the *n*-3 long-chain PUFA, respectively) increases the concentration of *n*-3 PUFA in the beef (*5*, *7*, *11*). The high content of C18:3 *n*-3 in the lipids of grass and wilted silage, typically 50−65% of total fatty acids, is partly deposited into the muscle lipids despite a high extent of biohydrogenation of dietary PUFA in the rumen (*5*, *11*). Itoh et al. (*32*), Lorenz et al. (*12*), and Dannenberger et al. (*7*) reported that the total muscle PLs of pasture-fed bulls contained significantly higher concentrations of total and individual *n*-3 fatty acids compared with those fed on concentrates. The total phospholipid content of the longissimus muscle of German Holstein and German Simmental bulls varied between 0.54 and 0.68 g/100 g of muscle in animals fed on pasture and concentrate, respectively (*7*). There was no significant difference in PL content due to breed or diet, as already described in previous studies by Larick and Turner (*14*) and Yeo et al. (*15*).

The diet effects on the fatty acid composition of the seven PL classes PE, PC, PI, SM, CL, LPE, and LPC in longissimus muscle German Holstein and German Simmental bulls are presented in **Tables 1**−**7**.

Diet affected the proportions of total PUFAs in all PL classes of muscle lipids, except the PI fraction (**Tables 1**−**7**). Pasture-

Table 1. Fatty Acid Composition (Percent) of Phosphatidylethanolamine (PE) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM[a]	SEM[a]	LSM	SEM	LSM	SEM	LSM	SEM	significance ($P < 0.05$)
C12:0	0.03	0.004	0.03	0.004	0.03	0.004	0.03	0.005	
C14:0	0.16	0.04	0.39	0.04	0.38	0.04	0.16	0.05	D*B
C16:0	1.68	0.11	1.38	0.12	1.22	0.12	1.19	0.12	B
C18:0	18.79	0.58	18.21	0.60	21.43	0.62	17.54	0.64	D, D*B
C18:1 *cis*-9	13.42	0.54	11.56	0.55	12.77	0.57	11.78	0.59	D
C18:1 *cis*-11	1.26	0.08	1.09	0.09	1.14	0.09	0.81	0.09	D, B
C18:2 *n*-6	19.14	0.83	18.90	0.86	20.07	0.89	18.79	0.92	
C18:3 *n*-3	0.93	0.17	4.84	0.17	0.88	0.18	4.57	0.18	D
C20:4 *n*-6	25.21	0.70	17.25	0.72	26.22	0.74	17.75	0.77	D
C20:5 *n*-3	2.07	0.29	7.97	0.30	1.55	0.31	8.22	0.32	D
C22:5 *n*-3	4.77	0.24	8.09	0.24	3.34	0.25	8.02	0.26	D, B, D*B
C22:6 *n*-3	1.00	0.06	1.27	0.07	0.62	0.07	1.22	0.07	D, B, D*B
CLA *cis*-9,*trans*-11[c]	0.17	0.05	0.27	0.05	0.19	0.05	0.37	0.05	D
sum C18:1 *trans*[d]	1.00	0.08	1.20	0.08	0.90	0.08	1.56	0.09	D, D*B
sum SFA[e]	21.28	0.59	20.50	0.61	23.54	0.63	19.42	0.65	D, D*B
sum UFA[f]	78.72	0.59	79.49	0.61	76.46	0.63	80.58	0.65	D, D*B
sum PUFA[g]	60.02	0.75	61.90	0.77	58.89	0.80	61.80	0.82	D
sum *n*-3 FA[h]	9.04	0.49	22.36	0.51	6.66	0.53	22.17	0.54	D, B, D*B
sum *n*-6 FA[i]	47.66	0.64	38.41	0.66	49.10	0.68	38.50	0.71	D
n-6/*n*-3 ratio	5.41	0.23	1.72	0.23	7.68	0.24	1.78	0.25	D, B

