Exogenous effects of alpha-linolenic and linoleic acid on the fatty acid distribution and the regulation of lipid metabolism in ruminant tissues

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Preamble

Abbreviations

ACC	Acetyl-CoA carboxylase
ALA	Alpha-linolenic acid
AA	Arachidonic acid
CHD	Coronary heart disease
CLA	Conjugated linoleic acid
COX	Cyclooxygenase
CVD	Cardiovascular disease
CytP450	Cytochrome P450
Δ6d	Delta-6 desaturase
DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DTA	Docosatetraenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acids
FFA	Free fatty acids
GCB	German corned beef sausages
LA	Linoleic acid
LC PUFA	Long chain polyunsaturated fatty acids
LOX	Lipoxygenase
MLD	*Longissimus* muscle
MUFA	Monounsaturated fatty acids
PL	Phospholipids
PSB	ProSafeBeef
PUFA	Polyunsaturated fatty acids
SAT	Subcutaneous adipose tissue
SCD	Stearoyl-CoA desaturase
SDA	Stearidonic acid
SFA	Saturated fatty acids
TG	Triglycerides
TMR	Total mixed ration
TSS	Tea sausage spread
TVA	*Trans* vaccenic acid, C18:1*trans*-11

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List of Publications and Presentations

(1) Herdmann, A., Nuernberg, K., Martin, J., Nuernberg, G. and Doran, O. Effect of dietary fatty acids on expression of lipogenic enzymes and fatty acid profile in tissues of bulls. *Animal* **2010**, 4:5, 755-762.

(2) Herdmann, A., Martin, J., Nuernberg, G., Dannenberger, D. and Nuernberg, K. How do *n*-3 fatty acid (short-time restricted *vs.* unrestricted) and *n*-6 fatty acid enriched diets affect the fatty acid profile in different tissues of German Simmental bulls? *Meat Science* **2010**, 86, 712-719.

(3) Herdmann, A., Martin, J., Nuernberg, G., Dannenberger, D. and Nuernberg, K. 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. *Journal of Agriculture and Food Chemistry* **2010**, 58, 8314-8321.

(4) Herdmann, A., Martin, J., Nuernberg, G., Doran, O., Nuernberg, K. Effect of dietary fatty acids on expression of lipogenic enzymes and fatty acid profile in tissues of bulls. Abstract and poster presentation at the 9th congress of the International Society of the Study of Fatty Acids and Lipids (ISSFAL) in Maastricht, Proc ISSFAL, **2010**, 85.

(5) Herdmann, A., Martin, J., Nuernberg, G., Dannenberger, D., Nuernberg, K. Dietary *n*-6 and *n*-3 fatty acids alter the fatty acid composition of tissues and the fate of beneficial fatty acids during processing. Short communication and poster presentation at the 56th International congress of Meat Science and Technology (ICOMST) **2010** in Korea, Proc. 82.

(6) Herdmann, A. Recent research on CLA – An overview. Presentation at the EU-Project Meeting "ProSafeBeef" in Clermont-Ferrand, **2007**.

(7) Herdmann, A. Investigation of lipid metabolism in ruminants. Presentation at the Leibniz Institute for Farm Animal Biology in Dummerstorf, Germany at the 4th EU-Project Meeting "ProSafeBeef" **2008**.

(8) Herdmann, A. Investigation of lipid metabolism in ruminants. Presentation at the Leibniz Institute for Farm Animal Biology in Dummerstorf, Germany at PhD student day **2008**.

Chapter 1

General Introduction

1. General Introduction

Diet-related chronic diseases such as coronary heart diseases, metabolic syndrome and cancer represent the single largest cause of morbidity and mortality in the United States and most of the Western countries (Cordain *et al.*, 2005). Additionally, 60% of the 56.5 million deaths worldwide are caused by chronic diseases and half of the deaths are attributable to cardiovascular diseases (CVD) (Givens *et al.*, 2006). Many studies are ongoing to investigate the dietary and lifestyle factors that are the reason for the development of the so-called Western diseases. In the last decades the consumption of animal products has increased, and consequently the fat intake and the energy density increased (Givens *et al.*, 2006). A high intake of saturated fatty acids (SFA) is positively associated with coronary heart disease and mortality rate (Rioux and Legrand, 2007). In contrast to SFA, some unsaturated fatty acids are recognised as beneficial to health. A number of studies demonstrated hypo-cholesterolemic effects of monounsaturated fatty acids (MUFA) and some polyunsaturated fatty acids (PUFA) (Demaison and Moreau, 2002). The particular health benefit has been linked to *n*-3 PUFA which are essential for normal growth and development (Simopoulos, 1991). In addition to *n*-3 PUFA, an increasing attention has been paid to another PUFA, namely conjugated linoleic acids (CLA). CLA have been demonstrated to enhance the immune system, to reduce body weight and to decrease the risk of cancer (Tricon *et al.*, 2005; Bhattacharya *et al.*, 2006). The main natural source of *cis*-9,*trans*-11 CLA is meat and milk of ruminants (Song and Kennelly, 2003). According to national and international dietary guidelines, SFA contribution to dietary energy intake should be not more than 10% of total daily dietary energy, and the inclusion of MUFA into human diet should be up to 15% of total daily energy and to a maximum of 10% of total daily energy for PUFA (Wahrburg, 2004). The DACH-Association recommended a fat intake of 30% based on the total daily energy intake. Not more than 10% of these should be saturated fatty acids, approximately 7-10% should be PUFA and the remaining 10% are recommended to be monounsaturated fatty acids (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, und Schweizerische Vereinigung für Ernährung [DACH, 2008]). One way to achieve this is to decrease SFA and increase MUFA and PUFA content of food and of meat in particular.

Ruminant meat is higher in SFA when compared to pork or poultry meat (Valsta *et al.*, 2005) but beef is a high-quality product because it is a source of high biological value protein

and of a well-balanced ratio of essential amino acids. Furthermore, the essential minerals and trace elements such as iron, zinc and cooper are highly bioavailable (Biesalski, 2005). Moreover, beef is a source for the vitamins A, B_6, B_{12}, D, and E and for long-chain *n*-3 fatty acids (Nuernberg, 2009; Mahecha *et al.*, 2009).

In this work, the focus is on the exogenous effect of different PUFA in terms of an animal diet supplementation (linoleic acid enriched *vs.* α-linolenic acid enriched diet) on the fatty acid distribution, and the effect on protein expression of selected lipogenic enzymes in different tissues of bulls.

N-6 and *n*-3 fatty acids in human health and nutrition

It is well known that the amount and type of fat is closely related to human health. An intake of more than 15% SFA of the daily energy intake is positively associated with coronary heart disease (CHD), mortality rate, and reduced anti-inflammatory properties of the high-density lipoprotein (Rioux and Legrand, 2007) as well as increased blood cholesterol concentrations (Williams, 2000). It should be noted, however, that new systematic reviews recommend further evaluation of the relationship between dietary pattern and CHD (Siri-Tarino *et al.*, 2010, Mente *et al.*, 2009, Mozaffarian *et al.*, 2010).

However, unsaturated fatty acids, monounsaturated and polyunsaturated, are recognized as beneficial to human health and, in the case of PUFA, as essential. Populations consuming the Mediterranean diet, known to be high in MUFA and low in SFA, have clearly lower incidences of CVD compared with populations that have high intakes in SFA (Lada and Rudel, 2003). The essential PUFA are linoleic acid (C18:2*n*-6, LA) and α-linolenic acid (C18:3*n*-3, ALA) (Figure 1).

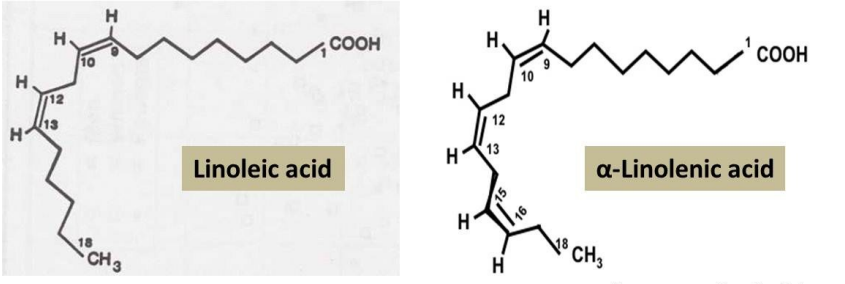

Figure 1: Chemical structure of linoleic acid and α-linolenic acid

The essentiality of LA and ALA as precursors for the physiologically important long-chain PUFAs is based on the lack among all mammals of enzyme systems to introduce a double bond in the fatty acid distal to C_{10} (Simopoulos, 2008; Palmquist, 2009). Figure 2 demonstrates the pathway of ALA and LA to the long-chain products and the enzymes that are involved in this process. The same enzyme system is used for the conversion of LA and ALA. but it prefers *n*-3 compared to *n*-6 fatty acids. Otherwise, a high intake of LA affects the elongation and desaturation of ALA (Simopoulos, 2008). Furthermore, the essentiality of both precursors is confirmed by the fact that an inter-conversion of the products is not possible (Palmquist, 2009).

