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ORIGINAL ARTICLE

Vaccenic Acid and *cis*-9,*trans*-11 CLA in the Rumen and Different Tissues of Pasture- and Concentrate-Fed Beef Cattle

Xiangzhen Shen · Karin Nuernberg · Gerd Nuernberg · Ruqian Zhao · Nigel Scollan · Klaus Ender · Dirk Dannenberger

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Abstract The objective of present study was the comparison of *trans*-11 18:1 (VA) and *cis*-9,*trans*-11 CLA concentrations in the rumen and different tissues in beef cattle, and to examine the diet and breed effects on the compound concentration and deposition. Sixty-four German Holstein and German Simmental bulls were randomly assigned to two dietary treatments, based on concentrate or pasture. The concentration of *cis*-9,*trans*-11 CLA and VA in rumen, duodenal digesta and different tissues was determined by gas chromatography. The results showed that pasture relative to concentrate feeding significantly increased the concentration of VA in duodenal digesta, plasma and erythrocyte phospholipids. Pasture-based feeding resulted in a significant enrichment of *cis*-9,*trans*-11 CLA in plasma lipids and erythrocyte phospholipids, but not in rumen and duodenal digesta, compared to concentrate-fed diet. Diet did not affect the *cis*-9,*trans*-11 CLA concentrations (mg/100 g fresh tissue) in semitendinosus muscle and subcutaneous fat. There was a breed effect on the deposition of *cis*-9,*trans*-11 CLA in longissimus muscle with lower concentration in pasture-fed German Simmental bulls compared to concentrate-fed bulls. However, pasture feeding significantly increased both, the VA and *cis*-9,*trans*-11 CLA concentrations in liver and heart tissues. Both diet and breed effects on Δ^9-desaturase index was observed in muscle and subcutaneous fat tissues. There was a linear relationship between the concentration of VA and *cis*-9,trans-11 CLA and the coefficients of determination (R^2) varied between 0.29 and 0.87 from rumen to the different tissues.

Keywords CLA · Vaccenic acid · Beef · Pasture · Rumen · Digesta · Blood · Muscle

X. Shen · R. Zhao
Nanjing Agricultural University,
Nanjing 210095, China

K. Nuernberg · K. Ender · D. Dannenberger (✉)
Department of Muscle Biology and Growth,
Research Institute for Biology of Farm Animals,
Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
e-mail: dannenberger@fbn-dummerstorf.de

G. Nuernberg
Department of Genetics and Biometry,
Research Institute for Biology of Farm Animals,
Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

N. Scollan
Institute of Grassland and Environmental Research,
Aberystwyth SY23 3EB, UK

Abbreviations

CLA	Conjugated linoleic acids
DM	Dry matter
FAME	Fatty acid methyl esters
GH	German Holstein
GC	Gas chromatography
GS	German Simmental
LSM	Least square mean
ME	Methyl ester
PL	Phospholipids
SEM	Standard error of LSM
PUFA	Polyunsaturated fatty acids
VA	Vaccenic acid
vol	Volume
wt	Weight

Introduction

In recent years, conjugated linoleic acid isomers have received much attention due their potential beneficial properties to human health [1–3]. CLA has been implicated in the prevention of carcinogenesis, atherogenesis, obesity and enhancement of the immune function in animal models; however, in human studies inconsistent effects have been reported [4–7]. Of the two physiologically important isomers, *cis*-9,*trans*-11 CLA is the most prevalent accounting for up to 80–90% of total CLA in ruminant products, whereas *trans*-10,*cis*-12 CLA comprises 3–5% of total CLA [8]. The main dietary source of CLA in food is ruminant meat, milk and their products [9, 10].

It is well known that *cis*-9,*trans*-11 CLA is formed from two sources, one originates from ruminal biohydrogenation of linoleic acid to stearic acid in the rumen by *Butyrinvibrio fibrisolvens* and other bacteria [11, 12]. The second source is the endogenous conversion of *trans*-11 18:1 by Δ^9-desaturase in the mammary gland of dairy cows and ruminant adipose tissue [10, 13]. VA is a common intermediate produced during ruminal biohydrogenation of linolenic acid (18:3n-3) and linoleic acid (18:2n-6) [14, 15]. Griinari et al. [10] demonstrated that endogenous synthesis of *cis*-9,*trans*-11 CLA from VA represents the primary source in milk fat of lactating cows. More recently, Mosley et al. [16] confirmed using ^{13}C-labeled VA that endogenous conversion of dietary VA to *cis*-9,*trans*-11 CLA in the mammary gland catalyzed by Δ^9-desaturase occurred. The authors [16] found that approximately 80% of milk fat *cis*-9,*trans*-11 CLA originated from VA. In the course of endogenous synthesis of *cis*-9,*trans*-11 CLA, VA and Δ^9-desaturase are the two primary prerequisites. Daniel et al. [17] demonstrated that the Δ^9-desaturase is active in sheep adipose tissues, and its mRNA is well expressed. The genetic basis for the individual variation in milk fat content of *cis*-9,*trans*-11 CLA and the Δ^9-desaturase index remains to be identified.

