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Analysis of Fatty Aldehyde Composition, Including 12-Methyltridecanal, in Plasmalogens from *Longissimus* Muscle of Concentrate- and Pasture-Fed Bulls

DIRK DANNENBERGER,† STEPHANIE LORENZ,† GERD NUERNBERG,‡ NIGEL SCOLLAN,§ KLAUS ENDER,† AND KARIN NUERNBERG*,†

Departments of Muscle Biology and Growth and Genetics and Biometry, Research Institute for Biology of Farm Animals, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany, and Institute of Grassland and Environmental Research, Aberystwyth, SY23 3EB, United Kingdom

In a large study, 64 German Holstein and German Simmental bulls were randomly allocated to either an indoor concentrate system or periods of pasture feeding followed by a finishing period on a concentrate containing linseed to enhance the contents of beneficial fatty acids in beef. This paper reports the diet effects on the concentration of 12-methyltridecanal (12-MT) and further fatty aldehydes released from plasmalogens in the phospholipids of *longissimus* muscle of the bulls. Because of the trace level of the important odorant 12-MT in beef, the determination of fatty aldehydes in phospholipids was done by acidic hydrolysis and the reaction of the aldehydes with 2,4-DNPH followed by high-pressure liquid chromatography (HPLC) analysis. The diet affected the 12-MT concentrations in the muscle phospholipids of both breeds. Pasture feeding significantly increased the 12-MT concentrations up to 350 μg/100 g fresh muscle in the muscle phospholipids of German Holstein and German Simmental bulls as compared with the concentrate-fed bulls. Furthermore, pasture feeding resulted in a significant increase of *n*-octadecanal in the muscle phospholipids of both breeds up to 39.5 mg/100 g fresh muscle. The concentration of *n*-hexadecanal was not affected by the diet. Pasture feeding as compared to concentrate feeding significantly decreased the concentration of *n*-octadec-9-enal in the muscle phospholipids. Summarizing, pasture feeding increased the 12-MT concentration, which can be associated with meat of more intensive aroma and better taste.

KEYWORDS: Fatty aldehydes; plasmalogens; phospholipids; 12-methyltridecanal; beef; pasture; HPLC; dinitrophenylhydrazones

INTRODUCTION

Plasmalogens are a special group of glycerophospholipids in which the *sn*-1 position of the glycerol backbone is linked with a long chain fatty aldehyde via a vinyl−ether bond. In the plasmalogen molecule, the *sn*-1 position is occupied by a saturated fatty aldehyde, *n*-hexadecanal or *n*-octadecanal, or a monounsaturated fatty aldehyde, octadecenal. A high proportion of plasmalogens contains esterified polyunsaturated fatty acids (PUFA) in the *sn*-2 position including oleic acid, linoleic acid, arachidonic acid, or docosahexaenoic acid (*1*−*3*). Plasmalogens containing a vinyl−ether bond (a double cis bond adjacent to an ether bond) show a high sensitivity to acids and to reactive oxygen substances (ROS), and several studies suggest that plasmalogens could act as antioxidant molecules (*3*−*6*). The vinyl−ether bond of plasmalogens could be among the first targets of free radicals attack (*4*, *5*). Therefore, because of their location in the cells membranes, plasmalogens represent a first shield against oxidative damage by protecting other macromolecules. However, the biological functions of plasmalogens are still not fully resolved, and the antioxidant properties are sometimes contradistinctively discussed (*7*). The decomposition of plasmalogens due to oxidative stress leads to the release of fatty aldehydes. The oxidation products are nonreactive stable compounds and, therefore, do not continue the free radical chain reaction (*3*−*5*). However, Stadelmann-Ingrand et al. (*6*) showed that the fatty aldehydes released from plasmalogens generated deleterious effects on cells due to the formation of covalent adducts with endogenous macromolecules.

Over the last 20 years, an increased interest has arisen with respect to the occurrence and properties of plasmalogens. This attention in plasmalogens has been inspired by their implication in several human diseases, like Alzheimer's disease or Down's syndrome, and the discovery of genetic disorders in which ether-phospholipids are deficient (*3*−*5*). Recently, Brites et al. (*5*)

* To whom correspondence should be addressed. Tel: +49(38208)68857. Fax: +49(38208)68852. E-mail: knuernbg@fbn-dummerstorf.de.
† Department of Muscle Biology and Growth, Research Institute for Biology of Farm Animals.
‡ Department of Genetic and Biometry, Research Institute for Biology of Farm Animals.
§ Institute of Grassland and Environmental Research.

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reviewed the current state of knowledge regarding the enzymatic synthesis of plasmalogens and their biological role.

Studies on plasmalogens in meat, the fatty aldehyde distribution of phospholipids, and factors influencing their occurrence and concentrations have been little described in the literature (*11*, *12*). Meat phospholipids consist largely of diacyl phosphatides (phosphatidyl choline and phosphatidyl ethanolamine) and their corresponding alk-1-enyl acyl phosphatides (cholin plasmalogen and ethanolamin plasmalogen). Guth and Grosch (*13*) first described the key component of the beef flavor, the 12-methyltridecanal (12-MT). 12-MT is released from plasmalogens of beef muscle and has been detected in muscle and stewed beef juice (*13*−*15*). 12-MT smells tallowy, beef-like, and is one of a group of substances that determines the characteristic odor of stewed beef. Because of the trace level of 12-MT in the lipids of beef [50−150 μg/g lipid (*15*)], the quantification of this compound and other aroma compounds has been done by means of stable isotope dilution assay (*13*, *16*, *17*). Further fatty aldehydes released from plasmalogens found in meat are *n*-hexadecanal, *n*-octadecanal, and *n*-octadec-9-enal (*18*). The determination of the dimethyl acetales (DMA) can be easily performed by gas chromatography (GC) with flame ionization detection (*8*, *19*). The analyses in our study were done by acidic hydrolysis and the reaction of the aldehydes with 2,4-dinitrophenylhydrazine followed by high-pressure liquid chromatography (HPLC) analysis (*12*, *20*).

Until now, the roles of plasmalogens in beef affected by different feeding systems have been only scarcely described in the literature (*12*). In a large study, 64 German Holstein and German Simmental bulls were randomly allocated to either an indoor concentrate system or periods of pasture feeding followed by a finishing period on a concentrate containing linseed to enhance the contents of beneficial fatty acids in beef. The results on aspects of meat quality and fatty acid composition in different lipid fractions (total lipids, triglycerides, and phospholipids) have been recently reported by Nuernberg et al. (*21*) and Dannenberger et al. (*22*). This paper reports the diet effects on the concentration of 12-MT, including further fatty aldehydes released from plasmalogens in the phospholipids of *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 ($n = 16$) and German Holstein ($n = 17$), were randomly assigned to two dietary treatments, concentrate or pasture. German Simmental ($n = 16$) and German Holstein ($n = 17$) bulls fed on concentrate were kept in a stable and fed semi-ad libitum 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). The remaining bulls, German Simmental ($n = 15$) and German Holstein ($n = 16$), were kept on pasture for two summer periods (approximately 190 days) followed by indoor periods 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 minerals and vitamins, and a special concentrate diet containing 12% barley, 10% coarsely cracked linseed, minerals, and a mixture minerals and vitamins.

All bulls were slaughtered at 620 kg live weight in the research abattoir of the Research Institute for the Biology of Farm Animals, and the carcasses were chilled (4 °C). The age of animals at slaughter varied between 17 and 22 months. Samples of the *longissimus* muscle were taken at the 6th rib of the left carcass side 24 h postslaughter and stored frozen at −70 °C until lipid extraction and further sample preparation.

Methods. *Synthesis of Long-Chain Aldehydes.* The synthesis of the long-chain aldehydes (*n*-hexadecanal, *n*-octadecanal, and *n*-octadec-9-enal) was performed by selective oxidation of the corresponding alcohols (*n*-hexadecanol, *n*-octadenanol, and *n*-octadec-9-enol) with sodium hypochloride and 2,2,6,6-tetramethylpiperidin-1-oxyl as the catalyst. Ten millimoles of corresponding alcohols (2.42 g of *n*-hexadecanol, 2.71 g of *n*-octadenanol, and 2.69 g of *n*-octadec-9-enol) was treated with 1 mmol (15.5 mg) of 2,2,6,6-tetramethylpiperidin-1-oxyl, 25 mL of dichloromethane, and 1 mol of potassium bromide solution. The mixture was cooled to 0 °C. Under stirring, 11 mL of 1 mol of sodium hypo chloride solution (ph 9.5) was added and stirred for 1 h. The organic phase was separated, dried over magnesium sulfate, and evaporated. Anelli et al. (*23*) described the method in more detail.

2,4-Dinitrophenylhydrazine Solution (2,4-DNPH). 2,4-Dinitrophenylhydrazine was dissolved in 1 M hydrochloric acid and stirred at 50 °C for 1 h. The solution was shaked out twice with *n*-hexane and can be stored under nitrogen atmosphere for approximately 2 months (*24*).

Derivatization with 2,4-DNPH. The derivatization of the aldehydes was performed according to Lorenz (*12*) and Esterbauer et al. (*20*) with 2,4-dinitrophenylhydrazine in an acidic environment. The corresponding aldehydes (*n*-decanal, *n*-dodecanal, *n*-tetradecanal, *n*-hexadecanal, *n*-octadecanal, *n*-octadec-9-enal, and 12-MT) were dissolved in ethanol, and 20 mL of 2,4-DNPH solutions was added. The precipitation (hydrazones) was filtered and washed two times with sodium hydrogen carbonate solution (5%) and water. The recystallization was performed with ethanol (95%).

Extraction of Muscle Lipids and Separation of Lipid Classes. The intramuscular fat of 2 g of muscle was extracted with chloroform/methanol (2:1, v/v) according to Folch et al. (*25*) by homogenization (Ultra Turrax, 3 × 15 s, 12000 revolutions per minute) at room temperature. The details of muscle lipid extraction were described previously (*19*, *26*). To obtain the lipid classes, the isolated lipids were separated by thin-layer chromatography (TLC) on precoated silica gel 60 plates (Merck, Darmstadt, Germany). Lorenz et al. (*19*) described the method of separation of the lipid classes in more detail.

Derivatization of Phospholipids with 2,4-DNPH. The derivatization of the phospholipids was completed with 2,4-dinitrophenylhydrazine in an acidic environment as described by Lorenz (*12*) and Esterbauer et al. (*20*). Ten milligrams of phospholipid was dissolved in 250 μL of methanol, and approximately 400 μL of 2,4-DNPH solution was added. The derivatization was performed for 12 h at room temperature overnight. The hydrazones formed were extracted two times with chloroform. After both extracts were combined, these were evaporated with a gentle N_2 stream. The samples were redissolved in 50 μL of methanol for purification by TLC.

TLC, Purification of the Hydrazones). The removal of impurities (e.g., spare 2,4-DNPH) was completed by TLC described by Lorenz (*12*) and Esterbauer et al. (*20*). For purification, the hydrazone extracts were applied to the TLC plate in a narrow band. The TLC plates were developed in chloroform for 5 min, dried, and then developed for 45 min in toluene. The hydrazon band was scraped off and eluted with 3 mL of chloroform.

HPLC Analysis. The separation of aldehyde hydrazones was performed using a HPLC system (Shimadzu, LC10a, Japan) equipped with a 50 μL injection loop, UV detection (SPD-10AV) operated at 365 nm, and an operating system (Shimadzu CLASS-VP, version 6.12 sp4). A Supelcosil LC-18 column (2.1 mm i.d. × 250 mm stainless steel, 5 μm particle size, Sigma-Supelco, Taufkirchen, Germany) combined with a Supelcoguard LC-18 precolumn (2.1 mm × 20 mm, 5 μm particle size, Sigma-Supelco) was used. Two mobile phase systems were applied. System I: 0−10 min acetonitrile/water (95:5, v:v), 10−35 min acetonitrile (100%), and 35−80 min acetonitril/water (95:5, v:v) and pumped at a flow rate of 0.3 mL/min. System II: 0−60 min acetonitrile (100%) and pumped at a flow rate of 0.15 mL/min. The injection volume ranged between 20 and 40 μL. The external calibration of the hydrazones of *n*-decanal, *n*-dodecanal, *n*-tetradecanal, *n*-hexadecanal, *n*-octadecanal, *n*-octadec-9-enal, and 12-methyltridecanal was used for quantification (*12*).

GC Analysis. The fatty acid concentrations of phospholipids were determined by capillary GC using a CP SIL 88 CB column (100 m ×

Table 1. Concentrations of Fatty Aldehydes (mg/100 g Fresh Muscle) in the Phopholipids of *Longissimus* Muscle of German Holstein Bulls and German Simmental Bulls (LSM and SEM)[a]

	German Holstein				German Simmental				
	concentrate		pasture		concentrate		pasture		
	$n = 17$		$n = 16$		$n = 16$		$n = 15$		significance ($P < 0.05$)
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	
N-decanal	ND		ND		ND		ND		
N-dodecanal	ND		ND		ND		ND		
12-methytridecanal	0.29	0.02	0.34	0.02	0.23	0.02	0.35	0.02	D
N-tetradecanal	0.45	0.04	0.42	0.04	0.32	0.04	0.48	0.04	D*B
N-hexadecanal	23.22	1.61	19.26	1.67	15.70	1.56	18.28	1.56	B, D*B
N-octadecanal	39.52	3.03	48.05	3.14	29.57	2.93	39.51	2.93	D, B
N-octadec-9-enal	14.36	0.77	9.37	0.80	10.76	0.74	8.12	0.74	D, B

[a] D, significant influence of diet; B, significant influence of breed; and D*B, significant interaction of D*B.

Table 2. Fatty Acid Concentration (mg/100 g Fresh Muscle) in the Phospholipids of *Longissimus* Muscle of German Holstein and German Simmental Bulls (LSM and SEM)[a]

	German Holstein				German Simmental				
	concentrate		pasture		concentrate		pasture		
	$n = 17$		$n = 16$		$n = 16$		$n = 15$		significance ($P < 0.05$)
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	
C14:0	2.15	0.31	1.82	0.29	2.07	0.30	1.27	0.31	
C16:0	85.53	11.52	97.90	11.16	108.76	11.16	72.59	11.52	B*D
C18:0	62.18	9.67	85.54	9.36	91.04	9.38	64.79	9.67	B*D
C18:1 *cis*-9	133.22	19.65	155.35	19.03	190.93	19.03	128.80	19.65	B*D
C18:2 *n*-6	100.86	17.58	138.28	17.02	169.86	17.02	103.28	17.58	B*D
C18:3 *n*-3	5.44	3.21	38.06	3.11	9.40	3.11	28.98	3.21	D, B*D
C20:4 *n*-6	77.28	7.87	47.76	7.62	74.32	7.62	34.66	7.87	D, B*D
C20:5 *n*-3	4.38	2.25	20.26	2.18	5.02	2.18	18.37	2.25	D
C22:6 *n*-3	2.46	0.45	4.01	0.43	2.12	0.43	2.81	0.45	D
CLA *cis*-9,*trans*-11[b]	1.15	0.28	2.54	0.28	2.09	0.28	2.64	0.29	D
sum C18:1 *trans*[c]	3.13	0.60	4.92	0.58	3.50	0.58	5.64	0.60	D
sum SFA[d]	158.46	23.03	202.96	22.30	216.93	22.30	150.46	23.03	B*D
sum UFA[e]	345.18	56.02	478.64	54.24	526.11	54.24	378.57	56.02	B*D
sum *n*-3 FA[f]	22.76	7.96	89.38	7.70	29.37	7.70	70.91	7.96	D
sum *n*-6 FA[g]	163.89	27.59	198.96	26.72	270.00	26.72	148.92	27.60	B*D
n-6/*n*-3 ratio	7.27	0.22	2.42	0.21	9.48	0.21	2.11	0.22	D, B, B*D

[a] D, significant influence of diet; B, significant influence of breed; and D*B, significant interaction of D*B. [b] Coelution (CLA-*trans*-7,*cis*-9 and CLA-*trans*-8,*cis*-10). [c] Sum of the isomers C18:1-*trans6-trans11*. [d] 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. [e] 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 + *cis*-9,*trans*-11-CLA + C18:3 *n*-6 + C20:2 *n*-6 + C20:3 *n*-3 + C22:2 *n*-6 + C24:1. [f] 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. [g] 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.

0.25 mm, Chrompack-Varian, United States) installed in an Perkin-Elmer gas chromatograph Autosys XL with an flame ionization detector (FID) and split injection (Perkin-Elmer Instruments, Shelton, United States). The initial oven temperature was 150 °C, held for 5 min, subsequently increased to 175 °C at a rate of 2 °C min^{-1}, held for 15 min, then to 200 °C at 7 °C min^{-1}, held for 20 min, then 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. Nonadecanoic acid methylester used as a internal standard was added to the phopholipids prior to saponification and methylation. The amounts were calculated using the internal standard method of Turbochrom workstation software. Fatty acid methyl esters (FAMEs) were identified by means of purified standards ("Sigma-FAME mixture", Sigma Aldrich, Deisenhofen, Germany).

Reagents. *n*-Decanal, *n*-dodecanal, and *n*-tetradecanal were purchased from Sigma-Aldrich. 2,4-Dinitrophenylhydrazine, sodium hypochloride, 2,2,6,6-tetramethylpiperidin-1-oxyl, and potassium bromide were obtained from Fluka (Buchs, Switzerland). 12-MT was from the German Research Centre for Food Chemistry (DFA, Garching, Germany). The TLC plates coated with 0.25 mm silica gel (20 cm × 20 cm) were obtained from Merck (Darmstadt, Germany). FAMEs were identified by means of purified standards (Sigma-FAME mixture, Sigma Aldrich). FAMEs of C18:1 *trans*-11, C18:1 *cis*-11, C22:5 *n*-3, C22:4 *n*-6, and C18:2 *cis*-9,*trans*-11 were purchased from Matreya (Pleasent Gap, United States). All solvents used were HPLC grade from Lab-Scan (Dublin, Ireland).

Statistical Analysis. All data were analyzed by the least squares method using the GLM procedures of the StatisticalAnalysis System (SAS Systems, Release 8.2, and SAS Institute Inc., Cary, NC). 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

The concentrations of fatty aldehydes (mg/100 g fresh muscle) and the fatty acid concentration (mg/100 g fresh muscle) in the phospholipids of *longissimus* muscle of German Holstein and German Simmental bulls are shown in **Tables 1** and **2.** 12-MT and *n*-tetradecanal were measured on an approximately 70-fold lower concentration level as compared to the other investigated aldehydes (**Table 1**).

The diet affected the 12-MT concentrations in the muscle phospholipids of both breeds. The concentration of 12-MT was

significantly increased from 0.23 to 0.35 mg/100 g fresh muscle and from 0.29 to 0.34 mg/100 g fresh muscle in pasture-fed German Holstein and German Simmental bulls as compared with concentrate-fed bulls, respectively (**Table 1**). The analysis of further fatty aldehydes released from plasmalogens revealed that *n*-octadecanal was the most abundant fatty aldehyde in both diet groups up to 48.0 mg/ 100 g fresh muscle, followed by *n*-hexadecanal up to 19.3 mg/ 100 g fresh muscle and *n*-octadec-9-enal up to 14.4 mg/100 g fresh muscle (**Table 1**). Pasture feeding resulted in a significant increase of *n*-octadecanal in the muscle phospholipids of German Simmental bulls and German Holstein bulls as compared with concentrate-fed from 29.6 to 39.5 mg/100 g fresh muscle and from 39.2 to 48.0 mg/ 100 g fresh muscle, respectively. The *n*-hexadecanal showed a significant influence of breed (**Table 1**). The concentration of *n*-hexadecanal in the phospholipids of *longissimus* muscle of concentrate- and pasture-fed German Holstein bulls was higher as compared with the *n*-hexadecanal concentration in the phospholipids of *longissimus* muscle of concentrate- and pasture-fed German Simmental bulls. Furthermore, the *n*-hexadecanal showed a significant interaction of diet and breed (**Table 1**). Pasture feeding increased the concentration of *n*-hexadecanal in the phospholipids of German Simmental bulls and decreased the *n*-hexadecanal concentration in the phospholipids of German Holstein bulls. The concentration of *n*-octadec-9-enal was significantly decreased by pasture as compared to concentrate feeding from 14.3 to 9.4 mg/100 g fresh muscle in the phospholipids of German Holstein bulls and from 10.8 to 8.1 mg/100 g fresh muscle in the phospholipids of German Simmental bulls, respectively. The concentration of *n*-tetradecanal in muscle phospholipids of both breeds showed significant interaction of diet and breed. The aldehydes, *n*-decanal and *n*-dodecanal, were below the detection limit in all samples investigated.

The phospholipid content of the *longissimus* muscle varied between 0.44 and 0.67 g/100 g muscle in animals of both breeds fed on pasture and concentrate (*22*). The sum of *n*-3 fatty acids was significantly increased from 22.8 to 89.4 mg/100 g fresh muscle and from 29.4 to 70.9 mg/ 100 g fresh muscle in concentrate- and pasture-fed German Simmental and German Holstein bulls, respectively (**Table 2**). The highest accumulation in the phospholipids of pasture fed bulls was detected for linolenic acid up to 38.1 mg/100 g fresh muscle. The sum of the *n*-6 fatty acids showed a significant interaction of diet and breed. Pasture feeding increased the sum *n*-6 fatty acid concentration in the phospholipids of German Holstein bulls and decreased the sum *n*-6 fatty acid concentration in the phospholipids of German Simmental bulls (**Table 2**). Consequently, the *n*-6/*n*-3 ratio was low in pasture-fed bulls. The *n*-6/*n*-3 ratio in the phospholipids of both breeds was 7.3 and 2.4 and 9.5 and 2.1, respectively. The sum saturated fatty acid (SFA) concentrations in the phospholipids of both breeds showed a significant interaction of diet and breed. Pasture feeding increased the sum SFA concentration in the phospholipids of German Holstein bulls and decreased the sum SFA concentration in the phospholipids of German Simmental bulls (**Table 2**). The detailed results of fatty acid composition in phospholipids and triglycerides of *longissimus* muscle in German Holstein bulls of our study were described recently (*22*).

DISCUSSION

The determination of the FAME and the DMA in muscle lipids of beef after reaction with borontrifluoride/methanol can be performed by GC with FID (*8*, *14*). However, the 12-MT could not be measured by gas chromatography with flame ionization detection, because of the trace level in beef muscle (*12*, *27*). The advantages of this HPLC method to determine the aldehydes of phospholipids in beef muscle are the sensitivity of UV detection and the stability of the dinitrophenylhydrazones. Furthermore, Wiesner (*27*) detected the occurrence of artifacts (1-alkenylmethyl ether) during reaction of lipids with borontrifluoride/methanol in the use of GC−mass spectroscopy. These artifacts make the GC identification of DMA in muscle lipids of beef more difficult. Two mobile phases HPLC systems were used in our study to optimize the resolution of all aldehydes investigated (**Figure 1**). The addition of 5% water to the acetonitrile resulted in a higher resolution of the hydrazones from *n*-decanal to *n*-tetradecanal. The hydrazones of the longer chain aldehydes (*n*-octadec-9-enal, *n*-hexadecanal, and *n*-octadecanal) were better separated by the use of pure acetonitrile (**Figure 1**). Because of that, system I was used to quantify *n*-decanal, *n*-dodecanal, 12-MT, and *n*-tetradecanal. System II was used to quantify *n*-octadec-9-enal, *n*-hexadecanal, and *n*-octadecanal.

Our results showed that diet affected the distribution of both fatty aldehydes and fatty acids. The effects of pasture feeding on fatty acid distribution in beef muscles have been well-documented (*26*, *28*, *29*). However, studies of diet effects on fatty aldehyde distribution released from plasmalogens in beef are very scarce (*12*, *27*). Recently, Lorenz (*12*) observed significant effects of pasture feeding on distribution of 12-MT, including further fatty aldehydes in the phospholipids of *longissimus* muscle of German Simmental bulls as compared with concentrate feeding. The distribution of plasmenyl-phospholipids (plasmalogens), plasmanyl-phospholipids, and diacyl-phospholipids in the *longissimus* muscle of pasture-fed animals showed that approximately 52% were plasmalogens and 47% were diacyl-phospholipids. The plasmanyl-phospholipids were detected up to 1.0% in the *longissimus* muscle of pasture-fed animals (*12*). The analysis of fatty aldehydes released from plasmalogens showed that *n*-octadecanal was the most abundant fatty aldehyde, followed by *n*-hexadecanal and *n*-octadec-9-enal in the muscle phopholipids of both feeding groups. The sum of these three fatty aldehydes represents approximately 90% of the total fatty aldehydes analyzed (*12*). Our results confirmed these observations. Fogerty et al. (*8*) found the same fatty aldehyde distribution in phospholipids of beef and lamb muscles. In contrast, in the phospholipids of pork muscle, the *n*-hexadecanal is the most abundant fatty aldehyde followed by *n*-octadecanal (*8*, *11*).

The main source of the branched aldehyde 12-MT in the phospholipids of beef muscle is the microorganisms occurring in the rumen (*15*). Only a small amount of it is resorbed by the animal and transported to the muscular tissues and is incorporated into the plasmalogens (*15*). However, 12-MT smells tallowy and beef-like and is one of the most important substances that determines the characteristic odor of beef (*14*, *15*). Our results showed that pasture feeding significantly increased the 12-MT concentrations up to 350 μg/100 g fresh muscle in the muscle phospholipids of both breeds as compared with concentrate-fed bulls (**Table 1**). Also, Lorenz (*12*) found an accumulation of 12-MT in the phospholipids of pasture-fed German Holstein steers and German Simmental bulls. One explanation of this enrichment of 12-MT by pasture feeding could be a higher activity of the microorganisms occurring in the rumen resulting in a formation of a higher amount of the branched aldehyde 12-MT, which is transported to *longissimus* muscle and incorporated into the plasmalogens to a significantly higher extent. Guth and Grosch (*14*) found similar levels of

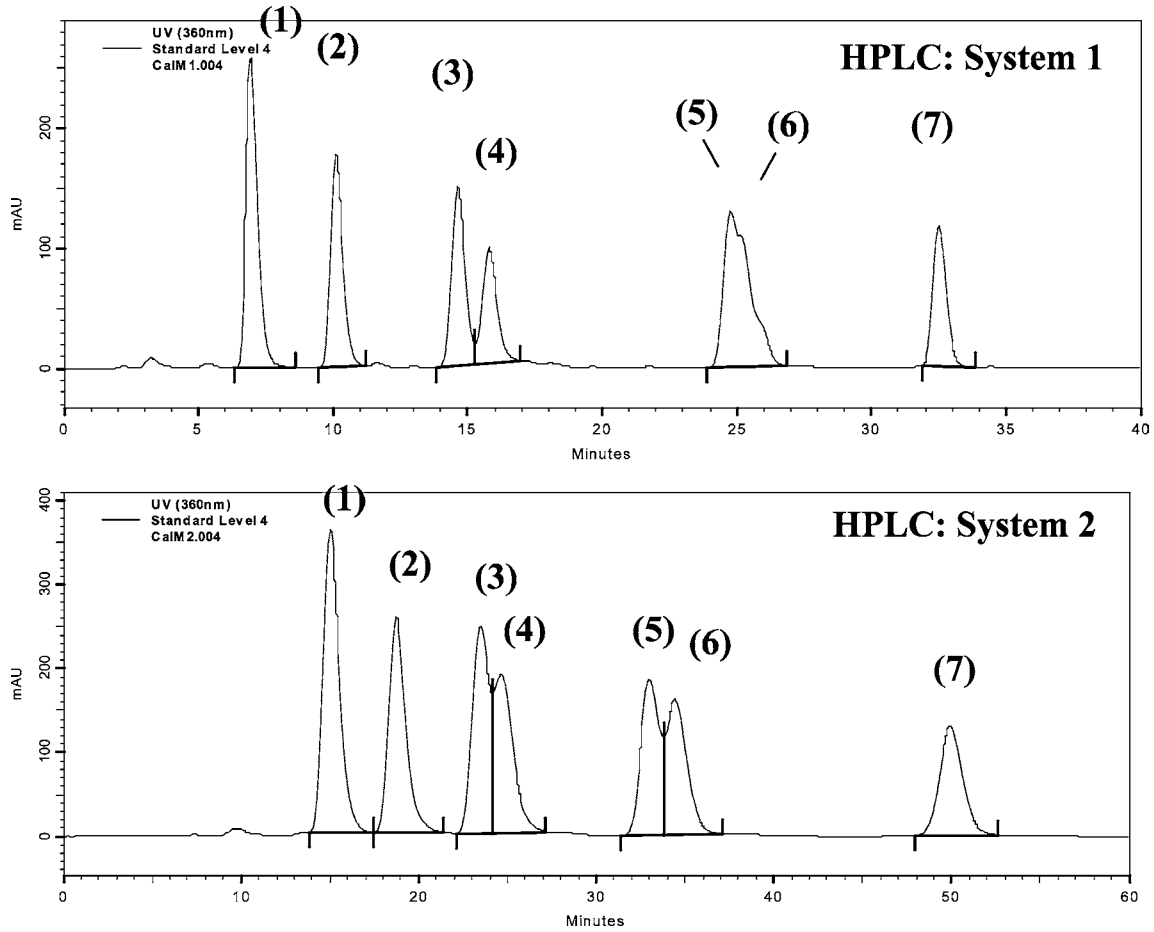

Figure 1. HPLC chromatograms of a standard solution of the aldehyde hydrazones (detected at 233 nm, UV) at two mobile phase systems (see Materials and Methods) Hydrazones of **1**, *n*-decanal; **2**, *n*-dodecanal; **3**, 12-MT; **4**, *n*-tetradecanal; **5**, *n*-octadec-9-enal; **6**, *n*-hexadecanal; and **7**, *n*-octadecanal.

12-MT concentrations up to 150 μg/g lipid in the beef muscle after treatment with hydrochloric acid using stable isotope dilution assay. The accumulation of the tallow smell 12-MT by feeding grass can result in beef meat with a more intensive flavor due to a higher proportion of vitamin E in grass improvements in color and shelf lives of beef (*12*, *14*, *21*).

In contrast, for the formation of further fatty aldehydes released from plasmalogens, long-chain fatty alcohol is required for plasmalogen biosynthesis as a substrate for the alkyl-dihydroxyacetone phophate (alkyl-DHAP). The second step of plasmalogen biosynthesis results in the formation of an ether bond by replacement of the sn-1 fatty acid with a long-chain fatty alcohol (*3*−*5*). The final step in the biosynthetic pathway involves the insertion of a double bond between C1 and C2 of the alkyl chain forming the "vinyl−ether" linkage in the plasmalogens (*3*−*5*). Nagan and Zoeller (*4*) discussed three possibilities of the origin of the fatty alcohols moiety utilized for plasmalogen biosynthesis. The first possibility is dietary. Long-chain fatty alcohols would come primarily in the form of wax esters found in certain vegetables (*4*, *30*). This could be one explanation for the partially increased fatty aldehyde concentrations measured in the muscle phospholipids of both breeds fed on pasture as compared with concentrate. Besides *n*-octadecanal, the *n*-hexadecanal and *n*-tetradecanal concentrations in the phospholipids of German Simmental bulls tended to be higher in the pasture-fed group also (**Table 2**).

The second possibility is that fatty alcohols can be formed from fatty acids by the reduction of fatty-acyl-CoA. This reaction is catalyzed by the long-chain fatty acyl CoA reductase, which uses NADPH as a cofactor (*3*, *4*). Snyder (*31*) postulated that the low activity of the long-chain fatty acyl CoA reductase accounts for this enzyme as the rate-limiting reaction for the biosynthesis of plasmalogens in animal tissue. In addition, this reductase is known to be specific for C16:0, C18:0, and C18:1 fatty acids. Therefore, the long-chain fatty acyl CoA reductase is believed to determine the distribution of vinyl−ether chains in tissues (*31*). In our study, the palmitic, stearic, and oleic acid concentrations in the muscle phospholipids showed not a uniform trend, despite the significantly higher levels of *n*-octadecanal in the phospholipids (**Table 2**). The concentrations of these fatty acids in German Holstein bulls tended to be higher, and the concentrations in the muscle phospholipids of German Simmental bulls tended to be lower in pasture-fed bulls as compared with concentrate (**Table 2**). The concentration of *n*-octadec-9-enal in the muscle phospholipids was significantly decreased in pasture-fed bulls of both breeds. However, the C18:1 *cis*-9 concentrations were not significantly affected by the diet and showed interactions of diet and breed (**Table 2**). Recently, Estevez-Garcia et al. (*11*) found a similar nonspecific linking of fatty aldehydes with the precursor fatty acid proportion between fatty and lean pigs. Hayashi and Hara (*32*) proposed a third possibility of the formation of long-chain fatty alcohols. The authors proposed that long-chain fatty alcohols, used for plasmalogen synthesis, are produced primarily within the peroxisomes during peroxisomal β-oxidation. Because of the literature results and from our study on the diet effect of the formation of plasmalogens, it seems to be that plasmalogen synthesis is under complex regulation in which different factors are included (*33*).

The presence of a vinyl−ether bond makes plasmalogens susceptible to oxidative attack as compared to their 1-acyl

analogues (*3*, *5*). This has prompted the hypothesis that plasmalogens may act as scavengers, protecting other phospholipids and lipids from oxidative reaction (*5*, *34*, *35*).

Our results showed that pasture feeding increased the total amount of aldehydes released plasmalogens in muscle lipids of German Holstein and German Simmental bulls. Taking into account that pasture feeding increased the total amounts of *n*-3 fatty acids in the phospholipids of muscles, then the plasmalogens, besides the higher vitamin E concentrations in pasture-fed beef, additionally may act as a protection shield to avoid a higher degree of peroxidation of PUFAs (*21*). Finally, it seems that meat from pasture-fed bulls can help to protect the PUFA in the lipids and color pigments and contribute to the production of high-quality and healthy meat. In conclusion, more investigations are required to control the mechanisms for the formation and function of plasmalogens in animal tissues.

ABBREVIATIONS USED

TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography; GC, gas chromatography; FID, flame ionization detector; ROS, reactive oxygen substances; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; DMA, dimethyl acetale; alkyl-DHAP, alkyl-dihydroxyacetone phophate; 2,4-DNPH, 2,4-dinitro-phenylhydrazine; LSM, least squares mean; SEM, standard error of LSM; FAME, fatty acid methyl ester; 12-MT, 12-methyltridecanal.

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