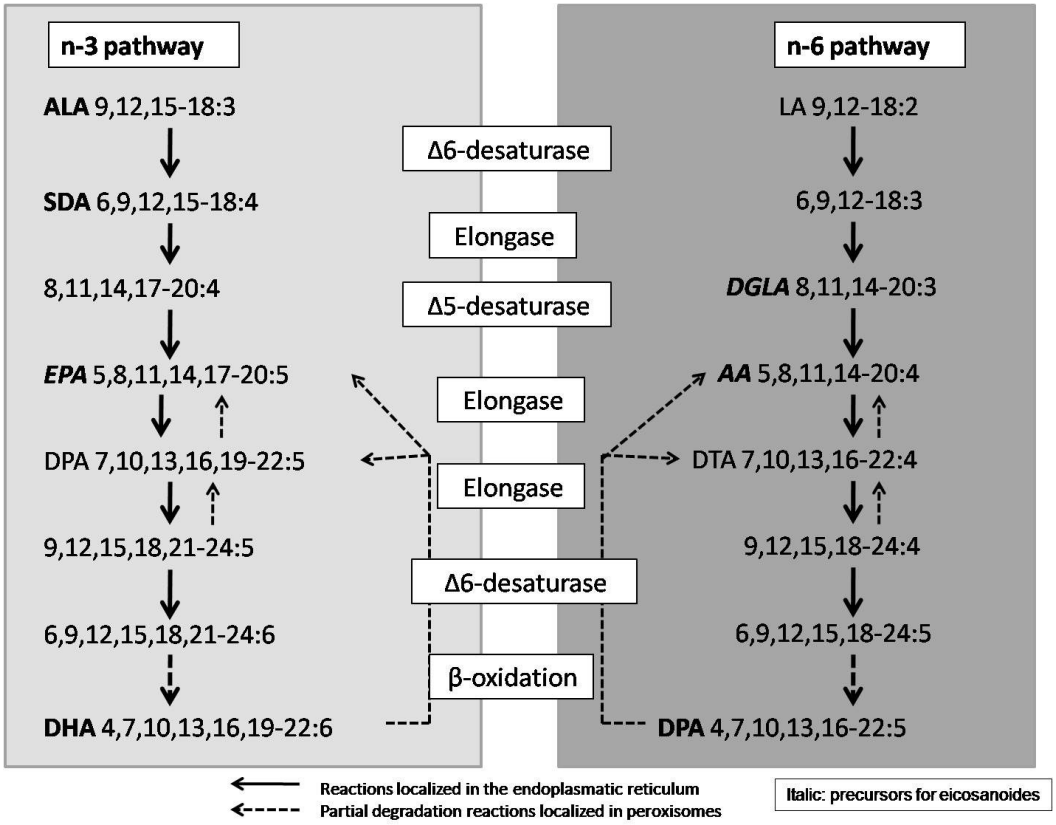

Figure 2: *N*-3 and *n*-6 pathway to form the physiologically important LC PUFAs (adapted from Palmquist, 2009)
ALA-alpha-linolenic acid; SDA-stearidonic acid; EPA-eicosapentaenoic acid; DPA-docosapentaenoic acid (*n*-3); DHA-docosahexaenoic acid; LA-linoleic acid; DGLA-dihomo-γ-linolenic acid; AA-arachidonic acid; DTA- docosatetraenoic acid; DPA-docosapentaenoic acid (*n*-6)

LC PUFA are primarily found in phospholipids at oil-water interfaces and are important components of cell membranes to determine the cell membrane fluidity, because of the unsaturation and therefore the influence on the transition temperature of a membrane. They

are mainly found on position two in membrane phospholipids, and the position-specific phospholipase PLA_2 releases the LC PUFA for the conversion to eicosanoids, biologically active mediators with 20 carbon residues.

Three classes of enzymes, COX, LOX, and CytP450, catalyse the reaction of DGLA, AA, and EPA (as precursors for eicosanoids) to prostaglandins, thromboxanes, and prostacyclines. These bioactive molecules are involved in different physiological processes whereas the eicosanoids of the *n*-3 series have often an opposing effect, or are less intense than the eicosanoids of the *n*-6 family (Palmquist, 2009). The main processes where eicosanoids of the *n*-3 and *n*-6 series are involved are the immune and inflammatory response, cardiovascular disease, carcinogenesis, development and function of the brain and behaviour, and skeletal growth (Palmquist, 2009; Simopoulos, 2008).

The cytokines formed from AA (*n*-6) are inflammatory cytokines stimulating white blood cells (Simopoulos, 2008). An increased intake of EPA and DHA replaces AA, and more cytokines from the *n*-3 series with anti-inflammatory effects will be produced. The positive effects are mainly localized when the disease is present, such as in the case rheumatoid arthritis (Palmquist, 2009) and asthma (Simopoulos, 2008).

EPA and DHA have favourable effects on CVD through anti-atherosclerotic actions. With more distinct evidence of *in vitro* studies than in human studies, (probably due to transient endothelial activations) DHA and EPA reduce the concentrations of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α as well as the mRNA concentrations of pro-atherosclerotic growth factors and monocyte chemotactic protein-1 in mononuclear cells. Cardioprotective effects of the dose dependent application of DHA and EPA in human studies are on arrhythmias, endothelial function, inflammation, thrombosis as well as the modulation of both the fasting and postprandial blood lipid profile (Palmquist, 2009). As a novel risk factor for death from CVD the *n*-3 index is proposed. It is the sum of EPA+DHA in erythrocyte membranes. This sum has been demonstrated to reflect the human myocardial EPA and DHA content.

Anti-carcinogenic effects of *n*-3 FA, and especially DHA and EPA were found in animal and *in vitro* studies. The following molecular mechanisms seem to be included in these pathways: the inhibition of AA-derived eicosanoid biosynthesis; influences on transcription factor activity, gene expression, and signal transduction pathways; alteration of estrogen metabolism; increased or decreased production of free radicals and reactive oxygen species; and mechanisms involving insulin sensitivity and membrane fluidity. Colomer *et al.*

(2007) published a systematic review about *n*-3 fatty acid supplementation in patients with advanced cancer (tumours of the upper digestive tract and pancreas) and weight loss. They concluded that the supplementation of more than 1.5 g EPA per day over a prolonged time period (at least 8 weeks) has beneficial effects on clinical, biochemical and quality of life parameters.

More research is needed to clarify the effect of *n*-3 PUFA on the brain function and behaviour. From present research data a subcommittee of the American Psychiatric Association endorsed the recommendation from the American Heart Association to supplement 1 g/d of EPA+DHA to patients with depression and bipolar disorder, especially because of the high rate of co morbid CVD in psychiatric patients (Palmquist, 2009).

Osteoporosis is a disease with increasing incidence in the elderly population. 70% of bone mass is under the direct and indirect control of genetic factors. Other factors are nutrition, physical activity and the body mass index. Animal experiments have given evidence that *n*-3 LC PUFA influence bone formation and bone loss, and in studies with elderly people an inverse relationship between the ratio of LA to ALA and the bone mineral density were found. In conclusion, *n*-3 fatty acids and the ratio of *n*-6 to *n*-3 fatty acids play an important role in affecting the skeletal growth (Simopoulos, 2008).

This preceding explication emphasises the importance of *n*-3 and *n*-6 fatty acids, especially the *n*-3 fatty acids, in the physiology of humans to prevent diseases and for all domestic and commercially important food species to increase the concentration of *n*-3 PUFA in the compartments of these species, which are important for the human nutrition. Table 1 represents recommendations of different institutions for human's daily intake of ALA, LA and the main *n*-3 LC PUFA EPA and DHA.

Considering the fatty acid cluster of the daily intake of infants, children and adolescents, the amount of SFA is excessive and the amount of MUFA and PUFA is considerably lower than the recommendation (DGE, Ernährungsbericht, 2008). The ratio of LA/ALA in the German diet is with more than 7:1 clearly above the recommended ratio of ≤5:1 but the intake of EPA+DHA is for men approximately 250 mg/d and for women 150 mg/d (DGE, Ernährungsbericht, 2004).

Table 1: Recommendations for the daily intake of LA, ALA and DHA+EPA of different institutions

	LA	ALA	*n*-6/*n*-3	DHA+EPA
DGE (2008)	2.5 E%	0.5 E%	5:1	-
EFSA (2010)	4.0 E%	0.5 E%	8:1	250 mg/d
Australia/New Zealand (2005) *Men:*	13 g/d	1.3 g/d	10:1	160 mg/d*
Women:	8 g/d	0.8 g/d		90 mg/d*
WHO (2003)	2.0 E%	≥ 0.5 E%	4:1	0.25-2.0 g/d[#]
ISSFAL (1999-2004)	4.4 g/d (2.0 E%) *Upper limit:* 6.7 g/d (3.0 E%)	1.7-2.4 g/d (0.7-1.0 E%)		220 mg/d[+]

* DHA+EPA+DPA
[#] for secondary prevention of CHD
[+] DHA
E% - percentage of daily energy intake

In the last decades the consumption of animal products, and consequently the fat and energy intake increased all over the world (Givens *et al.*, 2006); whereas in Germany meat consumption (59.5 kg/head/year in 2006 and assumed 60.5 kg/head/year in 2009) has been relatively constant since 2006 (Bundesverband der Deutschen Fleischwarenindustrie). The daily consumption of meat, sausages and meat products in Germany is for men 103 g and for women 53 g (Max Rubner-Institut, Bundesforschungsinstitut für Ernährung und Lebensmittel, 2008) and of fish only 15 g and 13 g, respectively. Therefore, it is important to enhance the beneficial fatty acids, *n*-3 PUFA and *n*-3 LC PUFA in meat, and to produce meat products and sausages without a loss of these beneficial fatty acids due to production processes.

Trans fatty acids (TFA) and conjugated linoleic acids (CLA) in human health and nutrition

Two major sources of TFA are known. Firstly, the concentration of TFA arises when vegetable oil is partially hydrogenated. This is the case in the industrial production of margarine (Gebauer *et al.*, 2007), snacks and fast food products (Bendsen *et al.*, 2011), for example. In such products the amount of TFA ranges between 10 and 40% (Gebauer *et al.*, 2007). The second source of TFA is the ruminant fat, found in meat, milk and products. Due to the incomplete biohydrogenation of dietary PUFA the amount of TFA ranges between 3 and 8% of total fat (Gebauer *et al.*, 2007; Field *et al.*, 2009; Brouwer *et al.*, 2010). The

species of TFA in industrial and ruminant fats are similar and the majority of TFA are isomers of the C18:1*trans* fatty acid with approximately 80-90% (Brouwer *et al.*, 2010; Gebauer *et al.*, 2007). The difference between industrial and ruminant TFA is found in the proportion of the isomers (Brouwer *et al.*, 2010). The major TFA in partially hydrogenated oil is the elaidic acid (C18:1*trans*-9) and the C18:1*trans*-10. Furthermore, the isomers C18:1*trans*-8, C18:1*trans*-11 (*trans*-vaccenic acid, VA) and the *trans* isomers of ALA are contained in smaller amounts (Brouwer *et al.,* 2010). The VA, positional and geometric isomer of the oleic acid, is with 50-80% the predominant TFA in ruminant fat. The conjugated linoleic acid *cis*-9,*trans*-11 is contained in small amounts in meat and milk of ruminants and could be also formed from VA in tissues (Brouwer *et al.*, 2010; Field *et al.*, 2009; Gebauer *et al.*, 2007).