[a] LSM, least-square means; SEM, standard error of LSM. [b] D, significant influence of diet; B, significant influence of breed; D*B, significant interaction of D*B. [c] Coelution (CLA *trans*-7,*cis*-9 and CLA *trans*-8,*cis*-10). [d] Sum of the isomers C18:1 *trans*-6,*trans*-11. [e] Sum of C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. [f] Sum of C14:1 + C15:1 + C16:1 + C17:1 + C18:1 *trans* + C18:1 *cis*-9 + C18:1 *cis*-11 + C18:2 *trans* + C18:2 *n*-6 + C18:3 *n*-3 + C18:4 *n*-3 + C20:3 *n*-6 + C20:4 *n*-6 + C20:5 *n*-3 + C22:1 + C22:4 *n*-6 + C22:5 *n*-3 + C22:6 *n*-3 + CLA *cis*9,*trans*11 + C18:3 *n*-6 + C20:2 *n*-6 + C20:3 *n*-3 + C22:2 *n*-6 + C24:1. [g] Sum of C18:2 *trans* + C18:2 *n*-6 + C18:3 *n*-3 + C18:4 *n*-3 + C20:3 *n*-6 + C20:4 *n*-6 + C20:5 *n*-3 + C22:1 + C22:4 *n*-6 + C22:5 *n*-3 + C22:6 *n*-3 + CLA *cis*-9,*trans*-11 + C18:3 *n*-6 + C20:2 *n*-6 + C20:3 *n*-3 + C22:2 *n*-6. [h] Sum of C20:3 *n*-3 + C22:6 *n*-3 + C22:5 *n*-3 + C20:5 *n*-3 + C18:4 *n*-3 + C18:3 *n*-3. [i] Sum of C22:2 *n*-6 + C20:2 *n*-6 + C18:3 *n*-6 + C22:4 *n*-6 + C20:3 *n*-6 + C18:2 *n*-6 + C20:4 *n*-6.

Table 2. Fatty Acid Composition (Percent) of Phosphatidylcholine (PC) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM[a]	SEM[a]	LSM	SEM	LSM	SEM	LSM	SEM	significance[b] ($P < 0.05$)
C12:0	0.07	0.01	0.10	0.02	0.08	0.02	0.11	0.02	
C14:0	0.21	0.02	0.31	0.02	0.28	0.02	0.15	0.02	B, D*B
C16:0	24.53	0.15	24.58	0.46	24.72	0.48	24.39	0.48	
C18:0	4.72	0.15	5.07	0.15	4.70	0.16	4.99	0.160	D
C18:1 *cis*-9	35.75	0.98	31.11	1.01	36.04	1.05	34.43	1.05	D
C18:1 *cis*-11	2.02	0.12	1.98	0.12	1.99	0.12	1.95	0.12	
C18:2 *n*-6	19.34	0.81	18.60	0.84	20.41	0.86	16.74	0.86	D
C18:3 *n*-3	1.14	0.16	5.67	0.17	1.12	0.17	4.90	0.17	D, B, D*B
C20:4 *n*-6	4.21	0.14	2.68	0.15	3.32	0.16	2.30	0.16	D, B
C20:5 *n*-3	0.48	0.07	1.86	0.07	0.29	0.07	1.66	0.07	D, B
C22:5 *n*-3	1.22	0.06	2.05	0.06	0.80	0.07	1.79	0.07	D, B
C22:6 *n*-3	0.20	0.01	0.23	0.01	0.10	0.01	0.19	0.02	D, B, D*B
CLA *cis*-9,*trans*-11[c]	0.21	0.02	0.28	0.02	0.25	0.02	0.41	0.02	D, B, D*B
sum C18:1 *trans*[d]	0.59	0.48	0.71	0.05	0.54	0.05	0.97	0.05	D, B, D*B
sum SFA[e]	30.52	0.41	31.11	0.42	30.74	0.43	30.73	0.43	
sum UFA[f]	69.48	0.41	68.89	0.42	69.26	0.43	69.27	0.43	
sum PUFA[g]	29.17	1.05	32.83	1.08	28.40	1.11	29.17	1.11	D, B
sum *n*-3 FA[h]	3.09	0.22	9.84	0.23	2.36	0.23	8.58	0.23	D, B
sum *n*-6 FA[gi]	25.04	0.91	22.35	0.94	24.93	0.97	19.90	0.97	D
n-6/*n*-3 ratio	8.18	0.21	2.28	0.21	10.68	0.22	2.33	0.22	D, B, D*B

[a] For footnotes see **Table 1**.