Furthermore, numerous investigations have demonstrated that diet has a substantial effect on the content of VA and *cis*-9,*trans*-11 CLA both in milk and intramuscular fat [1, 9, 13, 19]. When comparing pasture and concentrate diets there are several differences that relate to milk and intramuscular fat *cis*-9,*trans*-11 CLA [9]. Most research investigating the biohydrogenation of dietary fatty acids to VA, the desaturation of VA to *cis*-9,*trans*-11 CLA and the endogenous synthesis of *cis*-9,*trans*-11 CLA has focused on milk fat [1, 9]. However, there is a lack of information about the pathway of VA and *cis*-9,*trans*-11 CLA from the rumen, via duodenal digesta and blood transport into the muscle and subcutaneous fat.

A large study was carried out by Nuernberg et al. [19] to investigate the effect of feeding pasture versus concentrate to two different cattle breeds (German Holstein and German Simmental bulls) on meat quality and fatty acid composition of intramuscular fat of bulls. The diet effects of pasture feeding on carcass- and meat quality, total fatty acid composition, fatty acid composition of polar and neutral lipids, CLA and *trans*-18:1 isomers in muscles and subcutaneous fat had been published previously [20–22]. However, the experiment results of diet effects on fatty acid concentrations in the rumen, duodenal digesta and blood have not been published until now. The objective of this paper was to compare the VA and *cis*-9,*trans*-11 CLA concentrations in the rumen and different tissues in beef cattle fed different diets, and to determine the relationships between VA and *cis*-9,*trans*-11 CLA concentrations in different tissues.

Experimental Procedures

Materials

Sixty-four bulls (5–6 months old) were randomly assigned to two dietary treatments (concentrate vs. grass-based) in the experiment. For indoor housing the concentrate group consisted of 16 German Simmental (GS) and 17 German Holstein (GH) bulls. The animals were fed semi ad libitum maize silage, concentrate, hay, straw and a mixture of minerals and vitamins up to 620 kg live weight. The pelleted concentrate for the indoor concentrate group was a mixture of winter barley, molasses, and soybean meal (Vollkraft-Mischfutterwerk, Güstrow, Germany). The other animals, (15 GS and 16 GH bulls) were kept on pasture during the summer period. During the following winter period and 3 months before finishing these bulls were kept in a stable and were fed wilted silage, hay, a pelleted concentrate diet and a mixture of minerals and vitamins up to 620 kg live weight. The pelleted concentrate for the pasture group was a mixture of 76% sugar beet pulp (molasses), 12% barley, and 10% coarsely cracked linseed (Vollkraft Mischfutterwerk, Güstrow, Germany). This is termed a grass based feeding. The details of chemical and fatty acid composition of the diet were previously described by Nuernberg et al. [19].

All bulls were slaughtered as they reached 620 kg live weight in the abattoir of the Research Institute for the Biology of Farm Animals in Dummerstorf, Germany. The slaughter and dressing procedures were in accordance with EU specifications. The carcasses were chilled (4 °C) before muscle samples were removed. The blood, rumen content, duodenal digesta (1 m after pylorus), heart and liver tissue samples were taken immediately after slaughter. Samples of longissimus muscle (at the sixth rib of the left carcass side), subcutaneous fat and semitendinosus muscles (left

carcass side) were taken 24 h after slaughter. Rumen content and digesta samples were prepared on the slaughter day. All tissue samples were stored frozen at –70 °C until lipid extraction was carried out.

Methods

Extraction and Methylation of Lipids

Plasma and Erythrocytes For plasma and erythrocytes preparation EDTA blood was centrifuged at 2,750×*g* at 4 °C for 10 min into plasma and erythrocytes. Approximately 2 g plasma samples were taken for lipid extraction. Erythrocyte samples were washed two times by 0.9% sodium chloride solution, and then a 2 g sample was added to 5 ml methanol (cold, shaking by vortex). Waiting for 10 min, and then 10 ml chloroform was added to extract total lipids. After filtration the solution was dried under gentle nitrogen stream at room temperature. To obtain the phospholipid fraction (PL) of the isolated erythrocytes lipids were separated by thin layer chromatography on precoated silica gel 60 plates using the solvent mixture *n*-hexane/diethyl ether/acetic acid (70:30:2, vol/vol/vol). PL fraction was viewed under ultraviolet light after spraying with 2,7-dichlorofluoresceine (0.1% in ethanol, wt/vol). The PL bands were scraped off and eluted with chloroform/methanol (2:1, vol/vol), decanted after 1 h and eluted once more with chloroform/methanol (2:1, vol/vol). After combining both extracts, the solvent was removed under nitrogen at room temperature. The phospholipids were treated with 0.5 M methanolic sodium methylate for 20 min at room temperature. Methylation of fatty acids was performed with borontrifluride/methanol (14% wt/vol) for 20 min at room temperature. The fatty acid methyl ester (FAME) formed was extracted with *n*-hexane, then evaporate using a rotary evaporator and dried under a gentle nitrogen stream at room temperature. The FAME was dissolved in *n*-heptane for gas chromatography analysis (GC).

Tissue Samples For tissue samples (muscle approximately 2g, subcutaneous fat 1 g, heart 2 g, liver 2 g), total lipids were extracted with chloroform/methanol (2:1, vol/vol) by homogenisation (Ultra Turrax, 3 × 15 s, 12,000 revolutions per minute) at room temperature according to Folch et al. [23]. The details of methylation were described previously [24].