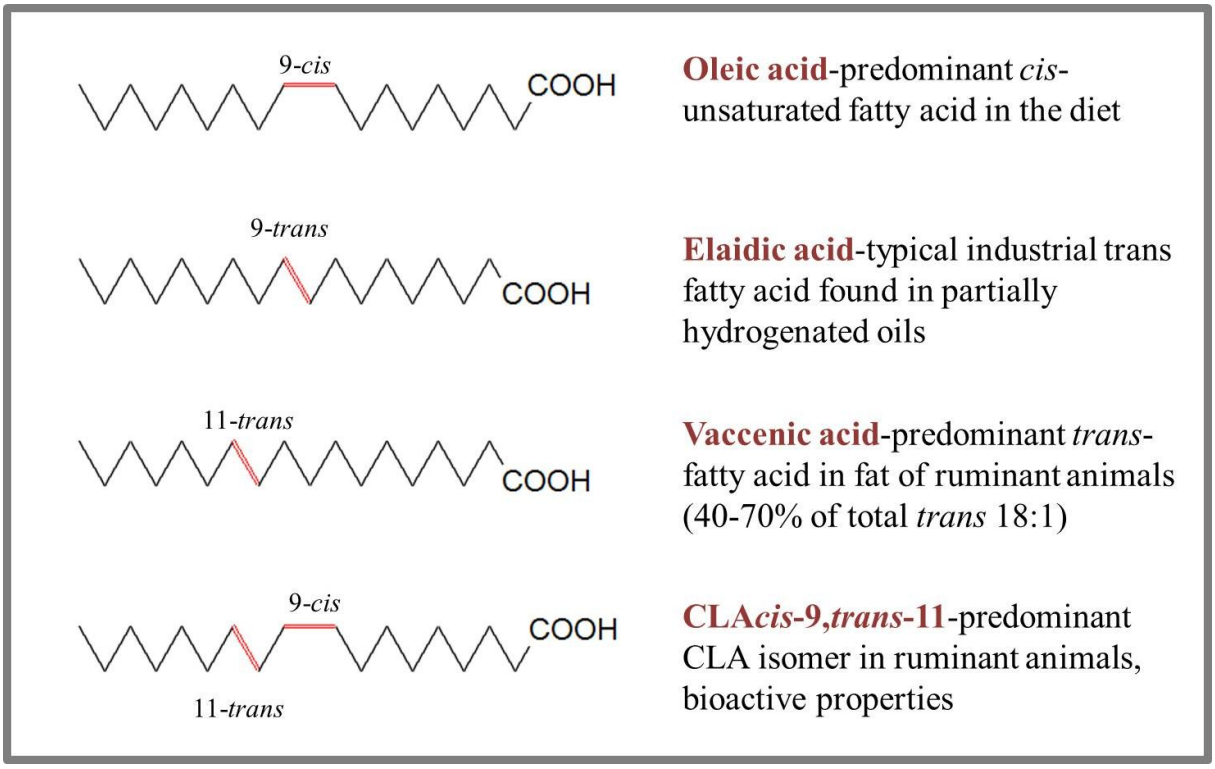

Figure 3: Oleic acid and major *trans* isomers in partially hydrogenated oil and ruminant tissues (adapted from Gebauer *et al.*, 2007 and Brouwer *et al.*, 2010)

Two pathways are known for the production and enrichment of CLA*cis*-9,*trans*-11 in tissues (Figure 4). After biosynthesis by bacteria in the rumen it can be directly incorporated into tissues. The other way is the desaturation of TVA on position 9 in tissues by the enzyme Δ-9 desaturase (SCD) in ruminants, rodents and humans (Field *et al.*; 2009; Tanaka, 2005).

This endogenous synthesis of CLA*cis*-9,*trans*-11 in tissues by SCD is more pronounced than the direct incorporation from rumen to tissues (Griinari *et al.*, 2000).

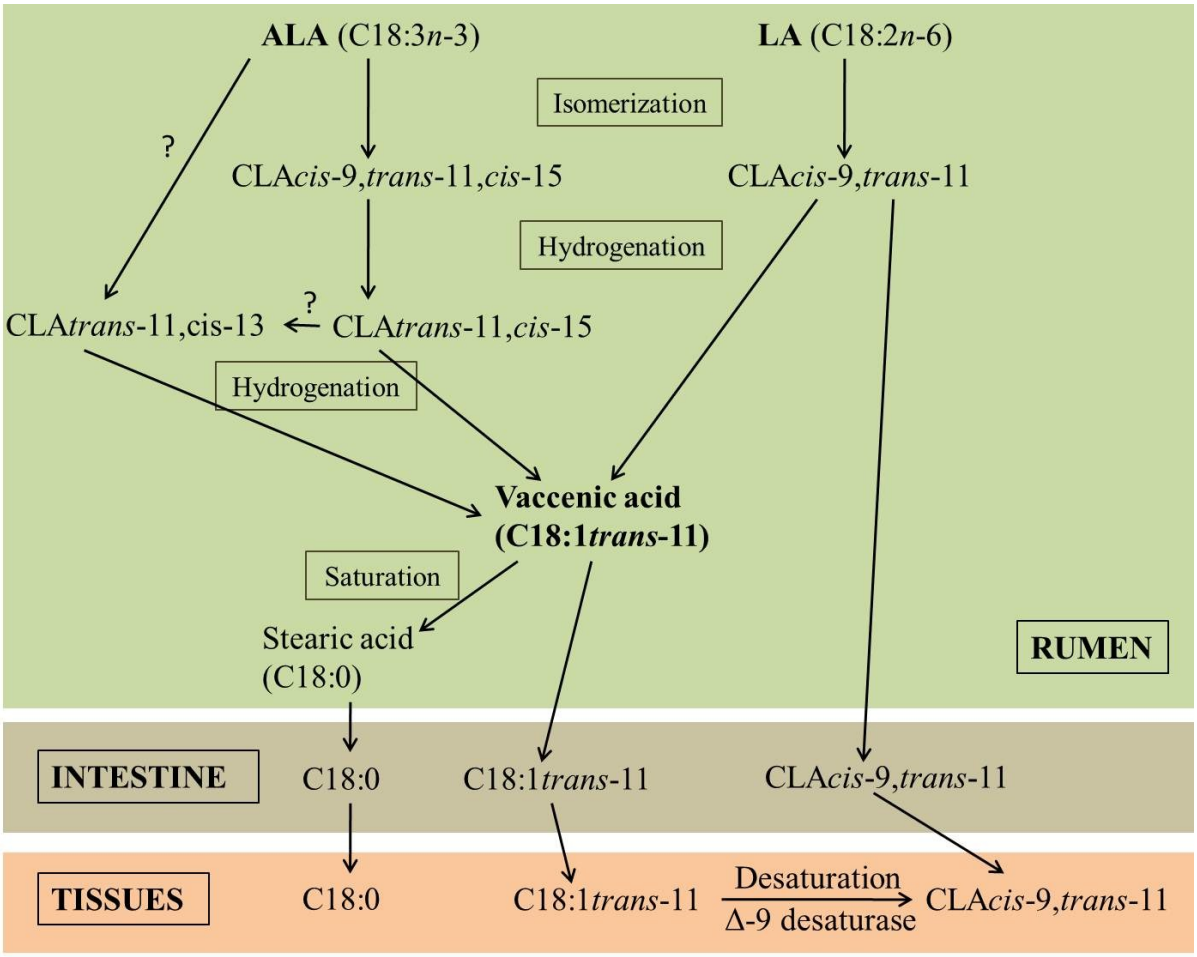

Figure 4: Formation and incorporation of VA and CLA*cis*-9,*trans*-11 outgoing from ALA and LA (adapted from Field, 2009; Tanaka, 2005; Collomb *et al.*, 2004; Kraft *et al.*, 2003)

The daily intake of *trans* fatty acids with the diet should be less than 1% of the energy intake (WHO/FAO, 2003; DGE, 2008). This recommendation is given based on unfavourable effects of TFA, but it should be differentiated between ruminant and industrial TFA. Varying results for the effects of industrial and ruminant TFA arise out of several studies. While Brouwer *et al.* (2010) concluded that, independent from the source, TFA raise the concentration of LDL and lower the concentration of HDL, resulting in a higher ratio of LDL:HDL and an increasing risk for the development of CHD. They also concluded that the effect of ruminant produced TFA is less compared to TFA industrial origins. Sanders (2009)

reviewed the metabolic effects of fatty acid intake in the human body. The results are supportive of TFA, showing with convincing evidence an increased risk for postprandial lipaemia and elevated concentrations of the coagulation factor VII (EC 3.4.21.21) as an endopeptidase, and an increased risk is possible for inflammation and an impaired endothelial function. Considering especially the ruminant TFA, in epidemiological, clinical, and rodent studies no relationship was found between VA or the sum of TFA and heart or cardiovascular disease, insulin resistance, inflammation (Field *et al.*, 2009) , or the risk of coronary heart disease (Bendsen *et al.*, 2011).

The effects of CLA should be considered in a separate way. They have been shown to have anti-inflammatory, anti-carcinogenic, anti-adipogenic, anti-atherogenic, and anti-diabetogenic properties (Bhattacharya *et al.*, 2006). Most of these effects were detected and investigated in animal models. Roy *et al.* (2007) found a decreased aortic lipid deposition in CLA*cis*-9,*trans*-11+TVA fed white rabbits, but also a significantly decreased concentration of HDL. This result is confirmed in humans by Steck *et al.* (2007) and Lambert *et al.* (2007). Ribot *et al.* (2007) investigated the effect of CLA*trans*-10,*cis*-12 feeding in hamsters and found a significant decrease in white adipose tissue and a decreased leptin mRNA expression. In humans a decrease in the body fat mass and an increase in the lean body mass were found by Gaullier *et al.* (2007).