based feeding resulted in a significantly higher proportion of PUFA in the PE, PC, CL, LPE, and LPC fraction as well as in the muscle lipids of German Holstein and German Simmental bulls compared with those fed concentrate. No diet effect on the PUFA proportions occurred in the PI fraction in both breeds, varying between 26.9 and 28.8% and between 27.2−27.8% in German Holstein and German Simmental bulls, respectively (**Table 3**). An interaction of diet × breed in PUFA proportions with lower values in pasture-fed German Holstein bulls and higher values in pasture-fed German Simmental bulls was identified in the SM fraction (**Table 5**). The highest PUFA proportions up to 74.5% were determined in the CL fraction, and the lowest values occurred in the SM fraction. Larick and Turner (*14*) found similar results investigating dietary effects

Table 3. Fatty Acid Composition (Percent) of Phosphatidylinositol (PI) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM[a]	SEM[a]	LSM	SEM	LSM	SEM	LSM	SEM	significance[b] ($P < 0.05$)
C12:0	0.08	0.01	0.08	0.01	0.06	0.01	0.06	0.01	
C14:0	0.50	0.08	0.83	0.08	0.52	0.08	0.22	0.08	D, D*B
C16:0	0.02	0.10	0.02	0.11	0.06	0.11	0.04	0.11	
C18:0	43.26	0.95	43.07	0.98	45.67	0.98	43.50	1.01	
C18:1 *cis*-9	12.41	0.59	11.35	0.61	10.96	0.61	12.28	0.63	
C18:1 *cis*-11	7.93	0.48	8.63	0.50	9.09	0.50	7.91	0.52	
C18:2 *n*-6	4.19	0.89	6.33	0.92	5.17	0.92	7.09	0.95	D
C18:3 *n*-3	0.33	0.23	1.92	0.23	0.45	0.23	2.03	0.24	D
C20:4 *n*-6	12.57	0.50	9.21	0.52	12.93	0.52	8.92	0.54	D
C20:5 *n*-3	0.71	0.12	2.72	0.12	0.63	0.12	2.36	0.13	D
C22:5 *n*-3	1.62	0.18	3.67	0.18	1.06	0.18	3.09	0.19	D, B
C22:6 *n*-3	0.35	0.04	0.48	0.04	0.19	0.04	0.31	0.04	D, B
CLA *cis*-9,*trans*-11[c]	0.15	0.02	0.29	0.03	0.23	0.03	0.53	0.03	D, B, D*B
sum C18:1 *trans*[d]	2.16	0.11	2.06	0.11	1.77	0.11	2.80	0.12	D, D*B
sum SFA[e]	49.0	0.95	47.38	0.98	49.71	0.98	46.83	1.01	D
sum UFA[f]	51.01	0.95	52.62	0.98	50.29	0.98	53.17	1.01	D
sum PUFA[g]	26.95	1.03	28.81	1.06	27.25	1.06	27.98	1.09	
sum *n*-3 FA[h]	3.35	0.31	9.04	0.32	2.69	0.32	8.06	0.33	D, B
sum *n*-6 FA[i]	20.45	0.86	18.16	0.89	21.68	0.89	18.58	0.92	D
n-6/*n*-3 ratio	6.20	0.24	2.04	0.26	8.16	0.26	2.37	0.26	D, B, D*B

[a] For footnotes see **Table 1**.

Table 4. Fatty Acid Composition (Percent) of Cardiolipin (CL) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM[a]	SEM[a]	LSM	SEM	LSM	SEM	LSM	SEM	significance[b] ($P < 0.05$)
C12:0	0.28	0.03	0.07	0.03	0.08	0.03	0.12	0.03	D, B, D*B
C14:0	0.68	0.13	1.11	0.13	0.66	0.13	0.41	0.13	B, D*B
C16:0	3.00	0.19	2.19	0.19	2.12	0.19	2.08	0.20	D, B
C18:0	2.32	0.13	1.83	0.13	1.75	0.13	1.61	0.14	D, B
C18:1 *cis*-9	11.34	0.59	9.70	0.59	10.88	0.59	11.00	0.61	
C18:1 *cis*-11	5.70	0.18	4.51	0.18	5.56	0.18	4.34	0.18	D
C18:2 *n*-6	62.91	1.13	61.76	1.13	67.32	1.13	59.89	1.17	D, D*B
C18:3 *n*-3	2.00	0.25	9.91	0.25	2.43	0.26	11.10	0.26	D, B
C20:4 *n*-6	1.59	0.09	0.83	0.09	0.99	0.09	0.72	0.09	D, B, D*B
C20:5 *n*-3	0.21	0.03	0.30	0.03	0.06	0.03	0.25	0.03	D, B
C22:5 *n*-3	0.30	0.03	0.36	0.03	0.11	0.03	0.27	0.03	D, B
C22:6 *n*-3	0.10	0.01	0.05	0.01	0.04	0.01	0.04	0.01	D, B, D*B
CLA *cis*-9,*trans*-11[c]	0.43	0.12	0.11	0.12	0.06	0.12	0.07	0.12	
sum C18:1 *trans*[d]	1.58	0.20	1.09	0.20	0.82	0.20	1.44	0.21	D*B
sum SFA[e]	7.04	0.36	5.54	0.36	4.93	0.36	4.55	0.37	D, B
sum UFA[f]	93.00	0.36	94.50	0.36	95.07	0.36	95.45	0.37	D, B
sum PUFA[g]	69.09	1.09	74.55	1.09	72.30	1.09	73.47	1.13	D
sum *n*-3 FA[h]	2.95	0.28	11.09	0.28	2.77	0.28	12.04	0.28	D, D*B
sum *n*-6 FA[i]	65.87	1.13	63.43	1.13	69.41	1.13	61.40	1.17	D, D*B
n-6/*n*-3 ratio	23.00	0.72	5.79	0.72	25.18	0.72	5.22	0.75	D

[a] For footnotes see **Table 1**.

in muscle PL classes of Angus and Angus/Hereford steers. Contrary to that, Wiesner (*13*) investigated the PC, PE, and CL fraction in muscle lipids of German Simmental and German Simmental/Angus bulls fed concentrate and pasture diet and detected no diet effect in PUFA proportions in the main PL classes PC and PE. The higher PUFA proportions in PL classes of pasture-fed bulls in our experiment may result in a higher extent of lipid oxidation combined with a rancid odor and undesirable flavor in the muscle (*14*). It is well-known that the relative rates of autoxidation of *n*-3 PUFA are higher compared with *n*-6 PUFA (*33*). However, the PUFAs derived from pasture feeding are associated with the occurrence of higher concentrations of antioxidants in the form of α-tocopherol and carotenoids. These antioxidants can stabilize the PUFAs in PLs and prevent a higher degree of lipid oxidation in the muscle lipids of pasture-fed bulls (*8, 11*).

Besides total PUFA, the diet affected the proportions of *n*-3 and *n*-6 fatty acids, C18:1 *trans* and conjugated linoleic acids (CLA), and saturated fatty acids (SFA) in the individual PL classes of the longissimus muscle lipids of both breeds. Pasture- relative to concentrate-fed bulls showed a significantly higher proportion of total and individual *n*-3 fatty acids in all PL classes of muscle lipids. The highest *n*-3 fatty acid proportions occurred in the PE fraction of pasture-fed bulls. The proportion of total *n*-3 fatty acids was increased 9.0−22.4 and 6.7−22.2% in pasture-fed German Holstein and German Simmental bulls compared with that of concentrate-fed bulls, respectively (**Table 1**). The total *n*-3 fatty acid proportions showed an average

Table 5. Fatty Acid Composition (Percent) of Sphingomyolin (SM) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	significance[b] ($P < 0.05$)
C12:0	4.46	0.52	4.10	0.53	3.51	0.53	6.75	0.55	D, D*B
C14:0	3.08	0.41	6.41	0.42	4.17	0.42	3.03	0.43	D, B, D*B
C16:0	21.93	0.53	22.34	0.55	20.12	0.55	21.31	0.57	B
C18:0	11.00	0.45	10.30	0.47	11.63	0.47	12.32	0.48	B
C18:1 *cis*-9	25.37	0.94	25.38	0.97	30.08	0.97	23.49	1.00	D, D*B
C18:1 *cis*-11	1.87	0.10	1.90	0.10	2.32	0.10	1.70	0.10	D, D*B
C18:2 *n*-6	7.20	0.48	8.31	0.49	8.81	0.49	7.04	0.51	D*B
C18:3 *n*-3	0.71	0.12	2.37	0.12	0.79	0.12	1.88	0.12	D, D*B
C20:4 *n*-6	1.92	0.34	1.22	0.35	2.00	0.35	3.04	0.36	B, D*B
C20:5 *n*-3	0.14	0.06	0.45	0.07	0.20	0.07	0.63	0.07	D, D*B
C22:5 *n*-3	0.56	0.13	0.72	0.13	0.58	0.13	0.99	0.14	D
C22:6 *n*-3	0.41	0.09	0.24	0.09	0.16	0.09	0.22	0.09	
CLA *cis*-9,*trans*-11[c]	0.27	0.08	0.45	0.08	0.50	0.08	0.51	0.08	
sum C18:1 *trans*[d]	2.95	0.21	2.62	0.21	2.41	0.21	2.77	0.22	
sum SFA[e]	50.40	1.06	50.39	1.09	46.08	1.09	50.50	1.12	D, D*B
sum UFA[f]	49.59	1.06	49.61	1.09	53.92	1.09	49.50	1.12	D, D*B
sum PUFA[g]	15.92	0.74	15.34	0.76	14.85	0.76	17.46	0.79	D*B
sum *n*-3 FA[h]	1.91	0.24	3.82	0.25	1.59	0.24	3.74	0.26	D
sum *n*-6 FA[i]	10.86	0.58	10.42	0.60	11.71	0.60	11.40	0.62	
n-6/*n*-3 ratio	6.47	0.40	2.94	0.41	7.55	0.41	3.31	0.43	D

[a] For footnotes see **Table 1**.

Table 6. Fatty Acid Composition (Percent) of Lysophosphatidylethanolamine (LPE) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM[a]	SEM[a]	LSM	SEM	LSM	SEM	LSM	SEM	significance[b] ($P < 0.05$)
C12:0	1.51	0.17	0.84	0.17	1.11	0.18	2.00	0.18	B, D*B
C14:0	0.87	0.18	1.65	0.18	1.24	0.18	0.85	0.18	D*B
C16:0	7.08	0.51	4.88	0.51	6.03	0.53	7.59	0.53	D*B
C18:0	27.36	1.48	30.72	1.48	22.79	1.523	19.75	1.53	B, D*B
C18:1 *cis*-9	21.07	0.54	18.18	0.54	22.26	0.56	18.40	0.56	D
C18:1 *cis*-11	2.70	0.29	3.22	0.29	2.22	0.30	1.75	0.30	B
C18:2 *n*-6	11.90	0.75	12.09	0.75	17.83	0.78	15.17	0.78	B
C18:3 *n*-3	0.68	0.17	2.86	0.18	0.93	0.18	4.12	0.18	D, B, D*B
C20:4 *n*-6	7.70	0.33	5.28	0.33	8.53	0.34	7.60	0.34	D, B, D*B
C20:5 *n*-3	0.67	0.22	2.34	0.21	0.66	0.22	3.69	0.22	D, B, D*B
C22:5 *n*-3	3.10	0.26	6.35	0.26	2.74	0.26	6.12	0.27	D
C22:6 *n*-3	0.90	0.08	1.20	0.08	0.69	0.09	0.84	0.09	D, B
CLA *cis*-9,*trans*-11[c]	0.15	0.02	0.28	0.02	0.22	0.02	0.46	0.02	D, B, D*B
sum C18:1 *trans*[d]	2.08	0.09	1.67	0.09	1.26	0.09	1.62	0.09	B, D*B
sum SFA[e]	39.76	0.98	39.89	0.98	33.00	1.01	33.32	1.01	B
sum UFA[f]	60.24	0.98	60.11	0.98	67.01	1.01	66.68	1.01	B
sum PUFA[g]	32.06	1.20	34.61	1.20	38.32	1.24	41.36	1.24	D, B
sum *n*-3 FA[h]	5.73	0.46	12.86	0.46	5.33	0.48	15.31	0.48	D, B, D*B
sum *n*-6 FA[i]	22.79	1.08	19.55	1.08	28.49	1.12	23.12	1.12	D, B
n-6/*n*-3 ratio	4.04	0.23	1.55	0.23	5.55	0.24	1.57	0.23	D, B, D*B

[a] For footnotes see **Table 1**.

increase of 2.9-fold in all PL classes of pasture-fed bulls, with highest accumulation rates up to a factor of 4.3 in the CL fraction of pasture-fed German Simmental bulls (**Table 4**). Also, all single investigated *n*-3 fatty acids were significantly increased in all seven PL classes by pasture-based diet as compared to concentrate diet, except docosahexaenoic acid (C22:6 *n*-3) in the CL, SM, and LPC fractions. Linolenic acid (C18:3 *n*-3) and eicosapentaenoic acid (C20:5 *n*-3) showed a higher accumulation rate in PL of muscle lipids in pasture-fed bulls up to a value of 5.3 compared with longer chain *n*-3 fatty acids docosapentaenoic acid C22:5 *n*-3 and C22:6 *n*-3 accumulated in PL muscle lipids up to a factor of 2.9 (**Tables 1**−**7**). In previous studies Larick and Turner (*14*) and Wiesner (*13*) reported similar results of increased *n*-3 fatty acid proportions in muscle PL classes of grass-based-fed Angus and Angus/Hereford steers and German Simmental bulls, respectively. Furthermore, the total *n*-6 fatty acid proportions in the individual PL classes differ widely from 10.4% in SM of pasture-fed German Holstein bulls to 69.7% in the CL fraction concentrate-fed German Simmental bulls. It is remarkable that in the CL fraction, linoleic acid (C18:2 *n*-6) occurred up to a proportion of 67.3% of total fatty acids (**Table 4**). As there are four different acyl residues in CL, the possibilities for complexity in the distribution within molecular species are enormous. However, the fatty acid compositions can be remarkably simple in animal tissues, very different from those of other PLs, and seem to be resistant to dietary changes (*17*).

Table 7. Fatty Acid Composition (Percent) of Lysophosphatidylcholine (LPC) in Longissimus Muscle of German Holstein and German Simmental Bulls

	German Holstein				German Simmental				
	concentrate ($n = 17$)		pasture ($n = 16$)		concentrate ($n = 16$)		pasture ($n = 15$)		
	LSM[a]	SEM[a]	LSM	SEM	LSM	SEM	LSM	SEM	significance[b] ($P < 0.05$)
C12:0	1.03	0.11	0.83	0.10	0.88	0.10	0.95	0.10	
C14:0	3.84	0.51	5.27	0.46	2.78	0.47	2.37	0.49	B
C16:0	21.82	0.74	19.64	0.67	17.92	0.69	22.17	0.72	D*B
C18:0	11.83	0.42	11.14	0.38	10.63	0.39	11.09	0.41	
C18:1 *cis*-9	26.44	0.93	27.30	0.84	32.92	0.87	28.22	0.89	D, B, D*B
C18:1 *cis*-11	1.76	0.09	1.79	0.08	2.00	0.09	1.52	0.09	D, D*B
C18:2 *n*-6	10.98	0.81	11.51	0.73	14.95	0.75	11.21	0.78	D, B, D*B
C18:3 *n*-3	0.94	0.17	3.00	0.15	1.08	0.16	3.16	0.16	D
C20:4 *n*-6	3.76	0.28	2.65	0.25	3.55	0.26	2.32	0.27	D
C20:5 *n*-3	0.23	0.08	1.16	0.07	0.24	0.07	1.09	0.08	D
C22:5 *n*-3	0.60	0.09	1.38	0.08	0.69	0.08	1.27	0.08	D
C22:6 *n*-3	0.27	0.08	0.26	0.07	0.13	0.07	0.42	0.08	
CLA *cis*-9,*trans*-11[c]	1.06	0.15	0.44	0.14	0.57	0.14	0.53	0.15	D
sum C18:1 *trans*[d]	3.87	0.33	3.03	0.29	2.21	0.30	2.02	0.31	B
sum SFA[e]	43.38	1.44	41.03	1.30	35.52	1.34	42.15	1.39	B, D*B
sum UFA[f]	56.62	1.44	58.97	1.30	64.48	1.34	57.85	1.39	B, D*B
sum PUFA[g]	20.05	1.23	22.92	1.11	23.93	1.14	22.08	1.19	D*B
sum *n*-3 FA[h]	2.85	0.36	6.00	0.32	2.34	0.33	6.42	0.34	D
sum *n*-6 FA[i]	16.00	0.99	15.47	0.90	19.80	0.93	14.95	0.96	D, D*B
n-6/*n*-3 ratio	6.56	0.46	2.63	0.41	8.83	0.43	2.40	0.44	D, B, D*B

[a] For footnotes see **Table 1**.

It is typical in animal tissues that CL contains almost exclusively C18 fatty acids and roughly 80% of C18 fatty acids is C18:2 *n*-6 (*17*).

In our experiment, diet effected the *n*-6 fatty acid composition of the CL fraction. Pasture feeding significantly decreased the C18:2 *n*-6 proportion from 62.9 and 67.3% to from 61.7 and 59.9% in the CL fraction of muscle lipids of German Holstein and German Simmental bulls, respectively, compared with concentrate-fed bulls (**Table 4**). In animal tissues, CL is believed to be an important cofactor for cholesterol translocation from the outer to the inner mitochondrial membrane and appears to have a functional role in the regulation of gene expression (*17*, *34*). Until now, little has been known about the relationships between dietary changes and the fatty acid composition of the CL and biological function in the animal tissues. Furthermore, the proportion of the longer chain arachidonic acid (C20:4 *n*-6) was significantly decreased by pasture feeding in the CL fraction of muscle lipids ranging between 0.77 and 1.6% (**Table 4**). In animal tissues, PE and PI are the primary sources of C20:4 *n*-6 required for the biosynthesis of eicosanoids, so high proportions of C20:4 *n*-6 are characteristic in the fatty acid composition of the PE and PI fraction (*18*). The C20:4 *n*-6 proportions in the PE and PI fraction were affected by the diet and ranged between 17.2 and 24.2% and between 8.9−12.9%, respectively (**Tables 1** and **3**). Pasture compared to concentrate feeding significantly decreased the C20:4 *n*-6 proportions in the PE and PI fractions of muscle lipids. In contrast to the *n*-3 fatty acids, pasture-feeding resulted in a significant decrease in the proportion of total *n*-6 fatty acids in PL classes, except in SM (**Table 5**). This contributed to very favorable *n*-6/*n*-3 ratios in PL fractions pasture-fed bulls. For example, the *n*-6/*n*-3 ratios in the PE fraction were 5.4 and 7.7 and 1.7 and 1.8 for concentrate- and pasture-fed bulls, respectively (**Table 1**). The *n*-6/*n*-3 ratios in the CL fraction were 23.0 and 25.2 and 5.8 and 5.2 for concentrate- and pasture-fed bulls, respectively (**Table 4**).

The results on the effect of diet on proportions of C18:1 *trans* fatty acids and CLA *cis*-9,*trans*-11 in the PL classes of muscle lipids are based on analysis by GC only. The distribution pattern and concentrations of individual C18:1 *trans* and CLA isomers in total muscle lipids of pasture- and concentrate-fed German Holstein and German Simmental bulls using Ag^+-TLC/GC and Ag^+-HPLC were recently reported by Dannenberger et al. (*7*, *27*). In the main PL classes PE and PC and in the PI fraction pasture relative to concentrate feeding resulted in significantly higher proportions of C18:1 *trans* fatty acids (sum of the isomers C18:1 *trans*-6−*trans*-11) (**Tables 1**−**3**). The highest C18:1 *trans* fatty acid proportions were found in the PI fraction, ranging between 1.8 and 2.2% and between 2.1 and 2.8% in concentrate- and pasture-fed bulls, respectively. No diet effect on C18:1 *trans* fatty acid proportions was detected in the SM and LPC fraction (**Tables 5** and **7**). An interaction of diet × breed with lower C18:1 *trans* fatty acid proportions in the PL classes for pasture-fed German Holstein bulls and higher proportions for pasture-fed German Simmental bulls compared with those on concentrate-fed was identified for the CL and LPE fractions. Furthermore, pasture feeding affected the CLA *cis*-9,*trans*-11 proportions (sum of CLA *cis*-9,*trans*-11, CLA *trans*-7,*cis*-9, and CLA *trans*-8,*cis*-10, because of coelution on GC) in PL classes of muscle lipids of bulls. Pasture feeding resulted in significantly higher CLA *cis*-9,*trans*-11 proportions in the PE, PC, PI, and LPE fractions compared with concentrate feeding (**Tables 1**−**3** and **6**). In contrast, pasture feeding decreased the CLA *cis*-9,*trans*-11 proportions in the LPC fraction. No diet effect for CLA *cis*-9,*trans*-11 was detected in the CL and SM fractions. The highest CLA *cis*-9,*trans*-11 proportions were found in the LPC fraction and ranged between 0.6 and 1.1% and between 0.4 and 0.5% in concentrate- and pasture-fed German Holstein and German Simmental bulls, respectively. The results agreed with the diet effects on C18:1 *trans* fatty acids and CLA *cis*-9,*trans*-11 in total PL of beef muscle reported by Dannenberger et al. (*7*). However, investigations into diet effects of C18:1 *trans* fatty acids and CLA *cis*-9,*trans*-11 in PL classes of beef muscle lipids have not been previously reported in the literature.

The total SFA proportions in the individual PL classes of muscle lipids of bulls fed both diets were different and ranged from 4.5% in the CL fraction to 50.5% in the SM fraction

(**Tables 1**−**7**). The diet effects on the SFA composition in the individual PL classes differ widely. Pasture compared to concentrate feeding significantly decreased the total SFA proportions in the PE, I,I and CL fractions of muscle lipids of both breeds. Also, Dannenberger et al. (*7*) found that the total SFA proportions in the total PLs of German Holstein and German Simmental bulls were significantly lower in the animals fed on pasture relative to concentrate groups. On the contrary, the total SFA proportions were increased in SM fraction of muscle lipids of pasture- compared to concentrate-fed bulls (**Table 5**). No diet effect was detected for the sum of SFA proportions in the PC, LPE, and LPC fractions. The diet effect on the composition of the individual SFA was inconsistent within the different PL classes. Typically, the fatty acids of the SM fraction in animal tissue are very long chain SFA components found in bovine brain and bovine milk (*19*, *35*). The proportions of palmitic and stearic acids in the SM fraction of the muscle lipids of bulls ranged between 20.1 and 22.2% and between 10.3 and 12.3%, respectively, with no diet effect (**Table 5**). Also, higher proportions of C23:0 up to 4.4% were detected in these PL fractions. These results are consistent with the composition of SFA in bovine milk and bovine brain (*35*). In bovine heart and liver, the characteristic feature of the PI fraction is the high content of stearic acid up to 40% of total fatty acids, and the stearic acid is linked to the *sn*-1 position of the glycerol backbone (*18*, *36*). This is in accordance with our results. The C18:0 proportions in the PI fraction of muscle lipids varied between 43.0 and 45.7% of the total fatty acids, with no diet effect (**Table 3**). Furthermore, stearic acid was significantly increased in the PC fraction by pasture feeding and significantly decreased in the PE and CL fractions compared with concentrate feeding (**Tables 1**, **2**, and **4**). No significant diet effect was detected in the PI, LPE, and LPC fractions (**Tables 3**, **6**, and **7**). Also, Larick and Turner (*14*) found higher C18:0 proportions in the PC/LPE fraction (coelution) of muscle lipids of Angus and Angus/Hereford steers finished on a grain-or-grass system, whereas Wiesner (*13*) detected no diet effect on C18:0 proportions in PC, PE, and CL fractions of German Simmental bulls. The proportions of palmitic acid (C16:0) showed no diet effect in all PL classes, except in CL (**Tables 1**−**7**). The highest C16:0 proportions were detected in the PC fraction of muscle lipids, varying between 24.4 and 24.7% in concentrate- and pasture-fed bulls (**Table 2**).

In conclusion, the results have shown that PL class profiles and the fatty acid composition in the individual PL classes can be affected by different feeding systems. Pasture- relative to concentrate-based feeding resulted in an accumulation of total and individual *n*-3 fatty acids in all PL classes of muscle lipids of German Holstein and German Simmental bulls. In contrast, pasture feeding significantly decreased the proportion of total and individual *n*-6 fatty acids in PL classes, except in the SM fraction. Because of the higher PUFA proportions in PL classes of pasture-fed bulls, further investigations on enhancing the contents of beneficial fatty acids in the beef have to include the occurrence of individual antioxidants and determinations into the antioxidative capacity. Additionally, further information is needed on the effects of dietary changes in the fatty acid composition of the individual PL class on their different biological functions in the muscle cells.

ABBREVIATIONS USED

HP-TLC, high-performance thin-layer chromatography; HPLC, high-performance liquid chromatography; GC, gas chromatography; FID, flame ionization detector; TAG, triacylglycerol; PL, phospholipid; FA, fatty acids; FAME, fatty acid methyl ester; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid; CLA, conjugated linoleic acid; D, diet; B, breed; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; CL, cardiolipin; SM, sphingomyolin; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine.

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