Rumen and Duodenal Digesta Approximately 60 g of rumen content (representative mix of whole rumen content) and 60 g duodenal digesta were freeze-dried, and then 1.2 g freeze-dried rumen or 1.2 g freeze-dried duodenal digesta content were taken for lipid extraction (duplicates). For extraction and direct fatty acid methylation of the rumen and digesta, a modified method from Sukhija and Palmquist [25] was used. The samples were treated with 3.5 ml toluene (containing 19:0 methyl ester as internal standard) and 4 ml of 5% methanolic HCl. The mixture was shaken in a water bath at 60 °C for 2 h. After cooling, methyl esters of total fatty acids were extracted with 3.5 ml toluene in the presence of 8.75 ml 6% K_2CO_3 solution and stirred with a vortex stirrer. After centrifugation (1,200*g* for 5 min at 4 °C) the toluene phase was separated and 1 g Na_2SO_4 and activated charcoal were added and stored overnight until the organic phase was colourless. After filtration, an aliquot of the toluene extract was taken and analysed by GC.

Gas Chromatography (GC) Analysis

An aliquot of this FAME extract was used for the gas chromatographic analyses of total fatty acids. The fatty acid composition of the samples was determined by GC on a CP SIL 88, 100 m × 0.25 mm × 0.25 μm capillary column (Chrompack-Varian, USA) installed in a Perkin Elmer gas chromatograph Autosys XL with a flame ionisation detector and split injection. The initial oven temperature was 120 °C, held for 5 min, subsequently increased to 170 °C at a rate of 2 °C min^{-1}, held for 15 min, then to 200 °C at 5 °C min^{-1}, held for 5 min, then to 235 °C at 2 °C min^{-1} and held for 10 min. Hydrogen was used as the carrier gas at a flow rate of 1 ml min^{-1}. The split ratio was 1:20; the injector was set at 260 °C and the detector at 280 °C. A reference standard mix added with *trans*-11 18:1 methyl ester, *cis*-9,*trans*-11 CLA methyl ester, 22:5n-3 methyl ester, *cis*-11 18:1 methyl ester, 18:4n-3 methyl ester and 22:4n-6 methyl ester was used for calibration and correction factors for individual fatty acids. The proportion and concentrations were calculated using the internal standard method of Turbochrom workstation software. 19:0 methyl ester was used as internal standard. The *cis*-9, *trans*-11 CLA concentrations includes the isomers *trans*-7,*cis*-9 CLA and *trans*-8,*cis*-10 CLA, because the separation of these CLA isomers is not possible by GC under these conditions [26]. The calculation for the index of Δ^9-desaturase activity was calculated according to Malau-Aduli et al. [27] Δ^9-Desaturase index = 100 × [(14:1 + 16:1+18:1)/(14:1 + 16:1 + 18:1 + 14:0 + 16:0 + 18:0)].

Reagents FAMEs were purchased from Sigma-Aldrich (Deisenhofen, Germany) and Matreya (Pleasant Gap, PA,

USA). The TLC plates coated with 0.25 mm silica gel (20 × 20 cm) were obtained from Merck (Darmstadt, Germany). FAMEs were identified by means of purified standards (''Sigma-FAME mixture'', Sigma Aldrich Deisenhofen, Germany). Fatty acid methyl esters of *trans*-11 18:1, *cis*-11 18:1, 22:5n-3, 22:4n-6, *cis*-9,*trans*-11 18:2 were purchased from Matreya (Pleasnet Gap, USA). All solvents used were HPLC grade from Lab-Scan (Dublin, Ireland). The 0.5 M methanolic sodium methylate were purchased from Fluka (Switzerland) and borontrifluride/methanol (14% wt/vol) from Sigma-Aldrich (Deisenhofen, Germany).

Statistical analysis. All data were analysed by the least-squares method using the GLM procedures with fixed factors feeding and breed (SAS® Systems, Release 8.2, SAS Institute Inc., Cary, NC). All tables contain the least squares means (LSM) and the standard error (SE) of the LSM. All statistical tests of LSM were performed for a significance level of $P \leq 0.05$. Relationships between *cis*-9,*trans*-11 CLA and TVA in different tissues were examined by regression analysis using REG procedure (SAS® Systems).

Results

The total fatty acid composition of longissimus and semitendinosus muscles, subcutaneous fat, liver and heart in German Holstein and German Simmental bulls fed different diets have been previously published [19–22].

Pasture-fed beef generally had lower intramuscular fat contents compared to concentrate-fed beef; therefore, when CLA proportions of the beef were calculated, the differences were less pronounced between pasture- and concentrate-fed beef [19]. Consequently, the results in the present paper base on concentrations (mg/100 g fresh tissue or mg/100 g dry matter). The concentrations of *cis*-9,*trans*-11 CLA, VA and Δ^9-desaturase index in rumen and duodenal digesta of German Holstein and German Simmental bulls are presented in Table 1. The diet had no effect on *cis*-9,*trans*-11 CLA concentration in both rumen and duodenal digesta. There was a significant interaction between diet and breed for the VA concentration in the rumen. Grazing of German Holstein bulls decreased the VA content but in grass-fed German Simmental bulls the VA concentration was higher compared with concentrate-fed bulls. However, compared with concentrate feeding, pasture feeding significantly increased the VA concentration in duodenal digesta (Table 1).

The *cis*-9,*trans*-11 CLA and VA concentration in plasma and erythrocyte phospholipids of German Holstein and German Simmental bulls is shown in Table 2. The results demonstrated that diet significantly affected the VA and *cis*-9,*trans*-11 CLA contents. The level of VA and *cis*-9,*trans*-11 CLA in plasma and erythrocyte PL was much lower ($P < 0.05$) by concentrate feeding compared to pasture feeding of both breeds. The concentrations of *cis*-9,*trans*-11 CLA and VA in German Simmental bulls were more than two times higher with pasture feeding as compared with concentrate feeding. In contrast, the differences of *cis*-9,*trans*-11 CLA and VA contents in plasma and erythrocyte PL of German Holstein bulls were much smaller between both diets (Table 2).

The concentrations of *cis*-9,*trans*-11 CLA and VA (mg/100 g tissue) and Δ^9-desaturase index in longissimus muscle, semitendinosus muscle and subcutaneous fat of German Holstein and German Simmental bulls are given in Table 3. There was a breed effect on the deposition of *cis*-9,*trans*-11 CLA in longissimus muscle, and the *cis*-9,*trans*-11 CLA concentration in muscle of German Holstein bulls

Table 1 Concentration of *cis*-9,*trans*-11 CLA, VA and in rumen content (mg/100 g DM) and duodenal digesta (mg/g DM) of German Holstein and German Simmental bulls

	German Holstein				German Simmental				Significance[a] ($P < 0.05$)
	Concentrate $N = 17$		Pasture $N = 16$		Concentrate $N = 16$		Pasture $N = 15$		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
Rumen (mg/100 g DM[b])									
cis-9,*trans*-11 CLA	2.25	0.64	0.86	1.38	2.60	0.72	1.93	1.07	
VA	87.32	9.86	53.40	14.90	42.07	10.18	58.58	10.54	D*B
Duodenal digesta (mg/g DM)									
cis-9,*trans*-11 CLA	0.06	0.01	0.07	0.01	0.07	0.01	0.09	0.01	
VA	0.25	0.05	0.31	0.05	0.23	0.06	0.42	0.06	D

[a] *D* Significant influence of diet; *B* significant influence of breed; *D***B* significant influence of interaction *D***B*

[b] *DM* dry matter

Table 2 Concentration of *cis*-9,*trans*-11 CLA, VA in plasma (μg/g) and erythrocyte phospholipids (μg/g) of German Holstein and German Simmental bulls

	German Holstein				German Simmental				Significance[a] ($P < 0.05$)
	Concentrate $N = 17$		Pasture $N = 16$		Concentrate $N = 16$		Pasture $N = 15$		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
Plasma (μg/g)									
cis-9,*trans*-11 CLA	2.12	0.20	2.57	0.18	1.40	0.19	3.11	0.22	D, D*B
VA	12.54	1.44	16.61	1.30	9.87	1.34	23.78	1.56	D, D*B
Erythrocytes pl (μg/g)									
cis-9,*trans*-11 CLA	1.94	0.22	1.78	0.25	1.46	0.22	3.17	0.22	D, D*B
VA	1.04	0.15	1.30	0.17	0.77	0.15	2.28	0.15	D, B, D*B

[a] For footnotes see Table 1

Table 3 Concentration of *cis*-9,*trans*-11 CLA, VA (mg/100 g fresh tissue) and Δ^9-desaturase index in longissimus muscle, semitendinosus muscle and subcutaneous fat of German Holstein and German Simmental bulls

	German Holstein				German Simmental				Significance ($P < 0.05$)
	Concentrate $N = 17$		Pasture $N = 16$		Concentrate $N = 16$		Pasture $N = 15$		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
Longissimus muscle									
cis-9,*trans*-11 CLA	17.12	1.72	17.34	1.77	13.32	1.77	11.51	1.83	B
VA	70.84	8.23	83.73	9.57	86.24	9.57	76.69	8.95	
Δ^9-desaturase index	50.87	0.66	46.06	0.68	48.64	0.68	44.92	0.70	D, B
Semitendinosus muscle									
cis-9,*trans*-11 CLA	4.91	0.63	4.23	0.65	4.85	0.65	3.19	0.67	
VA	18.66	2.77	11.50	2.86	13.69	2.86	9.02	2.95	
Δ^9-desaturase index	53.47	0.65	48.61	0.67	50.72	0.67	47.11	0.69	D, B
Subcutaneous fat									
cis-9,*trans*-11 CLA	423.09	23.88	397.23	24.62	354.40	26.32	375.69	25.43	
VA	1187.63	91.81	1739.09	94.64	917.94	91.17	1320.01	97.74	D, D*B
Δ^9-desaturase index	55.57	0.82	48.42	0.85	54.55	0.91	45.78	0.88	D, B

[a] For footnotes see Table 1

was significantly higher than that in German Simmental bulls. Furthermore, diet did not affect the deposition of *cis*-9,*trans*-11 CLA in semitendinosus muscle and subcutaneous fat. Between the different tissues longissimus muscle, semitendinosus muscle and subcutaneous fat) *cis*-9,*trans*-11 CLA accumulation in subcutaneous fat was the highest, up to 397.2 mg/100 g tissue in German Holstein bulls and 375.7 mg/100 g tissue in German Simmental bulls by pasture feeding (Table 3). The VA concentrations in subcutaneous fat were significantly higher with pasture compared to concentrate feeding.

The concentration of *cis*-9,*trans*-11 CLA, VA (mg/100 g tissue) and Δ^9-desaturase index (calculated) in splanchnic tissue of liver and heart of German Holstein and German Simmental bulls is shown in Table 4. In contrast to the muscle and subcutaneous tissue, pasture feeding significantly increased CLA deposition in liver and heart tissue compared with concentrate feeding. The *cis*-9,*trans*-11 CLA concentrations in heart tissue of German Simmental bulls was higher than that in German Holstein bulls, indicating breed difference. The accumulation of VA by pasture feeding was also enhanced significantly both in heart and liver tissue of German Holstein and German Simmental bulls. A diet effect on Δ^9-desaturase index was detected, showing that the index was higher feeding concentrate relative to pasture, except in the heart of German

Table 4 Concentration of *cis*-9,*trans*-11 CLA, VA (mg/100 g fresh tissue) and Δ^9-desaturase index in liver and heart tissue of German Holstein and German Simmental bulls

	German Holstein				German Simmental				Significance ($P < 0.05$)
	Concentrate $N = 17$		Pasture $N = 16$		Concentrate $N = 16$		Pasture $N = 15$		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
Liver									
cis-9,*trans*-11 CLA	10.46	1.00	14.02	1.03	6.98	1.03	15.94	1.07	D, D*B
VA	27.79	1.85	36.26	1.90	28.03	1.90	50.20	1.97	D, B, D*B
Δ^9-Desaturase index	24.20	0.63	21.36	0.65	24.40	0.65	22.55	0.67	D
Heart									
cis-9,*trans*-11 CLA	2.90	0.23	5.26	0.24	3.17	0.24	6.54	0.25	D, B, D*B
VA	19.94	1.50	25.52	1.55	23.85	1.55	36.41	1.60	D, B, D*B
Δ^9-Desaturase index	35.55	0.65	33.06	0.67	34.90	0.67	34.45	0.69	D

[a] For footnotes see Table 1

Simmental bulls. The relationships between the concentration of *cis*-9,*trans*-11 CLA and VA in ruminal content, duodenal digesta, plasma, erythrocytes, longissimus muscle, semitendinosus muscle, subcutaneous fat, liver and heart are presented in Fig. 1. There was linear trend between the concentration of VA and *cis*-9,*trans*-11 CLA and this correlation was shown to vary from rumen to different tissues. The R-Square values of linear equation

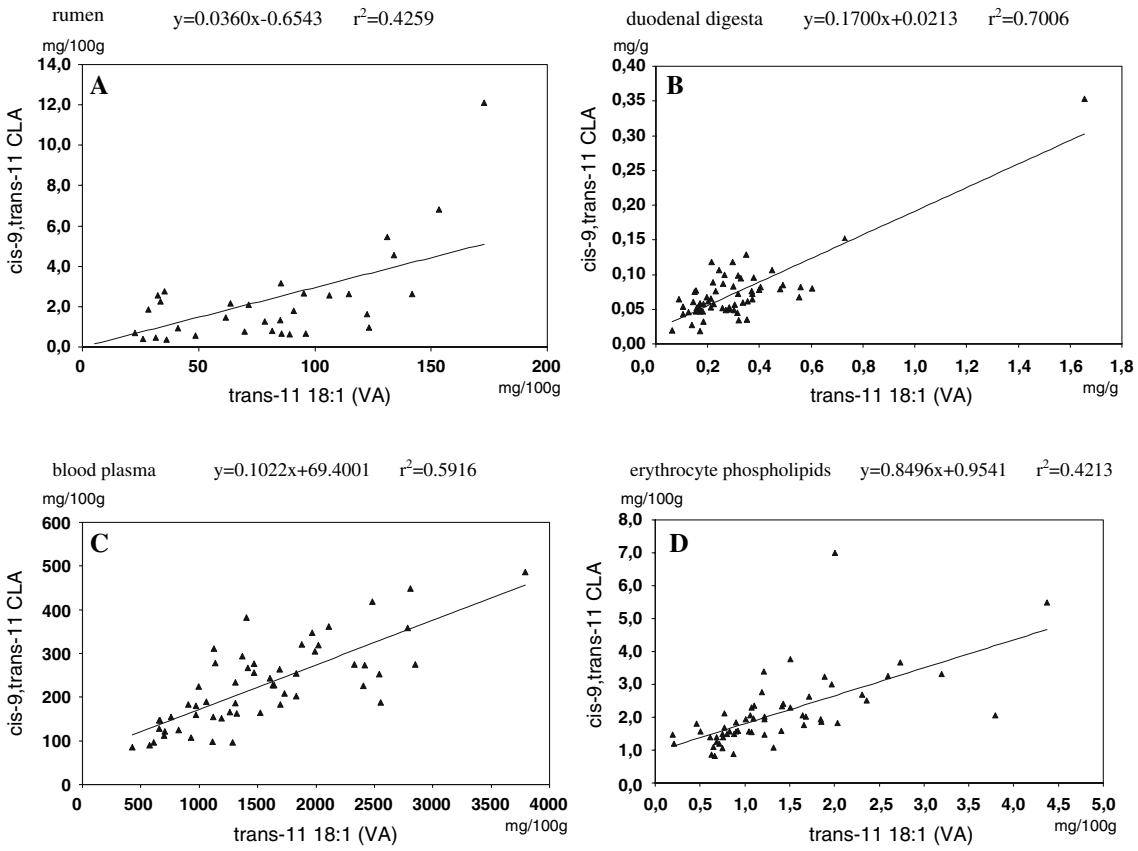

Fig. 1 Relationship between VA and *cis*-9,*trans*-11 CLA in **a** rumen content, **b** duodenal digesta, **c** plasma, **d** erythrocyte phospholipids, **e** longissimus muscle, **f** semitendinosus muscle, **g** subcutaneous fat, **h** liver and **i** heart in German Holstein and German Simmental bulls fed different diets

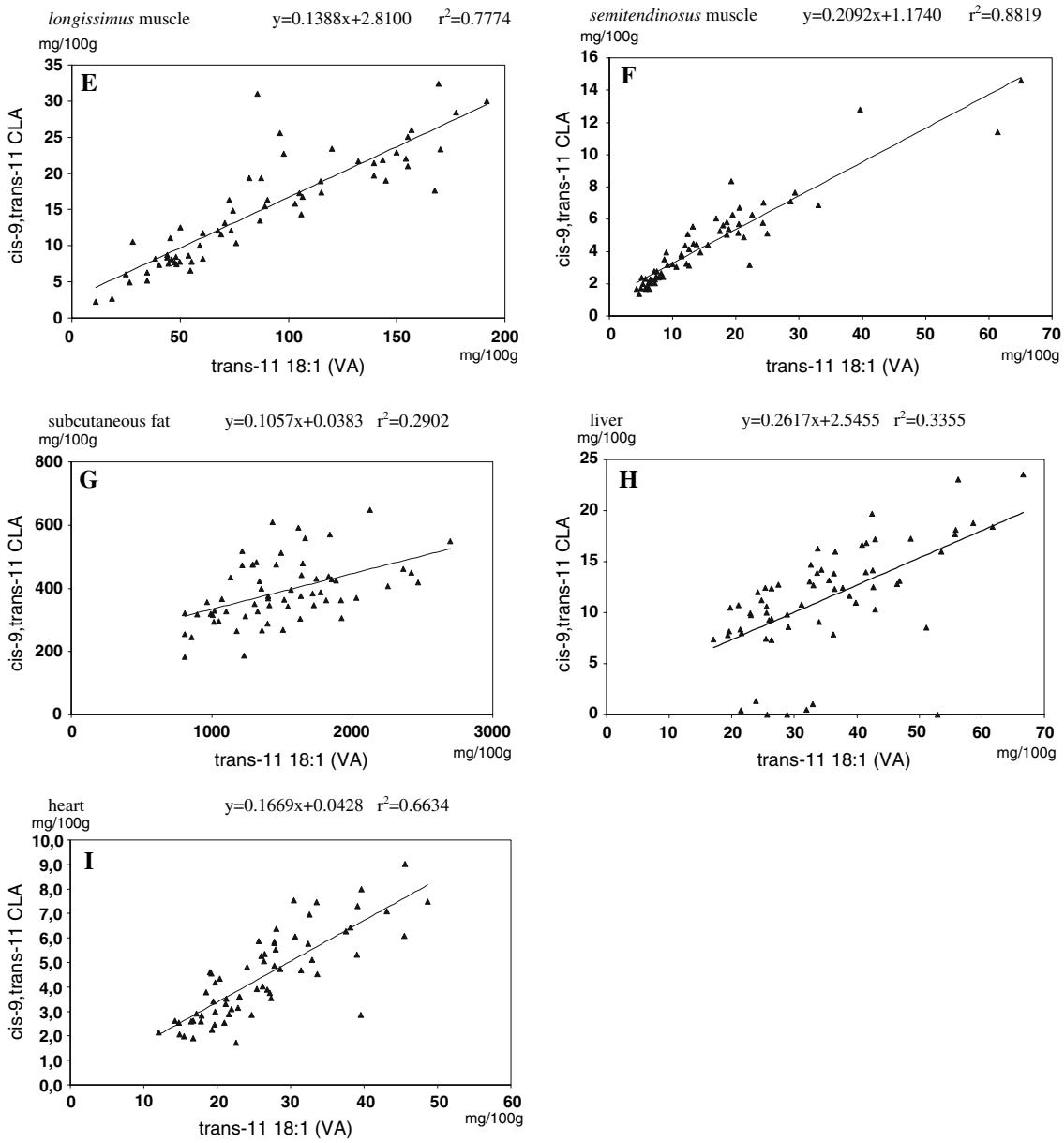

Fig. 1 continued

between *cis*-9,*trans*-11 CLA and VA in rumen content, duodenal digesta content, plasma, erythrocyte phospholipids, longissimus muscle, semitendinosus muscle, subcutaneous fat, liver and in heart lipids were 0.43, 0.70, 0.59, 0.42, 0.78, 0.88, 0.29, 0.34 and 0.66, respectively (Fig. 1).

Discussion

The majority of research examining the biohydrogenation of dietary fatty acids to VA, the desaturation of VA to *cis*-9,*trans*-11 CLA, the absorption and the de novo synthesis of *cis*-9,*trans*-11 CLA has been focused on milk fat [1, 8, 10, 13–16]. Only a few papers reported the pathway of a *cis*-9,*trans*-11 CLA and VA from the formation in the rumen, via duodenal digesta, and blood transport into the different muscle tissues [4, 8]. The present paper discusses the concentration of the two compounds VA and *cis*-9,*trans*-11 CLA in the rumen, duodenal digesta, plasma, erythrocyte phospholipids and different tissues of beef at one sampling point (after slaughter) under two different feeding conditions. Increasing *cis*-9,*trans*-11 CLA content in meat and milk from ruminants depends on the understanding of the sequential processes involved in the biohydrogenation of dietary unsaturated fatty acids in the

rumen. This process could be modified to increase *cis*-9,*trans*-11 CLA and VA output from the rumen by manipulating the ruminant diet [1, 4, 8]. In the rumen, *cis*-9,*trans*-11 CLA is formed from isomerization of dietary 18:2n-6 during the first step of the biohydrogenation process [28]. Once the *cis*-9,*trans*-11 CLA is formed, biohydrogenation of the *cis*-9 bond occurs by microbial reductase (group A microorganisms) to form VA. The extent, to which VA is hydrogenated to stearic acid (18:0; group B microorganisms) depends on the conditions in the rumen [28–30]. Dietary 18:3n-3 also undergoes biohydrogenation by being first isomerized to a conjugated triene (*cis*-9,*trans*-11,*cis*-15 18:3), followed by reductions of double bonds at carbons 9, 15, and 11 to yield *trans*-11, *cis*-15 18:2, VA, and 18:0, respectively [14, 30]. The lipids in the pasture diet are high in 18:3n-3, and the biohydrogenation by rumen micro-organisms does not include *cis*-9,*trans*-11 CLA as an intermediate [28]. VA is formed during the biohydrogenation of 18:3n-3 and 18:2n-6 to 18:0, and therefore VA is a common intermediate in the metabolic pathway of both PUFAs [28]. In the present study, feeding pasture-based diets to German Holstein and German Simmental bulls resulted in significantly higher VA concentrations in duodenal digesta, plasma, erythrocyte PL compared to concentrate-fed animals. Furthermore, these accumulations of VA by pasture feeding compared to concentrate feeding bulls were also measured in liver and heart tissues, and subcutaneous fat. The significantly higher VA concentrations in the rumen and duodenal digesta on a pasture diet indicates, that ruminal biohydrogenation of the predominant fatty acid in pasture diets, 18:3n-3, led to the production of high amounts of VA in the rumen and duodenal digesta. However, the *cis*-9,*trans*-11 CLA concentrations in the rumen and duodenal digesta were not affected by the diet. Very recently, Fukuda et al. [31, 32] found two new strains of *Butyrivibrio fibrosolvens.* One strain (MDT-10) does not have a great ability to hydrogenate 18:2n-6 to VA, and the other strain (MDT-5) rapidly isomerizes 18:2n-6 and 18:3n-3 to *cis*-9,*trans*-11 CLA and to *cis*-9,*trans*-11,*cis*-15 18:3. The authors assumed that the introduction of MDT-10 to the rumen might increase the amount of absorbed VA and therefore increase the conversion of VA to *cis*-9,*trans*-11 CLA in tissues [31, 32]. Harfoot and Hazlewood [28] have shown that *cis*-9,*trans*-11 CLA is rapidly metabolized, while VA accumulates during biohydrogenation of PUFA in the rumen. Piperova et al. [33] confirmed these results and demonstrated that the ratio of total *trans* 18:1 to *cis*-9,*trans*-11 CLA was high, approximately 60:1, in the duodenal flow of cows fed low and high levels of forage. It is known that pasture feeding resulted in significantly higher concentrations of *cis*-9,*trans*-11 CLA in milk and intramuscular fat [1, 13, 20, 34, 35]. With pasture feeding, the duodenal flow of VA is higher, compared with low forage-containing diets, in which lipids are higher in linoleic acid [33, 36, 37]. Furthermore, Piperova et al. [33] and Sackmann et al. [37] demonstrated that the duodenal output of *trans* 18:1 fatty acids was high in *trans*-10 18:1 in diets with low forage contents. Under these conditions, shifts in the ruminal biohydrogenation pathway have occurred resulting in *trans*-10 18:1 replacing VA as predominant *trans* 18:1 fatty acids isomer leaving the rumen [33, 36, 38]. Finally, the amount of duodenal *trans*-10,*cis*-12 CLA isomer was significantly increased in cows fed diets with a low forage content [33].

Plasma is the transportation system of nutritional ingredients, and erythrocyte phospholipid fatty acids status reflects the overall long-term (erythrocytes 120 days half-time) lipid metabolism influenced by diet and rumen microbial activity. For blood lipids, phospholipids and cholesterol esters are the main components and account for over 95% of the total lipids in the plasma of ruminant animals. However, triglycerides and free fatty acids only represent $< 5\%$ and 1% of total plasma lipids, respectively [36]. PUFA that escape ruminal biohydrogenation are preferentially esterified to the plasma cholesterol esters and phospholipids [39]. The current study demonstrated that pasture feeding significantly increased the concentrations of *cis*-9,*trans*-11 CLA and VA both in the plasma and erythrocyte PL compared to concentrate feeding, with one exception *cis*-9,*trans*-11 CLA in erythrocyte PL of German Holstein bulls. The *cis*-9,*trans*-11 CLA and VA concentration was significantly higher (up to two times) on pasture in comparison with concentrate feeding, in German Simmental bulls. Loor et al. [40] investigated the rumen, blood and milk of Holstein cows fed diets with a high ratio of concentrate to forage (grass, hay) (65:35) supplemented with fish oil, linseed oil or sunflower oil. The authors also observed that significantly higher *cis*-9,*trans*-11 CLA contents in blood plasma with linseed oil supplements (high in 18:3n-3) compared with sunflower or fish oil supplements. However, the highest VA contents were detected in the blood plasma with fish oil compared with the other supplements. It is well known that a major proportion of the *cis*-9,*trans*-11 CLA found in ruminant tissues is formed through the activity of Δ^9-desaturase on VA [12, 30]. Kinetic studies of rumen biohydrogenation of 18:2n-6 acid to 18:0 have shown that *cis*-9,*trans*-11 CLA is a transient intermediate in the rumen, whereas VA is the intermediate that accumulates under these certain dietary conditions [30, 41, 42]. Furthermore, dietary addition of plant oils containing 18:3n-3 also increases the *cis*-9,*trans*-11 CLA content of ruminant fat, and intermediates in its pathway of biohydrogenation include VA but not *cis*-9,*trans*-11 CLA [10, 41]. Griinari's results [10] demonstrated that an estimated 64% of the CLA in milk fat was of

endogenous origin, and endogenous synthesis of CLA from *trans*-11 18:1 represented the primary source of CLA in the milk fat of lactating cows [13]. Kay et al. [13] reported the first study to examine the importance of endogenous synthesis of *cis*-9,*trans*-11 CLA in the milk fat of pasture-fed cows. The authors confirmed these results and showed that approximately 90% of *cis*-9,*trans*-11 CLA was produced endogenously. Several investigations confirmed that pasture feeding to dairy cows doubles the *cis*-9,*trans*-11 CLA content of milk fat [41]. Kraft et al. [34] and Collomb et al. [35] showed differences between the individual CLA isomer distributions in milk lipids of cows fed diets with concentrates rations in PUFA indoors and grazing cows in the Alps (Switzerland). For intramuscular fat, Dannenberger et al. [21] concluded that pasture feeding affected the distribution of individual CLA isomers in the muscle lipids. No related investigations for milk fat have been reported to establish the importance of endogenous synthesis of *cis*-9,*trans*-11 CLA in the intramuscular fat of ruminant tissues. It could be assumed that the contribution from endogenous synthesis is similar to that reported for milk because VA and Δ^9-desaturase are necessary requirements.

Martin et al. [43] and Ward et al. [44] showed that mammary gland and adipose tissue of ruminants have substantial Δ^9-desaturase activity. A linear relationship between VA and *cis*-9,*trans*-11 CLA has been observed for milk fat in a number of studies and across a wide range of diets reviewed by Griinari and Bauman [41]. This relationship has also been observed over a wide range of TVA concentrations, indicating a high capacity for endogenous synthesis of *cis*-9,*trans*-11 CLA [41]. Madron et al. [45] and Enser et al. [46] observed a linear relationship between the concentration of *cis*-9,*trans*-11 CLA and VA in muscle adipose tissue in beef. The extent, to which Δ^9-desaturase is responsible for *cis*-9,*trans*-11 CLA production in the muscle adipose tissue showed no differences in the index of Δ^9-desaturase activity in muscle fat among diet treatments. Meanwhile, Ntambi [47] showed that there was a repressive effect of long-chain PUFA on the activity of the Δ^9-desaturase. The results of our study confirmed that there was a correlation between *cis*-9,*trans*-11 CLA and VA concentrations in the rumen, duodenal digesta, blood and in the different tissues (Fig. 1). Piperova et al. [33] assumed that tissue level of *cis*-9,*trans*-11 CLA may not be a simple reflection of the proportions of these compound that escaped rumen biohydrogenation. The major source of tissue *cis*-9,*trans*-11 CLA should be the post absorptive synthesis via Δ^9-desaturation of TVA produced during rumen biohydrogenation of dietary PUFA. However, in our experiment, pasture feeding led to different accumulation rates of *cis*-9,*trans*-11 CLA in the tissues. Although pasture feeding significantly increased the *cis*-9,*trans*-11 CLA concentrations in liver and heart, diet had no effect on the *cis*-9,*trans*-11 CLA concentrations in the muscles and subcutaneous fat. The Δ^9-desaturase index was significantly lower in all investigated tissues and gave no additional information about the cause for the different accumulation rates of *cis*-9,*trans*-11 CLA in the different tissues. Smith et al. [48] concluded that Δ^9-desaturase index was not an indicator of absolute enzyme activity. Further research is needed to investigate the reasons of different accumulation of *cis*-9,*trans*-11 CLA in the beef tissues by pasture feeding and include the absolute enzyme activity measurement of Δ^9-desaturase. Very recently J.M. Griinari and K. Shingfield (2006, private communication) established that the *cis*-9,*trans*-11 CLA accumulation in ruminant lipids can be increased up to ten fold by feeding diets that enhance the production of VA in the rumen. The focus should be on ruminal formation of VA rather than *cis*-9,*trans*-11 CLA. It suggests that the most effective methods to enhance *cis*-9,*trans*-11 CLA concentration in ruminant products is to feed supplements containing VA or manipulate rumen biohydrogenation to increase the formation of VA. So, understanding the mechanism involved in the biosynthesis of *cis*-9,*trans*-11 CLA and other CLA isomers will allow scientists to design feeding strategies for enhancing the CLA concentration in ruminant products.

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