Possibilities to alter the fatty acid composition in farm animal tissues by diet

The awareness of consumers considering the relationship between health and diet is increasing. Therefore the interest in the nutritional quality of food is becoming more important. Since *n*-3 PUFA have been demonstrated to have important roles in reducing health risks, such as for CHD and cancer, strategies are developed to increase the amount in beef as an important food source, because the consumption of fish and fish products as the main animal source for *n*-3 fatty acids is low (Scollan *et al.*, 2006).

There are different sources of fat in beef: the membrane fat (as phospholipid), the intermuscular fat between the muscles, intramuscular fat (IMF) and subcutaneous adipose tissue (Scollan *et al.*, 2006). The IMF content of meat is determined by the synthesis and degradation of fat within muscle, rather than from one specific energy metabolic pathway (Gondret *et al.*, 2004). Meat with an intramuscular fat content between 1-5% is denoted as lean meat and is being accepted as ‘low in fat’ (Nuernberg, 2009). The IMF is determined by a high genetic variability (Scollan *et al.*, 2006).

Diet, species, fatness, age, weight, depot site, gender, breed, season and hormones can affect the fatty acid composition of adipose and muscle tissues but the most effective way to manipulate the lipid and its fatty acid composition is through the strategic use of forages and dietary lipids in the nutrition of farm animals (Nuernberg, 2009). Table 2 shows the fatty acid composition of muscle and fat tissues as normally consumed. The species differences are clearly demonstrated, especially for LA and ALA. Both fatty acids are found in higher concentrations in pig tissues because they pass unchanged through the pig's stomach; whereas the biohydrogenation by bacteria in the rumen leads to an almost complete degradation of these fatty acids into saturated and monounsaturated fatty acids. Additionally, the biohydrogenation is with 85-100% for ALA higher than for LA with 70-95% (Wood *et al.*, 2008). It is obvious that the ratio of *n*-6/*n*-3 PUFA in pig muscle is higher than in cattle, despite the higher relative concentration of LA and ALA (Wood *et al.*, 2008).

Table 2: Fatty acid composition (%) and content (% total fatty acids in adipose tissue and muscle) of loin steaks/chops in pigs and cattle (adapted from Wood *et al.*, 2008)

	Adipose tissue		**Muscle**	
	Pigs	**Cattle**	**Pigs**	**Cattle**
C14:0	1.6[a]	3.7[b]	1.3[a]	2.7[b]
C16:0	23.9[a]	26.1[b]	23.2[a]	25.0[b]
C16:1*cis*-9	2.4[a]	6.2[b]	2.7[a]	4.5[b]
C18:0	12.8	12.2	12.2[a]	13.4[b]
C18:1*cis*-9	35.8	35.3	32.8[a]	36.1[b]
C18:2*n*-6	14.3[a]	1.1[b]	14.2[a]	2.4[b]
C18:3*n*-3	1.4[a]	0.5[b]	0.95[a]	0.70[b]
C20:4*n*-6	0.2	ND	2.2[a]	0.63[b]
C20:5*n*-3	ND	ND	0.31[a]	0.28[b]
Ratio of *n*-6/*n*-3	7.6	2.3	7.2	2.1
Total	65.3	70.0	2.2	3.8

a,b-Means with different superscripts are significantly different ($P<0.05$)

Different nutritional strategies are investigated to enhance the amount of beneficial *n*-3 PUFA, the precursor ALA as well as the long-chain *n*-3 products, in tissues of cattle.

Fish oil and algae:

Fish oil and algae are good sources for *n*-3 LC PUFA because algaes are the primary producers of LC PUFA, and are the food for fish (Givens *et al.*, 2006; Scollan *et al.*, 2006). Feeding supplemented concentrates with fish oil or micro-algae to steers increased the content of EPA and DHA in muscle lipids (Nuernberg, 2009). Probably due to the lower biohydrogenation of LC PUFA descending from algae compared to fish oil, the supplementation of the diet with algae led to a higher deposition of EPA and DHA in tissues (Givens *et al.*, 2006). The disadvantage of meat from animals that received diets enriched with fish oil and/or algae could be the lower shelf life based on the lower oxidative capacity, because of the high content of PUFA and the impaired flavour, and there is also a discussion about the sustainability of fish oil supplies (Givens *et al.*, 2006).

Fresh grass and grass silage:

In the terrestrial ecosystem, plants are the primary source of *n*-3 PUFA due to their ability to produce ALA *de novo* (Scollan *et al.*, 2006). Fresh grass is an important feed for beef cattle in northern Europe. Compared to the grass silage, where 27-73% of the fatty acids are presented as free fatty acids in fresh grass, there are 98% available in esterified form (Nuernberg, 2009). The amount of ALA, the main fatty acid in grass, depends on several factors: species, cutting date, cutting interval, growth stage and conservation. The amount of ALA in young and fresh grass is very high in addition to the amount of protein, which leads to problems in the optimization of a balanced diet for cattle (Givens *et al.*, 2006). A decreasing effect on the proportion of SFA and an increase of *n*-3 fatty acids in the muscle fat of steers is reached with a decreased amount of concentrate and an increased amount of grass intake with the diet. Heifers fed a diet with an increasing grazing phase before slaughter had a significantly decreased SFA concentration and increased *n*-3 PUFA proportion in muscle and subcutaneous fat (Nuernberg, 2009).

Plant oil and oil seed supplements:

Different dietary lipid sources were used to influence the meat quality of pigs, for example soybean oil (high in LA), palm kernel oil (high in lauric and myristic acid), palm oil (high in palmitic and palmitoleic acid) and linseed/flaxseed as oil seed or plant oil supplement (Wood *et al.*, 2008). The fatty acid composition of pig tissues can easily be manipulated, because in monogastric animals the dietary lipids pass unchanged through the digestive tract

and, as a result, can be absorbed and deposited into tissues. Therefore, an intake of linseed oil, high in ALA, leads to an increased deposition in tissues of pigs (Wood *et al.*, 2008).

In cattle it is unlikely to be more difficult to change the lipid profile in tissues due to the biohydrogenation by bacteria in the rumen. Nevertheless, many studies have investigated the supplementation of concentrates with plant oils, especially with linseed oil or seeds to enhance the concentration of *n*-3 PUFA despite the biohydrogenation, and the fact that these essential fatty acids are incorporated preferentially into muscle, rather than storing them in adipose tissue (Wood *et al.*, 2008) is particularly advantageous to improve the nutritional quality of beef.

Linseed oil is the plant oil with the highest amount of ALA. Higher ALA level in tissues lead to an increase in *de novo* production of EPA, but the contribution is low because of the very low conversion rate (Givens *et al.*, 2006). The deposition of ALA can be improved by additional concentrate supplementation with rapeseed oil or rapeseed cake because it contains approximately 8-10% ALA. A positive effect of enhancing the *n*-3 PUFA content in ruminant muscle is lowering the ratio of *n*-6/*n*-3 which is recommended for the human nutrition (Nuernberg, 2009).

In summary, there are different opportunities to accumulate ALA in ruminant tissues by feeding plants, like fresh grass or grass silage, and the supplementation of linseed and rapeseed in form of oil, seeds or cake. The *de novo* formation of *n*-3 LC PUFA proceeding from ALA to its accumulation in meat has been observed, despite the low conversion rate. A high intake of LA and ALA results in a higher production of *trans* fatty acids, especially VA and the CLA*cis*-9,*trans*-11. More research is needed on single *trans* fatty acid isomers to add clarity, considering the health impact of ruminant *trans* fatty acids.

Feed restriction and compensatory growth:

Compensatory growth seems to have profound effects on the rate of growth and body composition of animals (Papstein *et al.*, 1991; Hornick *et al.*, 1998). Different factors influence the compensatory growth: the nature of restricted diet, the severity and duration of undernutrition, the stage of development of the animal body at the beginning of undernutrition, the genetic background, gender, and the pattern of realimentation. Studies have shown that changing the diet type as well as the amount of feed tends to result in a greater compensatory growth, especially in cattle (Lawrence and Fowler, 2002).

Studies in pigs revealed that compensatory growth influences the deposition of adipose and lean tissue and by this, the carcass composition (Donker *et al.*, 1986; Hornick *et al.*, 2000). An undernutrition in cattle caused an underexpression of genes encoding muscle structural proteins, extracellular matrix and muscle metabolic enzymes, which especially belong to the metabolic glycolytic pathway. After refeeding the animals, the expression of most of the investigated genes was restored (Hocquette *et al.,* 2010). An increased intramuscular fat content is associated with an improved eating quality of meat (Wood *et al.*, 2008). Therefore, it is of interest to increase the IMF in beef meat and simultaneously the concentration of beneficial fatty acids, as *n*-3 PUFA. The aim of the short restriction time in the experiment with German Simmental bulls was to induce a higher deposition of intramuscular fat.

Lipogenic enzymes and fatty acids

The tissue fatty acid biosynthesis is regulated by different lipogenic enzymes. The focus of the following section is given to three key enzymes in the tissue fatty acid synthesis.

Acetyl-CoA carboxylase (ACC, EC 6.4.1.2):

ACC is the key enzyme controlling the *de novo* biosynthesis of SFA by catalyzing the first step from acetyl-CoA to malonyl-CoA (Hardie, 1989). It is a biotin-containing multifunctional enzyme with several subunits and contains the catalytic function of biotin carboxylase, transcarboxylase, biotin carboxyl carrier protein, and the regulatory allosteric site. ACC is regulated by phosphorylation, citrate and fatty acyl-CoA and is localized in the cell cytosol. Figure 5 illustrates major pathways of fatty acid and cholesterol synthesis in mammals. The connection between citrate cycles and the *de novo* formation of fatty acids is demonstrated. The ACC is next to the synthesis of fatty acids for the formation of triacylglycerol, also involved in the formation of cholesterol esters.

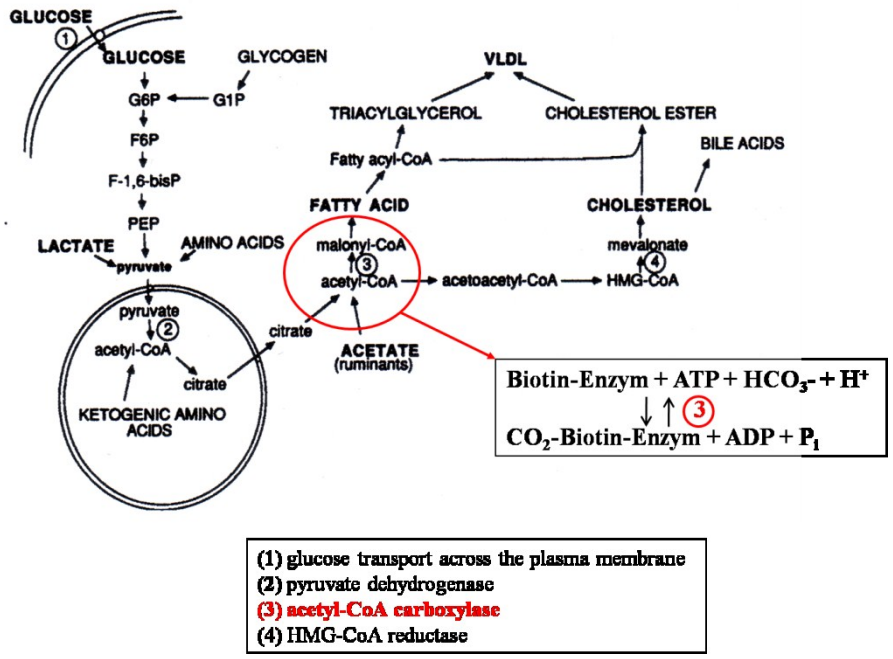

Figure 5: Major pathways of fatty acid and cholesterol synthesis in mammals with special consideration to the ACC pathway (adapted from Hardie, 1989)

Stearoyl-CoA desaturase (SCD, EC 1.14.19.1):

SCD as the key enzyme in the *de novo* synthesis of monounsaturated fatty acids is a microsomal enzyme which catalyses the insert of a *cis*-double bond in the Δ9 position between carbon C_9 and C_{10} in a saturated fatty acid in tissues. The preferred substrate for SCD is palmitoyl-CoA and stearoyl-CoA, and they will be converted to palmitoleoyl-CoA and oleoyl-CoA (Ntambi and Miyazaki, 2003; Kim *et al.*, 2002). Another substrate for the insertion of a *cis*-double bond in the Δ-9 position is the C18:1*trans*-11 (TVA), the product of bacterial biohydrogenation in the rumen. The product is the CLA*cis*-9,*trans*-11 (Smith *et al.*, 2009).

Four isoforms of SCD are known in mice with different tissue distributions. SCD-1 is regulated by dietary factors. The importance of SCD is founded in the physiological potential to affect a variety of key physiological variables such as insulin sensitivity, metabolic rate and adiposity due to the formation of MUFA in tissues. MUFA regulate the food intake in the brain, signal transduction, and cellular differentiation including neuronal differentiation (Ntambi and Miyazaki, 2003; Miyazaki *et al.*, 2003). For cattle, two isoforms of SCD are identified (Chung *et al.*, 2000; Lengi and Corl, 2007). Bovine SCD-1 was detected in adipose tissue with a threefold higher mRNA expression in female than in male animals (Chung *et al.*, 2000). The second isoform in cattle appears to be an ortholog of human SCD-5 rather than a

homolog of the other known SCD isoforms in different species and in cattle (Lengi and Corl, 2007).

In previous studies PUFA, like LA and ALA, have shown an inhibitory effect on SCD. These studies were made with mice, and the inhibitory effect was the repression of SCD mRNA expression, especially of SCD-1 (predominantly expressed in lipogenic tissues (Ntambi *et al.,* 1988) and the decrease of mRNA stability, especially for SCD-2 (predominantly expressed in brain and neuronal tissues) (Ntambi *et al.,* 1988; Clarke and Jump 1994, Jump and Clarke 1999, and Ntambi, 1999). Other FA such as SFA and MUFA did not show similar effects (Nakamura *et al.* 2004). It could be possible that *n*-3 is a more potent inhibitor of SCD compared to *n*-6. Waters *et al.* (2009) 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.

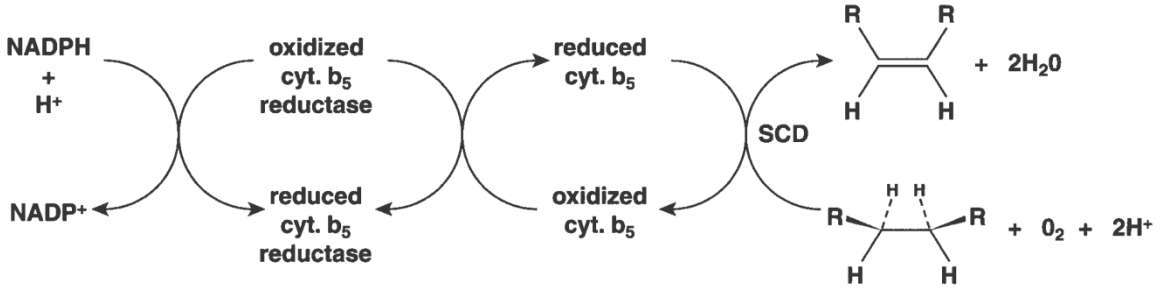

Figure 6: The pathway of electron transfer in the desaturation of fatty acids by SCD (Ntambi, 1999)

Δ-6 Desaturase (Δ6d, EC 1.14.19.3):

The Δ6d is a membrane-bound enzyme that catalyses the first step and a later step in the synthesis of LC PUFA products from the precursors ALA and LA in tissues (Figure 2). This enzyme was cloned from different species, such as Synechocystis, Borago officinalis, Caenorhabditis elegans, human, mouse, and rat. It introduces a double-bond between the pre-existing double bond and the carboxyl end of the fatty acid (Nakamuru and Nara, 2004).

Many studies found out that dietary and tissue related PUFA regulate lipogenic gene expression in several tissues, such as brain, liver, heart, and adipose tissue. Especially the fatty acids of the *n*-6 and *n*-3 series are in the focus of research because of their key role in the progression or prevention of human diseases. The regulation seems to occur at transcriptional level and the affected transcription factors are the sterol regulatory element binding proteins (SREBPs) (Nakamura *et al.*, 2004; Sessler and Ntambi, 1998; Ntambi and Bené, 2001; Nakamura and Nara, 2004).

A plethora of information is available about the activity and the gene expression of ACC, SCD and Δ6d; but not on the protein expression of these enzymes in muscle and adipose tissue of cattle.

Importance of tissues considering the fatty acid synthesis in cattle

SAT represents the main tissue for *de novo* fatty acid and triacylglycerol synthesis in cattle. The amount of stored triacylglycerides in adipocytes is equilibrium between *de novo* FA synthesis, FA uptake, FA esterification, triglyceride hydrolysis, and reesterification of FA produced by lipolysis (Chilliard, 1993). The liver in ruminants has less effect on *de novo* synthesis (Bauchart *et al.,* 1996) and disposal of lipids than adipose tissue or the lactating mammary gland (Bell, 1980). Availability of acetate partially depends on the uptake of LC PUFA in the liver, and is used for synthesis of glycerides, complex lipids, oxidation and a relative high rate of ketogenesis (Bell, 1980). Therefore, adipose tissue plays the major role in *de novo* synthesis of FA (Chilliard, 1993). Nevertheless, there is a low rate of *de novo* biosynthesis where acetate is the main carbon source.

Phospholipid composition of erythrocytes is a marker for long-term fat intake because of their lifespan of 120 days (Hodson *et al.,* 2008). The enrichment of diet with C18:2*n*-6 increased deposition of this fatty acid in erythrocytes, which demonstrates that the fatty acid composition of erythrocytes is dynamic and exchange occurs with other lipid pools (Hodson *et al.*, 2008). While the lipids in serum reflect the current status in blood transported lipids, the membrane fluidity of erythrocytes must be maintained (Smith, 1987).

For human nutrition, the skeletal muscle of ruminants as source of meat is of major interest. Due to the deposition of PUFA mainly in phospholipids, where they maintain the permeability of cell membranes, the proportion of PUFA in muscle is higher compared to SAT, and the fatty acid composition can be affected by diet (Lorenz *et al.* (2002) and Dannenberger *et al.* [2004]). Additionally, analysis of triglyceride and phospholipid fraction of muscle afford a more exact answer to how muscle fatty acid composition responds to diet of ruminant animals (Aurousseau *et al.*, 2007).

Chapter 2

Questions To Answer

2. Questions To Answer

Two animal experiments, one with German Simmental bulls and the other with German Holstein bulls, were conducted to investigate the effects of diets supplemented with LA as *n*-6 fatty acid and ALA as *n*-3 fatty acid. Arising from afore mentioned theoretical part the following questions will be answered:

a. Is there a tissue specific effect of LA and ALA supplementation in ruminant diet on different tissues as muscle, subcutaneous adipose tissue, liver, erythrocytes, and serum?
b. Is there an effect on lipid classes (triglycerides, phospholipids, and free fatty acids) of intramuscular fat?
c. Does a short-time feed restriction at the beginning of the feeding experiment and the following compensatory growth have an effect on the intramuscular fat content and is this restriction able to enhance essential fatty acids (*n*-6 and *n*-3) in tissues?
d. Does the supplemented diet have an effect on lipogenic enzyme protein expression (acetyl-CoA carboxylase, stearoyl-CoA desaturase and delta-6 desaturase) in subcutaneous adipose tissue and *longissimus* muscle?
e. Is there a dietary effect especially on the stearoyl-CoA desaturase activity in subcutaneous adipose tissue and *longissimus* muscle?
f. Are the effects of a PUFA enriched diet on the fatty acid composition of subcutaneous adipose tissue and *longissimus* muscle mediated by the regulation of lipogenic enzymes?
g. Are there tissue specific effects of the protein expression of acetyl-CoA carboxylase, stearoyl-CoA desaturase and delta-6-desaturase in subcutaneous adipose tissue and *longissimus* muscle?
h. Is there a loss of especially the *n*-3 PUFA and *n*-3 LC PUFA during the production process of beef products?

Chapter 3

Animal Experiments

3. Animal Experiments

Feeding experiment with German Simmental bulls (MPA Laage) – Experiment 1

In total, 25 animals at the age between 3 and 4 months were randomly assigned to different dietary treatments. The first group, henceforth referred to as control group, consisted of 9 German Simmental bulls. The animals were fed a diet composed of silage (70% maize silage and 30% grass silage) and concentrate. The concentrate was supplemented with 20% soybean meal and 2% soybean oil. The treatment diet contained only grass silage and the concentrate was supplemented with 32% rapeseed cake and 2% rapeseed oil (Table 3). The fatty acid composition of the total mixed rations (TMR) in % is represented in Table 4.

Table 3: Composition of the concentrate for the animal experiment MPA Laage

Concentrate compounds	**Control group (%)**	**Treatment group (%)**
Soybean meal	20	-
Triticale	63	39
Barley	10	22
Rapeseed cake	-	32
Soybean oil	2	-
Rapeseed oil	-	2
Minerals	5	5

Furthermore, the treatment animals were divided into two groups. Either of these groups received a feed restriction over a period of 112 days directly at the beginning of the trial. During this time the animals received 50% less concentrate compared to the unrestricted group. The unrestricted group comprised 7 and the restricted group 9 German Simmental bulls. The duration of the trial was also subdivided into three sections. More details are shown in Table 5.

All bulls were kept indoor and were individually fed. The point of slaughter was defined at a weight of approximately 635 kg. They were slaughtered at the facilities of the Institute for the Biology of Farm Animals, Dummerstorf, Germany and were killed by captive bolt stunning followed by exsanguinations in accordance with EU regulations.

Tissue samples were taken immediately after slaughter from the right side of the carcass. MLD for analysing lipid classes were taken at $13^{th}/14^{th}$ rib and stored at −80 °C, as well as erythrocytes and serum after separation from blood. The MLD for total fatty acid composition was collected at $9^{th}/10^{th}$ rib of carcasses after 24 h cooling. Liver samples and

subcutaneous adipose tissue (SAT) for analysing the total fatty acid composition were frozen and stored at −20 °C.

Table 4: Fatty acid composition of the total mixed ration in %

	TMR of	
	Control group (%)	**Treatment group (%)**
C16:0	12.06	7.97
C16:1*cis*-9	0.44	1.43
C18:0	3.03	1.56
C18:1*cis*-9	23.07	35.60
C18:2*n*-6	46.58	29.96
C18:3*n*-3	7.25	8.06
Σ of SFA	17.18	12.04
Σ of MUFA	28.72	52.65
Σ of PUFA	54.10	35.31
Σ of *n*-3 fatty acids	7.36	8.21
Σ of *n*-6 fatty acids	46.66	27.02
Ratio *n*-6/*n*-3	6.34	3.29

Table 5: Periods and the amount of feed for the animal groups of the experiment

		Control group n=9	**Restricted group n=9**	**Unrestricted group n=7**
Amount of feed		**(kg/animal and day)**	**(kg/animal and day)**	**(kg/animal and day)**
First Period	Concentrate	2.0	**1.0**	2.0
	Sugar beet pulp	1.0	1.0	1.0
	Maize silage	ad libitum (70 %)	-	-
	Grass silage	ad libitum (30 %)	ad libitum	ad libitum
	Hay/Straw	1.0	1.0	1.0
Second Period	Concentrate	2.5	2.5	2.5
	Sugar beet pulp	1.0	1.0	1.0
	Maize silage	ad libitum (70 %)	-	-
	Grass silage	ad libitum (30 %)	ad libitum	ad libitum
	Hay/Straw	1.0	1.0	1.0
Third Period	Concentrate	3.0	3.0	3.0
	Sugar beet pulp	1.0	1.0	1.0
	Maize silage	ad libitum (70 %)	-	-
	Grass silage	ad libitum (30 %)	ad libitum	ad libitum
	Hay/Straw	1.0	1.0	1.0

Feeding experiment with German Holstein bulls (ProSafeBeef) – Experiment 2

29 animals at the age between 8 and 9 months were assigned to two dietary treatments. The first treatment corresponds to a commercial diet for ruminant finishing in Germany. The animals received maize silage and a concentrate supplemented with soybean meal, resulting in a diet high in *n*-6 PUFA. The group fed the commercial diet is called "Control group" and is consisted of 15 German Holstein bulls. The second diet consisted of grass silage and concentrate supplemented with linseed oil and rapeseed cake, resulting in high *n*-3 PUFA content. The complete composition of the concentrate is shown in Table 6.

Table 6: Composition of the concentrate for the animal experiment ProSafeBeef

Concentrate compounds	Control diet	Experimental diet
Crushed wheat	40	58
Crushed maize	10	20
Soybean meal	41	-
Rapeseed cake	-	12
Minerals	5	4.7
Linseed oil	-	3
Molasses	2	2
Straw	2	-
Feed chalk (contained vitamin E)	-	0.3

Table 7 presents the chemical composition of the TMR in % and Table 8 the fatty acid composition. The content of LA in the control group was 1.4-times higher compared to the experimental group whereas the concentration of ALA was 4-times higher in the experimental compared to the control group.

Table 7: Chemical composition of the total mixed ration in %

Chemical composition (%, DM)	Control group	Experimental group
Crude protein	15.3	14.9
Crude fat	3.1	4.0
Crude ash	7.0	12.3

Table 8: Fatty acid composition of the total mixed ration in %

	TMR of	
	Control group (%)	**Treatment group (%)**
C12:0	0.24	0.17
C14:0	0.84	0.42
C14:1*cis*-9	0.01	0.00
C16:0	20.51	16.67
C16:1*cis*-9	0.26	0.28
C18:0	2.55	2.57
C18:1*cis*-9	19.14	15.69
C18:2*n*-6	39.96	21.51
C18:3*n*-3	10.79	35.48
Σ of SFA	28.01	23.68
Σ of MUFA	20.61	17.44
Σ of PUFA	51.33	58.77
Σ of *n*-3 fatty acids	11.08	36.47
Σ of *n*-6 fatty acids	40.23	22.30
Ratio *n*-6/*n*-3	3.68	0.61

The amount of concentrate and silage was fed according to age of the animals. The bulls were kept in groups. The animals were slaughtered weight dependent with approximately 625 kg at the facilities of the Institute for the Biology of Farm Animals, Dummerstorf, Germany and were killed by captive bolt stunning followed by exsanguinations in accordance with EU regulations.

Samples of MLD and SAT for protein expression, SCD activity and fatty acid composition analyses (approximately 50 g) were collected from the right side of carcass, between the thirteenth and fourteenth rib, within 30 min after slaughter. All the samples were snap-frozen in liquid nitrogen and stored at -70°C until analysed. The carcasses have been transported after 48 h to Greifenfleisch GmbH (Greifswald, Germany). Three different sausages have been produced by using the beef from these experimental bulls. Samples from sausages, produced by Greifenfleisch GmbH, were taken after delivery by the company Greifenfleisch GmbH and stored at -20°C until the analysis of fatty acid composition.

Chapter 4

Effect of dietary fatty acids on expression of lipogenic enzymes and fatty acid profile in tissues of bulls

A. Herdmann, K. Nuernberg, J. Martin, G. Nuernberg and O. Doran

Animal **2010**, 4:5, 755-762

Content

The effect of *n*-3 and *n*-6 fatty acids in the diet of ruminants on the protein expression of lipogenic enzymes in tissues is not described in the literature. Therefore animals were fed diets high in ALA or LA for a long-time period. In *longissimus* muscle (MLD) and subcutaneous adipose tissue (SAT) of German Holstein bulls the protein expression of ACC, SCD and Δ-6 desaturase was analyzed. There was no effect on the protein expression of ACC. Furthermore, the *n*-3 feeding was related to a significantly decreased protein expression of SCD in MLD and SAT with a significantly lower amount of oleic acid in the SAT. The Δ-6 desaturase protein expression was decreased in MLD associated with a lower *n*-6 PUFA level.

Work	**Contribution**
Study accomplishment	90%
Analyses (protein expression, fatty acids, diets)	70%
Statistics	30%
Preparation of the manuscript	100%

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Effect of dietary fatty acids on expression of lipogenic enzymes and fatty acid profile in tissues of bulls

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*This study investigated the effects of dietary linolenic acid (C18:3*n*-3) v. linoleic acid (C18:2*n*-6) on fatty acid composition and protein expression of key lipogenic enzymes, acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD) and delta 6 desaturase (Δ6d) in longissimus muscle and subcutaneous adipose tissue of bulls. Supplementation of the diet with C18:3*n*-3 was accompanied by an increased level of* n*-3 fatty acids in muscle which resulted in decrease of* n*-6/*n*-3 ratio. The diet enriched with* n*-3 polyunsaturated fatty acids (PUFAs) significantly inhibited SCD protein expression in muscle and subcutaneous adipose tissue, and reduced the Δ6d expression in muscle. There was no significant effect of the diet on ACC protein expression. Inhibition of the Δ6d expression was associated with a decrease in* n*-6 PUFA level in muscles, whereas repression of SCD protein was related to a lower oleic acid (C18:1* cis*-9) content in the adipose tissue. Expression of ACC, SCD and Δ6d proteins was found to be relatively higher in subcutaneous adipose tissue when compared with longissimus muscle. It is suggested that dietary manipulation of fatty acid composition in ruminants is mediated, at least partially, through the regulation of lipogenic enzymes expression and that regulation of the bovine lipogenic enzymes expression is tissue specific.*

Keywords: cattle, diet, enzyme expression, fatty acid, lipogenic enzyme

Implication

This study has demonstrated that regulation of fatty acid composition by the dietary n-3 and n-6 fatty acids in cattle is mediated, at least partially, through tissue-specific regulation of lipogenic enzymes expression. This finding is important for understanding the mechanisms underlying the accumulation and tissue distribution of n-3 and n-6 polyunsaturated fatty acid. Therefore, this research is an important step in the development of a complex (diet- and genetic-based) approach for improvement of eating quality of beef. Improving of beef fatty acid composition would be of direct benefit to human health and to the beef-producing industry.

Introduction

It is well known that the amount and type of fat in the diet is closely related to human health. A high intake (more than 15% of daily energy intake) of saturated fatty acids (SFAs) (mainly myristic, lauric and palmitic acid) is positively associated with coronary heart disease and mortality rate (Rioux and Legrand, 2007). The negative effect of SFA is mediated through increase in blood cholesterol concentrations, especially plasma total and low-density lipoprotein cholesterol (Williams, 2000). Moreover, the diets high in SFA reduce anti-inflammatory properties of high-density lipoprotein (Rioux and Legrand, 2007). In contrast to SFA, unsaturated fatty acids are recognised as health beneficial. A number of studies demonstrated hypo-cholesterolemic effects of monounsaturated fatty acids (MUFAs) and some polyunsaturated fatty acids (PUFAs) (Demaison and Moreau, 2002). MUFA have also been reported to possess anti-thrombogenic properties (Smith *et al.*, 2003). In countries with the Mediterranean diet (low content of SFA and high content of MUFA) the incidence of coronary heart disease is much lower when compared with the countries with the diets which are high in SFA (Lada and Rudel, 2003). The particular health benefit has been linked to n-3 PUFA which are essential for normal growth and development (Simopoulos, 1991). Long chain n-3 PUFA (docosahexaenoic acid and eicosapentaenoic acid) demonstrated anti-atherogenic, anti-thrombotic, anti-inflammatory and immunosuppressive properties in experimental studies in man (Williams, 2000; Garg *et al.*, 2006). In addition to n-3 PUFA an increasing attention has been paid to

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another PUFA, namely conjugated linoleic acids (CLAs). CLA is a group of isomers, which have been demonstrated to enhance immune system, to reduce body weight and to decrease the risk of cancer (Tricon *et al.*, 2005; Bhattacharya *et al.*, 2006). The main natural source of *cis*-9, *trans*-11 CLA is meat and milk of ruminants (Song and Kennelly, 2003).

According to national and international dietary guidelines, SFA contribution to dietary energy intake should be not more than 10% of dietary energy (Wahrburg, 2004), and the inclusion of MUFA and PUFA into human diet should be up to 15% of total daily energy for MUFA (Wahrburg, 2004) and to a maximum of 10% of total daily energy for PUFA (Wahrburg, 2004). One way to achieve this is to decrease SFA and increase MUFA and PUFA content in food and in meat in particular. This is particularly related to ruminant meat which is higher in SFA when compared with pig or poultry (Valsta *et al.*, 2005).

The fatty acid composition of adipose and muscle tissues depend on a number of factors such as diet, fatness of animals, age/weight, gender, breed, season and others (Nuernberg *et al.*, 1998; Scollan *et al.*, 2006; Wood *et al.*, 2008). In monogastric animals the quality of meat can be relatively easy improved by dietary manipulations. However in ruminant's dietary manipulations of fatty acid composition proved to be difficult because of rumen biohydrogenation. Therefore, there is an increasing interest in manipulation of fatty acid composition in ruminant tissues through the regulation of tissue fatty acid biosynthesis. The key enzymes involved in SFA, MUFA and PUFA biosynthesis are acetyl-CoA carboxylase (ACC) (EC 6.4.1.2), stearoyl-CoA desaturase (SCD) (EC 1.14.19.1) and delta 6 desaturase (Δ6d) (EC 1.14.19.3), respectively. These enzymes have been extensively studied in a number of species, including pigs. However, there is very limited information about tissue distribution and regulation of expression of these enzymes in cattle (Chang *et al.*, 1992; Smith *et al.*, 2002; Archibeque *et al.*, 2005).

The aim of this study was to investigate effects of the diet enriched in linolenic acid (as a source of n-3 PUFA) *v.* linoleic acid (source of n-6 PUFA) on fatty acid composition and protein expression of the key lipogenic enzymes in cattle tissues. The particular questions which we aim to answer in this study are: (i) whether the effects of PUFA-enriched diets on fatty acid composition of cattle muscle and adipose tissue is mediated through the regulation of lipogenic enzyme expression and (ii) whether the effects of the diet on enzyme expression are tissue-specific.

Material and methods

Animals and diets

Fourteen German Holstein bulls were assigned to two dietary treatments: (i) maize silage with soybean-based concentrate (control group) result in a 1.4 times higher content of C18:2n-6 and (ii) grass silage with linseed oil and rapeseed cake supplemented concentrate (experimental group) with a four times higher concentration of C18:3n-3 compared with control group. There were eight animals in control group and six animals in experimental group (initial number of animals in experimental group was eight but two animals died at the beginning of the trial). Bulls were kept in-door in groups over the whole fattening period and were fed a total mixed ration (TMR). Table 1 presents the composition of the two concentrates used. The chemical and fatty acid composition of the TMRs, the feed and the energy intake is given in Table 2. The animals received feed two times a day adapted to fit the needs of bulls in every state of growth. The feed was weighed and prepared with a fodder-mixing trailer and the daily feed intake was recorded through the car computer in the fodder-mixing trailer for each group during the trial. The metabolisable energy of the TMRs was 11.6 and 11.3 MJ/kg DM for the control and experimental groups, respectively. Daily feed intake was 9.7 and 9.6 kg DM/day for the control and experimental groups, respectively. The 14 bulls were fed the control and experimental ration for 209 ± 7 and 216 ± 8 ($P = 0.5073$) days, respectively. The average live weight of bulls at the slaughter was 621.1 ± 10.4 kg in control group and 627.7 ± 12.0 kg ($P = 0.6871$) in experimental group, respectively. The bulls were slaughtered at the facilities of the Institute for the Biology of Farm Animals, Dummerstorf, Germany and were killed by captive bolt stunning followed by exsanguinations in accordance with EU regulations.

Table 1 *Composition of the concentrate compound of the diets (wt %)*

Component	Control diet	Experimental diet
Crushed wheat	40	58
Crushed maize	10	20
Soybean meal	41	–
Rapeseed cake	–	12
Minerals	5	4.7
Linseed oil	–	3
Molasses	2	2
Straw	2	–
Feed chalk (contained vitamin E)	–	0.3

Samples of longissimus muscle and subcutaneous adipose tissue for protein expression analyses (approximately 50 g) were collected from the right side of carcass, between the thirteenth and fourteenth rib, within 30 min after slaughter. All the samples were snap-frozen in liquid nitrogen and stored at −70°C until analysed. It has been previously demonstrated that the storage conditions do not affect the lipogenic enzyme expression (Doran *et al.*, 2006).

Isolation of microsomal and cytosolic fractions

Microsomal and cytosolic fractions from muscle and subcutaneous adipose tissues were isolated by differential centrifugation. The tissue samples (10 g) were defrosted and homogenised (by using Potter homogenizer) in 20 ml of Tris-sucrose buffer composed of 10 mmol Tris-HCl and 250 mmol sucrose (pH 7.4) at room temperature. After centrifugation at 12 000×**g** for 10 min at 4°C, the supernatant (in the case of muscle) and the infranatant (in the case of subcutaneous adipose tissue) were collected. About 8 mmol of $CaCl_2$

Table 2 *Chemical and fatty acid composition (mg/100 g) of total mixed ration and intake parameters*

	Control group	Experimental group
Chemical composition (%, DM)		
Crude protein	15.3	14.9
Crude fat	3.1	4.0
Crude ash	7.0	12.3
Fatty acid profile (%)		
C12:0	0.2	0.2
C14:0	0.8	0.4
C16:0	20.5	16.7
C18:0	2.6	2.6
C18:1 *cis*9	19.1	15.7
C18:2n-6	40.0	21.5
C18:3n-3	10.8	35.5
Intake parameters		
Feed intake (kg DM/day)	9.7	9.6
Energy intake (MJ/ME/day DM)	112.5	108.5

DM = dry matter.

(final concentration) was added to the supernatant or to the infranatant to facilitate the microsomes sedimentation. Cytosolic and microsomal fractions were separated by centrifugation at 25 000×**g** for 30 min at 4°C. A microsomal fraction (the pellet) was re-suspended in Tris-KCl buffer, containing 10 mmol Tris-HCl, 250 mmol KCl (pH 7.4), and the inhibitors of proteolytic enzymes; antipain, pepstatin and leupeptin at final concentrations 1.5, 1.5, and 2 μM, respectively. The final protein concentration of the microsomal suspension was about 26 mg/ml for muscle and 2 mg/ml for subcutaneous adipose tissue. The protein concentration of the microsomal and cytosolic fractions was determined by the Bradford method using bovine serum albumin as standard.

Protein expression

Expression of ACC, SCD and Δ6d proteins was analysed by Western blotting. Microsomal and cytosolic proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, electro blotted onto nitrocellulose membrane at a constant 100 V for 1 h and incubated with one of the following primary antibodies: rabbit polyclonal anti-bovine adipose tissue SCD (custom-made at the University of Bristol, UK) or goat polyclonal anti-human (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The ACCα antibodies did not distinguish between the phosphorylated and non-phosphorylated enzyme forms. In the case of Δ6d the antibodies were custom-produced in rabbits against the synthetic peptides containing amino acid sequences from the regions which are conserved in the rat, pig and human, near C-terminus of the corresponding proteins (Sigma-Genosys Ltd, Cambridge, UK). The membrane was re-probed with appropriate commercial secondary antibody, which was either horseradish peroxide linked donkey anti-rabbit immune globuline G (IgG), or horseradish peroxide linked donkey anti-goat IgG. The blots were developed using an enhanced chemiluminescence reagent (Amersham, Buckinghamshire, UK). The films were scanned and the intensity of the corresponding bands was quantified by using the ImageQuant program (Molecular Dynamics, Sunnyvale, CA, USA). A microsomal or cytosolic preparation from one particular sample was present on all the blots (reference sample). The intensity of the signal on this sample was taken as 100 arbitrary units throughout, and the intensity of the signals from other samples was expressed as a fraction of the reference sample. Western blot analysis was performed with 6 μg of microsomal or cytosolic protein.

Fatty acid analysis

Fatty acids have been analysed as described previously (Dannenberger *et al*., 2004). Samples of longissimusmuscles and subcutaneous adipose tissue were thawed at 4°C. After blending and mincing in a grinding processor (IKA M20, Staufen, Germany), and adding C19:0 as an internal standard, the total lipids were extracted from 2 g of muscle and 1 g of subcutaneous fat (both in duplicates) with chloroform/methanol (2 : 1, v/v) by homogenisation (Ultra Turrax, 3 × 15 s, 12 000 r.p.m.) at room temperature. All the solvents contained 0.005% (w/v) of t-butylhydroxytoluene to avoid the oxidation of PUFA. The extraction mixture was stored at 5°C for 18 h in the dark and subsequently washed with 0.02% aqueous $CaCl_2$. The organic phase was dried with Na_2SO_4 and K_2CO_3 (10 : 1, wt/wt) and the solvent was subsequently removed under nitrogen at room temperature. The lipid extract was re-dissolved in toluene and an aliquot of 25 mg was used for methyl ester (ME) preparation. To prepare the fatty acid methyl esters (FAME**s**), 2 ml of 0.5 M sodium methanolate was added and the mixture was shaken in a water bath at 60°C for 10 min. After this, 1 ml of boron trifluoride in methanol (14%, wt/v) was added to the solution and the mixture was shaken in a water bath at 60°C for 10 min. MEs of the total fatty acids were extracted twice with 2 ml of n-hexane in the presence of 2 ml distilled saturated solution of $NaHCO_3$. The upper phases containing FAME were pooled and the solvent was eliminated by evaporation under nitrogen flow. FAME were resolved in 150 μl n-hexane and stored at −20°C until analysed by gas chromatography (GC).

An aliquot of the FAME mixture was used for analyses of total fatty acids. The fatty acid composition of longissimusmuscle, subcutaneous adipose tissue and animal feed were determined by capillary 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 (Waltham, MA, USA) with a flame ionisation detector and split injection. Initial oven temperature was 45°C (held for 4 min). It was subsequently increased to 150°C at a rate of 13°C min^{-1} (held for 47 min), and then to 215°C at 4°C min^{-1} and held for 35 min. Hydrogen was used as the carrier gas at a flow rate of 1 ml min^{-1}. In total 41 fatty acids were identified with a reference standard (Sigma FAME mixture no. 189-19, Sigma-Aldrich, Deisenhofen, Germany) to which the MEs of C18:1 *trans*-11, C22:5n-3, CLA *cis*-9, *trans*-11, C18:1 *cis*-11, C18:4n-3 and C22:4n-6 had been

Table 3 *Fatty acid composition (mg/100 g) of longissimus muscle of German Holstein bulls fed with different diets*

Fatty acids	Control group ($n = 8$) LSM ± s.e.m.	Experimental group ($n = 6$) LSM ± s.e.m.
C12:0	1.4 ± 0.2	1.2 ± 0.2
C14:0	56.6 ± 7.9	43.0 ± 9.2
C14:1	12.8 ± 1.8	10.4 ± 2.1
C16:0	549.3 ± 60.2	422.0 ± 69.5
C16:1	78.0 ± 9.4	54.6 ± 10.9
C18:0	304.4 ± 29.9	255.0 ± 34.5
C18:1 *trans*-9	4.3 ± 0.4	3.6 ± 0.5
C18:1 *trans*-10	5.3 ± 0.4[a]	2.5 ± 0.4[a]
C18:1 *trans*-11	12.5 ± 1.7	14.3 ± 1.9
C18:1 *cis*-9	746.8 ± 73.5	546.7 ± 84.8
C18:1 *cis*-11	24.4 ± 1.9	19.8 ± 2.2
C18:2n-6	104.6 ± 3.3[a]	90.6 ± 3.8[b]
C18:3n-3	12.6 ± 1.1[a]	32.1 ± 1.3[b]
C18:4n-3	1.2 ± 0.1	0.9 ± 0.2
C20:3n-6	6.9 ± 0.2[a]	4.8 ± 0.3[b]
C20:4n-6	27.2 ± 0.5[a]	23.8 ± 0.6[b]
C20:5n-3	3.7 ± 0.3[a]	7.8 ± 0.3[b]
C22:5n-3	8.0 ± 0.3[a]	10.6 ± 0.3[b]
C22:6n-3	1.0 ± 0.1[a]	1.3 ± 0.1[b]
cis-9, *trans*-11 CLA*	4.9 ± 0.5	4.9 ± 0.6
[a]Σ SFA	956.7 ± 100.0	760.1 ± 115.4
[b]Σ MUFA	910.6 ± 89.5	674.7 ± 103.4
[c]Σ PUFA	173.5 ± 5.1	178.5 ± 5.9
[d]Σ n-3 FA	26.4 ± 1.4[a]	52.7 ± 1.6[b]
[e]Σ n-6 FA	145.1 ± 3.8[a]	123.6 ± 4.4[b]
n-6/n-3	5.5 ± 0.1[a]	2.4 ± 0.2[b]
Sum of fatty acids	2040.9 ± 191.7	1613.3 ± 221.4

CLA = conjugated linoleic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; FA = fatty acid.
[a,b]indicates significant effect of the diet at $P \leq 0.05$.
* indicates coeluation with C18:2 *trans*-7, *cis*-9 and C18:2 *trans*-8, *cis*-10.
[a]Σ SFA = 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.
[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 PUFA = Σ n-3 fatty acids (FA) + Σ n-6 FA.
[d]Σ n-3 FA = C20:3n-3 + C22:6n-3 + C22:5n-3 + C20:5n-3 + C18:4n-3 + C18:3n-3.
[e]Σ n-6 FA = C22:2n-6 + C20:2n-6 + C18:3n-6 + C22:4n-6 + C20:3n-6 + C18:2n-6 + C20:4n-6.

Table 4 *Fatty acid composition (mg/100 g) of subcutaneous adipose tissue of German Holstein bulls fed different diets*

Fatty acids	Control group ($n = 8$) LSM ± s.e.m.	Experimental group ($n = 6$) LSM ± s.e.m.
C12:0	77.3 ± 7.3	63.9 ± 8.5
C14:0	2854.8 ± 251.8	2099.6 ± 290.7
C14:1	1126.0 ± 99.6	879.1 ± 115.0
C16:0	19232.2 ± 1332.7[a]	14725.4 ± 1538.8[b]
C16:1	4420.1 ± 334.4	3416.5 ± 386.1
C18:0	8510.2 ± 896.0	6826.8 ± 1034.6
C18:1 *trans*-9	169.8 ± 12.8	132.0 ± 14.8
C18:1 *trans*-10	302.8 ± 22.8[a]	159.2 ± 26.4[b]
C18:1 *trans*-11	514.5 ± 67.2	540.1 ± 77.6
C18:1 *cis*-9	24868.9 ± 1413.2[a]	18533.2 ± 1631.9[b]
C18:1 *cis*-11	837.1 ± 40.6[a]	626.2 ± 46.9[b]
C18:2n-6	1138.5 ± 58.0[a]	677.6 ± 66.9[b]
C18:3n-3	338.4 ± 42.5	468.6 ± 49.0
C18:4n-3	48.2 ± 6.2	29.2 ± 7.2
C20:3n-6	43.7 ± 4.0[a]	19.4 ± 4.7[b]
C20:4n-6	42.4 ± 2.6[a]	33.9 ± 3.0[b]
C20:5n-3	5.3 ± 2.6	13.3 ± 3.0
C22:5n-3	40.2 ± 3.4	42.6 ± 3.9
C22:6n-3	7.3 ± 4.5	7.5 ± 5.2
cis-9, *trans*-11 CLA*	288.3 ± 24.4	264.6 ± 28.2
[a]ΣSFA	32008.5 ± 2500.4	24874.6 ± 2887.2
[b]Σ MUFA	33000.7 ± 1774.7	24838.4 ± 2049.2
[c]Σ PUFA	2131.7 ± 137.4	1693.3 ± 158.6
[d]Σ n-3 FA	391.1 ± 46.5	532.0 ± 53.6
[e]Σ n-6 FA	1307.8 ± 64.5[a]	778.4 ± 74.5[b]
n-6/n-3	3.4 ± 0.1[a]	1.5 ± 0.1[b]
Sum of fatty acids	67253.3 ± 4230.6[a]	51488.4 ± 4885.0[b]

CLA = conjugated linoleic acid; SFA = saturated fatty acid; MUFA = mono unsaturated fatty acid; PUFA = polyunsaturated fatty acid; FA = fatty acid.
[a,b]indicates significant effect of the diet at $P \leq 0.05$.
* indicates coeluation with C18:2 *trans*-7, *cis*-9 and C18:2 *trans*-8, *cis*-10.
[a]Σ SFA = 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.
[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 PUFA = Σ n-3 fatty acids (FA) + Σ n-6 FA.
[d]Σ n-3 FA = C20:3n-3 + C22:6n-3 + C22:5n-3 + C20:5n-3 + C18:4n-3 + C18:3n-3.
[e]Σ n-6 FA = C22:2n-6 + C20:2n-6 + C18:3n-6 + C22:4n-6 + C20:3n-6 + C18:2n-6 + C20:4n-6.

added was used to determine recoveries and correction factors for the determination of individual fatty acids in intramuscular fat. FAMEs were purchased from Sigma-Aldrich and Biotrend (Köln, Germany); CLA *cis*-9, *trans*-11 was purchased from Matreya, (Biotrend, Köln, Germany). Twenty fatty acids are presented in Tables 3 and 4. The above conditions did not allow separating the CLA *cis-9, trans-11* from CLA *trans*-7, *cis-9* and CLA *trans-8, cis-10*. Therefore, the value for CLA *cis-9, trans-11* is presented as a sum of the values for the three above isomers. The sum of n-3 fatty acids was calculated as C18:3n-3 + C18:4n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3, the sum of n-6 fatty acids was calculated as C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:2n-6 + C22:4n-6, the sum of MUFA was calculated as C14:1 + C15:1 + C16:1 + C17:1 + ