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ARTICLE

Effect of Dietary *n*-3 and *n*-6 PUFA on Lipid Composition of Different Tissues of German Holstein Bulls and the Fate of Bioactive Fatty Acids during Processing

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The present study investigated the effects of dietary linolenic acid (ALA) versus linoleic acid (LA) on meat quality, fatty acid composition, and stearoyl-CoA desaturase (SCD) activity in longissimus muscle (MLD) and subcutaneous adipose tissue (SAT) of German Holstein bulls and the transfer of beneficial *n*-3 fatty acids into German corned beef sausages (GCB). Feeding LA- and ALA-enriched diets increased essential fatty acids in MLD and SAT. The ALA-supplemented diet decreased significantly the SCD activity in MLD and SAT, resulting in a reduced relative concentration of oleic acid in muscle. The relative proportion of CLA*cis*-9,*trans*-11 analyzed by HPLC was not different between groups in either tissue. GCB were produced by using the lean meat of bulls. Beef products of bulls fed the ALA-supplemented diet were rich in ALA and *n*-3 LC PUFA. Most importantly, there was no loss of *n*-3 fatty acids during processing under production conditions. Conclusively, the *n*-6/*n*-3 fatty acid ratio was beneficially low.

KEYWORDS: Cattle; diet; fatty acid; lipogenic enzyme; beef products; CLA

INTRODUCTION

Meat and meat products are important sources of protein, vitamins, and fat for humans. Consumers are more and more interested in meat with desirable nutritional quality and animal welfare. Consequently, demands for higher intrinsic quality increased the production of meat products with lower nitrate and fat content or enhanced long-chain polyunsaturated fatty acids (LC PUFA) (*1*). Precursors for LC PUFA are linoleic (C18:2*n*-6, LA) and α-linolenic acid (C18:3*n*-3, ALA). Both are essential, because all mammals cannot synthesize these precursors (*2*). Conclusively, these fatty acids must be provided by the diet (*2*).

Recommendations for the intake of fatty acids are given by the German Society of Nutrition (DGE) for Germany, Austria, and Switzerland and in 2010 by the European Food Safety Authority (EFSA), because quantity and quality of dietary fat are strongly related to human health (*3*, *4*). It is important to reduce dietary saturated (C12:0, C14:0, C16:0) fatty acids because different studies have shown an increasing effect on blood cholesterol concentrations and an increased risk of coronary heart disease (*5*). Human, animal, and in vitro studies have shown positive effects of single *n*-3 fatty acids in bone metabolism (*6*), breast cancer (*7*), and brain development (*8*) as well as antiarrhythmic effects (*9*). An adequate and sustained nutrient input and especially *n*-3 fatty acids in meat and meat products is recommended.

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Factors such as diet, gender, genetics, and breed fatness of animals affect the fatty acid composition of muscle and adipose tissue (*10*−*12*). The dietary fatty acids of monogastric animals will be directly deposited in tissues, whereby the fatty acid composition can easily be influenced. The biohydrogenation in ruminant animals makes it difficult to alter the tissue fatty acid composition. However, many studies were successful in enhancing essential *n*-6 and *n*-3 fatty acids in muscle and adipose tissue. Good results were achieved by feeding grass or concentrate containing linseed to accumulate ALA and the following LC PUFA (*13*). An important enzyme that catalyzes the introduction of a double bond into saturated fatty acids on the position between carbons 9 and 10 is stearoyl-CoA desaturase (SCD; EC 1.14.19.1). This introduction results in a monounsaturated fatty acid. Next to saturated fatty acids, the *trans*-vaccenic acid is also a substrate of SCD. After insertion of a double bond, the main CLA isomer in ruminant tissues is originated, CLA*cis*-9, *trans*-11 (*14*, *15*). Another source of CLA*cis*-9,*trans*-11 is biohydrogenation via bacterials in the rumen (*16*). The regulation of SCD is under research, but it is well-known that *n*-6 and *n*-3 fatty acids in dietary fat are able to inhibit the gene expression and catalytic activity of SCD (*15*).

The first aim of this study was to investigate the effects of diets enriched in linoleic (maize silage/concentrate with soybean meal) and α-linolenic acid (grass silage/concentrate with linseed oil/rapeseed cake) on fatty acid composition and stearoyl-CoA desaturase activity in longissimus muscle and subcutaneous adipose tissue of German Holstein bulls. To investigate the

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Table 1. Experimental Design

	control group (n = 15)	exptl group (n = 14)
diet	maize silage, soybean-based concentrate	grass silage, concentrate supplemented with rapeseed cake (12%) and linseed oil (3%)
feeding	indoor, group keeping, bedding on straw	
feeding duration (LSM ± SEM)	241.9 ± 10.2 days	244.4 ± 10.6 days
live weight at slaughter (LSM ± SEM)	622.6 ± 6.3 kg	629.6 ± 6.5 kg
breed and gender	German Holstein bulls	

transfer and fate of beneficial *n*-3 fatty acids of fresh beef into a product under production condition, German corned beef sausages were produced by using lean meat of the bull carcasses from this experiment, and the lipid profile of the sausages was investigated.

MATERIALS AND METHODS

Animals and Diets. In total, 29 German Holstein bulls were assigned to two dietary treatments. The control group was fed maize silage and a soybean meal based concentrate, whereas the experimental group got grass silage and a concentrate supplemented with linseed oil and rapeseed cake. The content of C18:2*n*-6 in the control group diet was 1.4 times higher than in the experimental group but the concentration of C18:3*n*-3 in the experimental group diet was 4 times higher compared to control. The composition of the rations was already described in Herdmann et al. (*17*), but a brief overview of the experimental design is shown in **Table 1**.

Chemical Composition and Marbling. Extra slices of longissimus muscle were taken at the 9th/10th rib of carcasses after 24 h of carcass cooling to measure the chemical composition (protein, fat, and water) with FoodScan Lab (FOSS) (*18*) and meat quality parameters as pH value using a pH-Star meter (Matthäus, Klausa, Germany).

Marbling was measured 24 h after slaughter and was scored using a 6-point scale with 1 as the lowest and 6 as the highest marbling grade. Muscle area has been estimated using planimetry software Scan-Star (Matthäus).

German Corned Beef Sausages. German corned beef sausages were produced by Greifenfleisch GmbH (Greifswald, Germany). Corned beef sausages contain 58% beef from bulls of this animal experiment (lean meat from joint and bug), 5% beef rind, and drinking water, gelatin, pickling salt, spices, yeast extracts, celeriac, and corn, soy, and plant proteins. The lean meat was scalded until an internal temperature of 68 °C. Then the cooked meat was cooled, minced, and mixed with spices and ingredients. The mass was filled in cleaned guts and scalded. After a central temperature of 78 °C was reached, the sausage was left for another 30 min in the scalding chamber at 82 °C. From each carcass single sausages were produced, and in total 29 sausages were analyzed.

Fatty Acid Analysis. The samples of longissimus muscle and subcutaneous adipose tissue for the fatty acid analysis were collected at the 13th/14th rib of carcasses immediately after slaughter and were stored at −70 °C. The samples of sausages were taken after delivery by Greifenfleisch GmbH, Germany and were stored at −20 °C. Intramuscular fat (IMF) of longissimus muscle (2 g sample), fatty acids of subcutaneous adipose tissue (1 g), and sausages (2 g) were extracted with chloroform/methanol (2:1, v/v) according to the method of Folch et al. (*19*) by homogenization at room temperature. The fatty acid composition for all tissues mentioned before was determined by the methodology of the extraction, esterification, and GC conditions described by Nuernberg et al. (*20*) and Herdmann et al. (*17*).

Analysis of CLA Isomers by Ag^+ HPLC. The separation of CLA isomers was done by using Ag^+ HPLC described in detail by Dannenberger et al. (*21*). Four ChromSpher 5 Lipids silver-impregnated columns were used to identify CLA isomers on the basis of retention time from a CLA isomer standard containing the following components: C18:2*cis*-9,*trans*-11; C18:2*trans*-9,*trans*-11; C18:2*trans*-10,*cis*-12; C18:2-*cis*-9,*cis*-11; and C18:2*cis*-11,*trans*-13. The other isomers were identified on the basis of these retention times in well-known elution order. In tissue and products of German Holstein bulls 13 isomers were identified.

Microsome Extraction and Stearoyl-CoA Desaturase (SCD) Assay. Microsomal fractions were isolated from subcutaneous adipose tissue and longissimus muscle, taken immediately after slaughter, shock frozen in liquid nitrogen, and stored at −80 °C according to Lozeman et al. (*22*). Tissues were homogenized with the homogenizer Homo 4/R (Edmund Bühler, Germany) in a 3:1 dilution of 1 g of tissue in 3 mL of ice-cold homogenization buffer consisting of 250 mM sucrose (Carl Roth GmbH, Germany), 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma-Aldrich, Germany), 4 mM ethylenediaminetetraacetate (Sigma-Aldrich), and 1 mM dithiothreitol (pH 7.0) (Sigma-Aldrich) in an ice bath at 6500 U/min for 10 s then for 30 s at 24000 U/min and at least 30 s at 6500 U/min. The homogenate was centrifuged at 3944*g* and 4 °C for 10 min. Then the supernatant (muscle) or infranatant (adipose tissue) was filtered through filter circles (Carl Roth GmbH). The last centrifugation step was at 100000*g* and 4 °C for 2 h. The supernatant was discarded, and the pellet was resuspended in 0.1 volume (v/wet weight of tissue) of ice-cold phosphate buffer (pH 7.4). Microsomes were stored at −70 °C.

The measured conversion rate of labeled SFA into MUFA indicates the SCD activity. The method was done as described by Doran et al. (*23*) and Yang et al. (*24*). The final incubation medium of 1.0 mL contains 70 mM potassium phosphate buffer at pH 7.4 (64 mM for muscle), 6 mM $MgCl_2$ (Sigma-Aldrich), 7.3 mM ATP (Sigma-Aldrich), 0.8 mM β-NADH (Sigma-Aldrich), and 24 μM [1-^{14}C]palmitoyl-CoA (GE Healthcare, Germany) with a specific activity of 56 μCi/mM. The assay was initiated by the addition of 0.1 mg of microsomal protein for subcutaneous fat (4.0 mg of microsomal protein for longissimus muscle) and was carried out in a shaking water bath at 37 °C for 5 min before termination by the addition of 2 mL of ice-cold 10% KOH in methanol containing hydroquinone (Sigma-Aldrich) as an antioxidant. Before hydrolysis at 60 °C for 2 h carrier fatty acids (0.1 mg of C14:0 and C14:1) were added to assay. After acidification with 5 M H_2SO_4, fatty acids were extracted three times with petroleum ether and the extracts were combined.

After evaporation of petroleum ether, 0.5 mL of boron trifluoride in methanol (14%, wt/v) (Sigma-Aldrich) was added, and the mixture was shaken in a water bath at 60 °C for 10 min. Methyl esters were extracted twice with 2 mL of *n*-hexane. The upper phases containing FAME were pooled, and the solvent was eliminated by evaporation under nitrogen flow. FAMEs were resolved in 100 μL of *n*-hexane. To quantify the incorporation of labeled substrate in labeled product, fatty acids were separated by thin layer chromatography (TLC) using silver nitrate-impregnated PolyGram Sil-G plates (Macherey-Nagel, Germany). *N*-Hexane-/diethyl ether (9:1) was used to develop the plates, and the bands (saturated and monounsaturated fatty acids) were visualized under UV light with 0.1% dichlorofluorescein (Sigma-Aldrich). The bands were cut out, and the radioactivity was counted using an LSA Tri-Carb liquid scintillation counter (Perkin-Elmer). The ratio of the activity in MUFA to SFA + MUFA wasdetermined, and this ratio multiplied by the amount of substrate added to the incubation was used to calculate the desaturase enzyme activities. It is expressed as nM of palmitoleic acid formed per milligram of protein per hour.

Protein Assay. Protein was determined according to the Bradford method using bovine serum albumin as standard and commercial Bradford reagent (VWR, Germany).

Statistical Analysis. The data were analyzed by the least-squares method using the general linear model procedures (GLM) of SAS (*25*) with the fixed factor feeding. The following model was used for traits: $Y_i = \mu + D_i + E_i$, where Y_i represents an observation, μ is the overall mean, D_i is the effect of the diet (i = 1, 2), and E_i is the residual error. A P value of ≤0.05 was considered to be statistically significant.

RESULTS

Performance, Carcass Composition, Chemical Composition and Meat Quality. Feeding German Holstein bulls with grass silage and concentrate supplemented with rapeseed cake and linseed oil

did not affect animal performance and meat quality parameters such as muscle area and marbling compared to maize silage and concentrate with soybean meal. IMF is not significantly different between feeding groups, nor were protein and ash contents. The dry matter of longissimus muscle is lower in the experimental group (**Table 2**).

Table 2. Animal Performance and Meat Quality Parameters of German Holstein Bulls and Quality Parameters of German Corned Beef Sausages[a]

	control group $n = 15$		exptl group $n = 14$		
	LSM	SEM	LSM	SEM	*P* value
live weight at slaughter (kg)	622.6	6.3	629.6	6.5	0.45
average daily gain (g)	1230.0	28.2	1164.0	29.2	0.12
liver (kg)	8.0	0.2	7.6	0.2	0.17
heart (kg)	2.7	0.1	2.7	0.1	0.80
pH_{24} longissimus muscle	5.6	0.02	5.6	0.02	0.21
muscle area (cm^2)	90.7	4.7	94.1	5.1	0.63
marbling	1.8	0.2	1.7	0.2	0.64
composition of longissimus muscle					
dry matter (%)	26.3	0.3	25.5	0.3	**0.05**
IMF (%)	2.8	0.3	2.1	0.3	0.16
protein (%)	22.5	0.1	22.4	0.1	0.21
ash (%)	1.1	0.01	1.0	0.01	0.62
composition of GCB					
dry matter (%)	26.1	0.6	26.7	0.7	0.49
fat (%)	2.3	0.1	2.0	0.1	0.09
protein (%)	20.9	0.6	22.1	0.7	0.20
ash (%)	2.9	0.1	2.7	0.1	**0.01**

[a] LSM, least-squares means; SEM, standard error of the mean.

Fatty Acid and CLA Composition of Longissimus Muscle. The sum of total fatty acids (mg/100 g) in longissimus muscle is >25% lower in the experimental group than in the control group (**Table 3**). Grass silage feeding induced a significantly higher sum of PUFA in muscle. The relative proportions of C18:1*cis*-9, C22:4*n*-6, and MUFA, and the ratio of *n*-6/*n*-3 fatty acids (FA) are significantly higher in the control than in the experimental group. Beneficial *n*-3 fatty acids, such as C18:3*n*-3, C20:5*n*-3, C22:5*n*-3, and C22:6*n*-3, are significantly increased in the experimental group, whereas the relative amount of C18:3*n*-3 in longissimus muscle of the experimental group is >3.0 times and the sum of *n*-3 LC PUFA 2.0 times higher compared to the control group. The relative proportions of C18:2*n*-6 are similar between both groups, but the concentration is 1.2 times significantly higher in the maize silage fed control group compared to the experimental group (data not shown). The amount of C18:1*trans*-11 and consequently the sum of *trans* C18:1 isomers in the experimental group are significantly increased compared to the control group.

In **Figure 1** selected CLA isomers of two different tissues and of German corned beef sausages are presented. In longissimus muscle 13 CLA isomers were identified. The proportion of CLA*cis*-9,*trans*-11 is not affected by feeding, whereas CLA*trans*-10,*cis*-12 and the sum of CLA*trans*-8,*cis*-10 and CLA*trans*-7,*cis*-9 are significantly decreased in the experimental compared to the control group. Feeding grass silage and concentrate enriched with *n*-3 fatty acids leads to an increased proportion of CLA*trans*-11, *cis*-13 compared to maize silage and *n*-6-enriched concentrate fed bulls.

Fatty Acid and CLA Composition of Subcutaneous Adipose Tissue. Animals of the experimental group have shown a

Table 3. Relative Fatty Acid Composition (Percent) of Longissimus Muscle and Subcutaneous Adipose Tissue of German Holstein Bulls[i]

	longissimus muscle					subcutaneous adipose tissue				
	control group $n = 15$		exptl group $n = 14$			control group $n = 15$		exptl group $n = 14$		
	LSM	SEM	LSM	SEM	*P* value	LSM	SEM	LSM	SEM	*P* value
C14:0	2.6	0.1	2.4	0.1	0.20	4.1	0.2	3.8	0.2	0.24
C16:0	26.3	0.4	25.2	0.4	0.07	28.1	0.4	28.2	0.4	0.85
C16:1*cis*-9	3.7	0.2	3.3	0.2	0.07	6.9	0.4	7.3	0.4	0.38
C18:0	14.6	0.4	15.7	0.4	0.08	12.1	0.6	11.9	0.6	0.83
C18:1*cis*-9	37.1	0.6	34.7	0.6	**0.01**	37.8	0.7	37.4	0.8	0.70
C18:1*trans*-11	0.6	0.03	0.8	0.04	**0.0005**	0.8	0.04	0.9	0.04	**0.03**
C18:2*n*-6	5.3	0.4	5.6	0.4	0.64	1.8	0.1	1.4	0.1	**0.00**
C18:3*n*-3	0.6	0.1	2.0	0.1	**<0.0001**	0.4	0.04	0.7	0.04	**<0.0001**
C20:4*n*-6	1.4	0.1	1.6	0.1	0.50	0.1	0.01	0.1	0.01	0.85
C20:5*n*-3	0.2	0.03	0.5	0.03	**<0.0001**	0.004	0.002	0.02	0.003	**0.0005**
C22:4*n*-6	0.2	0.02	0.1	0.02	**0.003**	0.03	0.00	0.02	0.01	0.10
C22:5*n*-3	0.4	0.04	0.7	0.04	**<0.0001**	0.04	0.01	0.1	0.01	0.15
C22:6*n*-3	0.05	0.00	0.08	0.01	**<0.0001**	0.01	0.00	0.01	0.00	0.99
Σ SFA[a]	45.5	0.6	45.5	0.6	0.96	46.8	0.9	46.7	0.9	0.92
Σ MUFA[b]	45.1	0.7	42.5	0.7	**0.01**	49.8	0.9	49.9	0.9	0.96
Σ *n*-3 FA[c]	1.3	0.1	3.3	0.1	**<0.0001**	0.5	0.1	0.8	0.1	**<0.0001**
Σ *n*-6 FA[d]	7.5	0.6	7.7	0.6	0.73	2.0	0.1	1.6	0.1	**<0.0001**
ratio *n*-6/*n*-3 FA	5.8	0.1	2.3	0.1	**<0.0001**	4.6	0.3	2.1	0.3	**<0.0001**
Σ *n*-3 LC PUFA[e]	0.6	0.1	1.3	0.1	**<0.0001**	0.05	0.01	0.1	0.01	0.06
Σ *n*-6 LC PUFA[f]	1.7	0.1	1.7	0.1	0.75	0.1	0.009	0.1	0.01	0.40
Σ *trans* FA[g]	1.3	0.1	1.4	0.1	**0.04**	1.9	0.08	1.9	0.08	0.67
Σ total FA[h]	2366.9	182.2	1764.5	188.9	**0.03**	66762.6	2541.7	58418.9	2630.9	**0.03**

[a] Σ SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. [b] Σ MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1*trans*-9 + C18:1*trans*-10 + C18:1*trans*-11 + C18:1*cis*-9 + C18:1*cis*-11 + C22:1 + C24:1. [c] Σ *n*-3 FA = C20:3*n*-3 + C22:6*n*-3 + C22:5*n*-3 + C20:5*n*-3 + C18:4*n*-3 + C18:3*n*-3. [d] Σ *n*-6 FA = 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. [e] Σ *n*-3 LC FA = C20:3*n*-3 + C22:6*n*-3 + C22:5*n*-3 + C20:5*n*-3. [f] Σ *n*-6 LC FA = C22:2*n*-6 + C20:2*n*-6 + C22:4*n*-6 + C20:3*n*-6 + C20:4*n*-6. [g] Σ *trans* FA = C18:1*trans*-6 + C18:1*trans*-7 + C18:1*trans*-8 + C18:1*trans*-9 + C18:1*trans*-10 + C18:1*trans*-11. [h] Σ FA = sum of all identified fatty acids. [i] LSM, least-squares means; SEM, standard error of the mean.

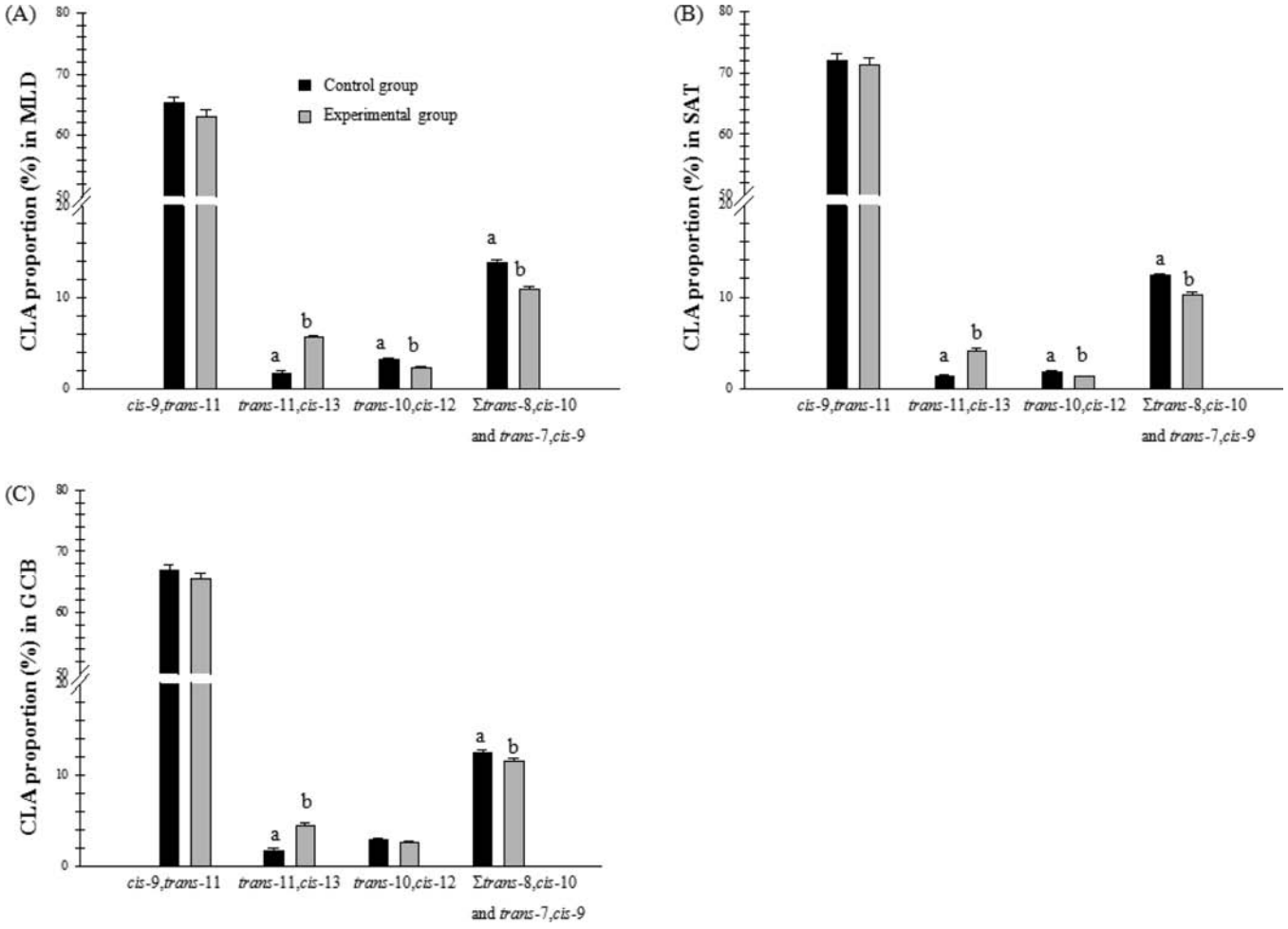

Figure 1. Relative composition of selected CLA isomers (% of total CLA isomers measured by HPLC) in longissimus muscle (**A**), subcutaneous adipose tissue (**B**), and German corned beef sausages (**C**) of German Holstein bulls (a and b indicate significant differences between groups within muscle, within subcutaneous fat, and within German corned beef sausages at $P \leq 0.05$).

significantly lower concentration of total fatty acids (mg/100 g) in subcutaneous adipose tissue compared to the control group (**Table 3**). The relative proportion of the sum of SFA, MUFA, PUFA, and especially C16:0, C16:1*cis*-9, C18:0, and C18:1*cis*-9 is not significantly different between groups. C18:2*n*-6, the sum of *n*-6 fatty acids, and the ratio of *n*-6/*n*-3 FA are significantly decreased in the experimental group. An increase was measured in the proportions of C18:1*trans*-11, C18:3*n*-3 (1.75 times), and C20:5*n*-3 and the sum of *n*-3 fatty acids in the experimental compared to the control group.

Similar to CLA content in MLD, the proportion of CLA*cis*-9, *trans*-11 in SAT is not significantly affected by the diet; however, it is between 6 and 7% higher than in MLD (**Figure 1**). CLA*trans*-10,*cis*-12 and the sum of CLA*trans*-8,*cis*-10 and CLA*trans*-7,*cis*-9 are significantly higher in the control group, whereas CLA*trans*-11,*cis*-13 is significantly higher in the experimental group.

Fatty Acid and CLA Composition of German Corned Beef Sausages. The relative proportion of C16:1*cis*-9 and the sum of MUFA are decreased in the experimental group compared to control (**Table 4**). PUFA such as C18:3*n*-3, C20:5*n*-3, C22:5*n*-3, C22:6*n*-3, the sum of *n*-3 PUFA, and *n*-3 LC PUFA are significantly increased in the experimental compared to the control group. The amount of C18:3*n*-3 is 1.5 times and the sum of *n*-3 LC PUFA 1.6 times higher in the experimental group, resulting in a lower ratio of *n*-6/*n*-3 FA (4.0 ± 0.4) in this group. No differences were found in C18:2*n*-6 proportion and concentration (data not shown).

The proportion of CLA in German corned beef sausages corresponds to the CLA level in longissimus muscle except for CLA*trans*-10,*cis*-12 (**Figure 1**).

Protein, fat, and dry matter are similar for both groups, whereas the ash content of German corned beef sausages in the experimental group is significantly lower than in the control group (**Table 2**).

SCD Enzyme Activity in Longissimus Muscle and Subcutaneous Adipose Tissue. The specific SCD enzyme activity in MLD and SAT is shown in **Figure 2**. The specific enzyme activity in MLD for the experimental group is 0.4 ± 0.1 nM of palmitoleic acid/mg of protein/h, significantly lower than in the control group with 0.8 ± 0.1 nM of palmitoleic acid/mg of protein/h. The SCD activity in SAT is much higher compared to the activity in muscle with 168.6 ± 18.6 nM of palmitoleic acid/mg of protein/h for the control group and 101.6 ± 21.7 nM of palmitoleic acid/mg of protein/h for the experimental group. The specific SCD activity in SAT of the experimental group is also significantly lower compared to the control group.

DISCUSSION

Fatty Acid Composition of Longissimus Muscle. In the present study 29 German Holstein bulls received two diets, which were different in the amounts of AL and ALA. Feeding did not influence animal performance and meat quality parameters. Feeding grass silage with supplementation of linseed, rapeseed, and algae or natural grazing to accumulate *n*-3 fatty acids in

Table 4. Relative Fatty Acid Composition (Percent) of German Corned Beef Sausages of German Holstein Bulls

	German corned beef sausage				
	control group $n = 15$		exptl group $n = 14$		
	LSM	SEM	LSM	SEM	*P* value
C14:0	1.8	0.1	1.7	0.1	0.28
C16:0	22.6	0.3	22.1	0.3	0.17
C16:1*cis*-9	3.8	0.1	3.4	0.1	**0.01**
C18:0	13.8	0.3	14.8	0.3	**0.04**
C18:1*cis*-9	38.7	0.5	37.3	0.5	0.07
C18:1*trans*-11	0.7	0.1	0.8	0.1	0.50
C18:2*n*-6	7.7	0.5	7.8	0.5	0.94
C18:3*n*-3	1.0	0.1	1.5	0.1	**0.01**
C20:4*n*-6	1.3	0.1	1.5	0.1	0.29
C20:5*n*-3	0.2	0.04	0.4	0.04	**0.002**
C22:4*n*-6	0.22	0.02	0.18	0.02	0.09
C22:5*n*-3	0.4	0.04	0.6	0.05	**0.003**
C22:6*n*-3	0.05	0.001	0.08	0.001	**0.001**
Σ SFA[a]	40.1	0.4	40.7	0.4	0.32
Σ MUFA[a]	47.2	0.6	45.4	0.6	**0.04**
Σ *n*-3 FA[a]	1.8	0.2	2.7	0.2	**0.003**
Σ *n*-6 FA[a]	9.9	0.5	10.1	0.5	0.79
ratio *n*-6/*n*-3 FA	5.9	0.4	4.0	0.4	**0.002**
Σ *n*-3 LC PUFA[a]	0.7	0.1	1.1	0.1	**0.002**
Σ *n*-6 LC PUFA[a]	1.6	0.1	1.7	0.1	0.44
Σ *trans* FA[a]	1.3	0.1	1.5	0.1	0.29
Σ total FA[a]	2165.7	118.0	1966.3	122.1	0.25

[a] See **Table 3** for explanation.

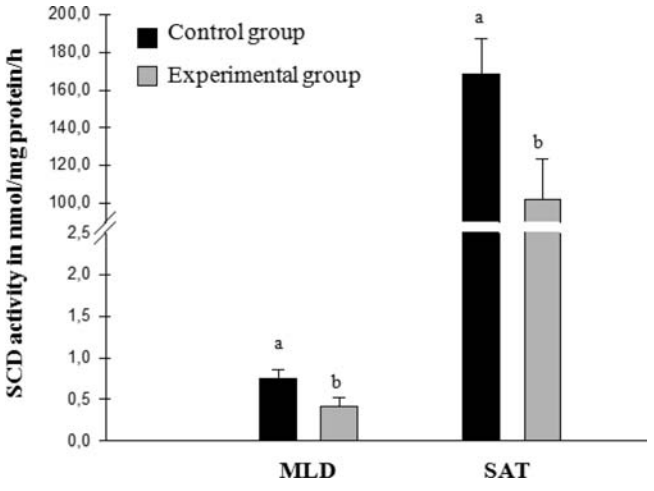

Figure 2. Specific SCD enzyme activity in longissimus muscle and subcutaneous adipose tissue of German Holstein bulls (a and b indicate significant differences between groups within muscle and within subcutaneous fat at $P \leq 0.05$).

ruminant tissues demonstrated successful results in enhancing *n*-3 fatty acids in tissues in several studies (*11*, *12*, *26*). In the present study feeding grass silage and concentrate supplemented with 3% linseed oil and 12% rapeseed cake resulted in enhancing essential and beneficial *n*-3 fatty acids such as ALA, C20:5*n*-3 (EPA), C22:5*n*-3 (DPA), and C22:6*n*-3 (DHA) in longissimus muscle of German Holstein bulls despite the 80−93% biohydrogenation of PUFA in the rumen and due to a clearly higher flow of ALA to the duodenum (*27*). The higher amount of *n*-3 fatty acids in MLD of experimental bulls caused a reduced ratio of *n*-6/*n*-3 FA (2.3 ± 0.1) compared to the ratio in muscle of the control group with 5.8 ± 0.1. The low *n*-6/*n*-3 FA ratio of MLD in the experimental group corresponds to the recommendation of the German Nutrition Society (*n*-6/*n*-3 FA ratio of ≤5:1) (*4*). Beef from bulls fed grass silage and C18:3*n*-3-enriched concentrate can be a source of *n*-3 fatty acids for human requirement apart from fish and fish products. Besides preventing coronary heart diseases (CHD) (*28*), substitution of PUFA for SFA and TFA has beneficial effects on insulin sensitivity, with stronger evidence for a potential protective effect from *n*-6 fatty acids (*29*).

Other fatty acids that are also critical in human nutrition are *trans* fatty acids (TFA). Generally, the dietary intake is directly related to the TFA content in human adipose tissue (*30*). Two major sources of TFA are industrially produced (partially hydrogenated vegetable oils) and products of biohydrogenation of dietary fatty acids in ruminants (*31*). TFA, for example, raise LDL and plasma total cholesterol and reduce HDL cholesterol (*32*). The proportion of C18:1*trans*-11 (TVA) in the present study, a hydrogenation product from LA and ALA and the major *trans* isomer in beef as a precursor for CLA*cis*-9,*trans*-11 (*13*), is increased in the experimental group, whereas the absolute amount is similar for both groups (data not shown). This is also attributed to the lower concentration of total fatty acids in the experimental group. Nuernberg et al. (*26*) also have found an increased proportion of TVA in MLD of bulls fed a grass-based diet. Further research is required to accurately assess the role of TVA as a risk factor of human health (*33*, *34*). After evaluating 39 scientific papers, Brouwer et al. (*35*) concluded that all *trans* FA (industrial and animal source) increase the LDL to HDL cholesterol ratio.

Not all fatty acids containing a *trans* conjunction have been shown to have negative effects on human health. The group of CLA isomers, positional and stereo isomers of octadecadienoic acid, is the focus of attention due to anti-inflammatory, anticarcinogenic, antiadipogenic, antiatherogenic, and antidiabetogenic properties (*16*). CLA*cis*-9,*trans*-11 is the main CLA isomer in beef, and the amount is mainly related to the biosynthesis in tissue from ruminally produced TVA and, to a lower extent, to the production in the rumen (*13*). A PUFA-enriched diet increases the level of this CLA isomer in beef (*13*, *25*, *36*, *37*). In the present experiment no significant differences were measured for the proportion of CLA*cis*-9,*trans*-11 between feeding groups. Very recently it has been shown that ALA has greater toxic effects on the bacterium *Butyrivibrio fibrisolvens*. This could induce a lower amount of CLA*cis*-9,*trans*-11 in ruminant tissues due to a reduced biohydrogenation (*38*). In the present study the inhibition of the protein expression of SCD (*17*) and a reduced CLA*cis*-9,*trans*-11 content in the rumen (*20*) of experimental bulls were probably the reasons for this result.

Another important CLA isomer is CLA*trans*-10,*cis*-12 with antiobesity effects (*39*). The muscle of experimental bulls contained a lower CLA*trans*-10,*cis*-12 proportion compared to the control group. CLA*trans*-10,*cis*-12 is a rumen fermentation product, and it has shown to block the conversion of TVA to CLA*cis*-9,*trans*-11 via the inhibition of SCD gene expression (*40*). Similar to our results in the muscle of dairy goats fed a diet based on grassland hay compared to maize silage, CLA*trans*-10,*cis*-12 in milk was decreased (*41*), and a substitution of grass silage for maize silage in the diet of late lactating British Holstein Friesian cows resulted also in a decrease of CLA*trans*-10,*cis*-12 in milk (*42*).

Fatty Acid Composition of Subcutaneous Adipose Tissue. Similar to the fatty acid composition of MLD, the proportion of TVA, C18:3*n*-3, C20:5*n*-3, and the sum of *n*-3 fatty acids are significantly increased and C18:2*n*-6, the sum of *n*-6 fatty acids, the sum of total fatty acids, and the ratio of *n*-6/*n*-3 FA are significantly decreased in subcutaneous adipose tissue of grass silage fed compared to the maize silage fed bulls. Our results are confirmed by the following researchers. Fincham et al. (*43*) investigated the

effect of pasture versus feedlot-finished cattle and observed an increase of TVA and ALA in the ruminal fluid and in SAT, whereas the amount of LA was decreased in rumen in pasture-fed cattle. Scollan et al. (*44*) demonstrated an increase of TVA and C18:3*n*-3 in SAT of steers fed a diet enriched with whole linseed. Contrary to our results, Bartoň et al. (*45*) found additionally an increase of LA and CLA in heifers fed a diet with extruded linseed supplementation. This increased LA amount could be explained by the higher LA than ALA concentration in the experimental diet used in that experiment, where the amount of LA was 16% greater and the amount of ALA 15% lower than in the experimental diet of the present study. In other studies feeding ruminants with a high-concentrate diet or a corn-based diet resulted in higher amounts of LA in SAT and muscle (*40*, *43*). One explanation is the lowering of rumen pH due to the high intake of corn, in particular the starch, which results in a lower biohydrogenation by microsomal organisms in rumen. This leads to an increase of LA and CLA*cis*-9,*trans*-11 in duodenal flow and results in an incorporation into tissues (*40*). The small particle size of LA-enriched concentrate diets result in a shorter rumen transit time than fibrous diets, such as grass silage. This has a limiting effect on the microbial biohydrogenation opportunities (*11*). As in muscle tissue, the ratio of *n*-6/*n*-3 FA in the experimental group is 2 times lower compared to the control group. This is based on the lower amount of LA and the greater amount of ALA in the experimental adipose tissue.

Fatty Acid Composition of German Corned Beef Sausages. Besides the fresh meat intake in Germany of 42 g/day for men and 23 g/day for women, the consumption of processed meat is about 61 g/day for men and 30 g/day for women (*46*). Therefore, one beef product was produced from the fresh lean meat of carcasses. German corned beef sausage was chosen because it contains 58% lean meat. It is of interest to know the alteration of the fatty acid composition during processing because the awareness of consumers of more nutritious and added-value meat products has increased and interest in pasture-based beef production systems is growing (*1*).

In the literature little information is available about the fatty acid composition of beef sausages made of fresh meat from cattle with *n*-6 and *n*-3 PUFA-supplemented diet. Carcasses from the present trial were used to produce German corned beef (GCB) sausage.

The chemical composition of GCB did not differ between groups except the ash content. The crude fat content of GCB (2−2.3%) is similar to that of the fresh meat (2.1−2.8%), therefore allowing it to be be considered a low-fat product (**Table 2**). Compared to GCB, the fat content of beef and lamb mortadella is approximately 21% (*47*). Comparison of the fatty acid composition of fresh MLD and GCB showed that the concentration of stearic acid tended to be higher and ALA is significantly increased in the experimental group, whereas the difference between both groups is approximately 3 times in MLD and 1.5 times in GCB. EPA, DHA, and DPA are nearly in the same concentration in GCB as in MLD. It seems there is no loss of LC *n*-3 FA from fresh meat during processing and production of German corned beef sausages. The short-time heating to internal temperatures of 68 °C did not cause changes in the FA profile. Lee et al. (*48*) found a loss of *n*-3 PUFA added to raw meat before heat processing due to cooking procedures to produce ham. During processing, lipids undergo an intense lipolysis (*49*) because heat catalyzes the initiation of lipid peroxidation and the formation of oxidation products (*50*). One study showed that the relative fatty acid concentration of grilled pig muscles was significantly affected and the PUFA concentration was significantly higher due to the water loss during heating (*51*). Frozen meatballs from beef meat, cooked in a gas oven for 10 min at 250 °C and then at a reduced temperature of 203 °C, did not show a loss of LA and ALA compared to the raw meat (*52*). Cooking meat of carcasses of grazing heifers at 140 °C for 30 min did not lead to detrimental changes of the fatty acid composition (*53*). The important result of the present experiment is that the beneficial *n*-3 fatty acids from the fresh muscle of the experimental bulls are also detected in the processed product GCB, which leads to an *n*-6/*n*-3 FA ratio of 4.0 ± 0.4. This ratio corresponds to the recommendation of the DGE to be < 5:1 (*4*). As in MLD CLA*cis*-9,*trans*-11 is the main isomer in GCB and the proportion is approximately 65−66% (HPLC data, **Figure 1**). In general, the CLA content seems not to be affected by the processing method. The transfer of beneficial fatty acids from fresh meat into processed product GCB is possible without important alterations.

SCD Enzyme Activity in Longissimus Muscle and Subcutaneous Adipose Tissue. Apart from diet and the microbial flora in the rumen, the fatty acid composition in muscle and subcutaneous adipose tissue of ruminants is also affected by the Δ^9-desaturase enzyme activity that introduces a double bound into SFA and TVA and results in MUFA, especially palmitoleic and oleic acid, and the CLA*cis*-9,*trans*-11 isomer (*15*). In the present study the specific SCD enzyme activity was measured in MLD and SAT. The experimental group revealed in both tissues a significantly lower SCD activity compared to control animals. Diets enriched with linseed also have decreasing effects on SCD in SAT of pigs compared to sunflower supplementation (*54*). Waters et al. (*55*) have shown that the ratio of *n*-6/*n*-3 FA in diet and tissues has an important effect on SCD mRNA regulation via SREBP-1c, whereas *n*-3 fatty acids are the main inhibitors of SREBP-1c. The significantly lower relative amount of C18:1*cis*-9 (control, 37.4%; experimental, 34.7%) in MLD, synthesized by SCD (*56*), and the significantly reduced concentration in SAT (control group, 25194.35 mg/100 g; experimental group, 21628.6 mg/100 g) of experimental bulls are in line with the significantly reduced SCD activity (**Figure 2**) and the lower SCD protein expression (*17*) in both tissues. Waters et al. (*57*) and Howell et al. (*58*) described that rather *n*-3 long-chain fatty acids (EPA, DHA) reduce the SCD mRNA expression via SREBP in different cell lines. The higher amounts of EPA and DHA in MLD and only EPA in SAT detected in German Holstein bulls of the present experiment fed grass silage confirm the results of Waters et al. (*57*) and Howell et al. (*58*). In general, the SCD activity in SAT is higher than the activity in muscle. Archibeque et al. (*59*) found that SAT has twice the SCD catalytic activity of marbling adipose tissue, which resulted in a higher concentration of MUFA in SAT. The measured SCD activity and the amount were greater compared to muscle but is not statistically verified, whereas the values for muscle are in nearly the same range as the activity in pig muscle (*23*). SCD also determines the amount of CLA*cis*-9, *trans*-11 in tissues. The proportion of TVA in MLD and SAT was significantly higher in the bulls of the experimental group; however, the product CLA*cis*-9,*trans*-11 proportion (HPLC data) was not affected by the diet because of the reduced SCD activity measured in the experimental group.

It is summarized that feeding ALA-enriched diets to German Holstein bulls induced enhancement of this essential fatty acid and the long-chain *n*-3 PUFA in MLD and SAT. The specific SCD activity was significantly reduced in MLD and SAT, resulting in a reduced proportion of oleic acid only in muscle. Most importantly, there was no loss of PUFA during processing of German corned beef sausage. The German corned beef sausage produced by using the fresh lean meat of the carcasses from the experimental group was rich in *n*-3 and *n*-3 LC PUFA, and the *n*-6/*n*-3 FA ratio corresponds to the recommendation.

ABBREVIATIONS USED

FA, fatty acids; ALA, linolenic acid; LA, linoleic acid; SCD, stearoyl-CoA desaturase, EC 1.14.19.1; SAT, subcutaneous adipose tissue; CLA, conjugated linoleic acids; MLD, longissimus muscle; TVA, *trans*-vaccenic acid; SFA, sum of saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; GCB, German corned beef sausages; GLM, general linear model; LSM, least-squares means.

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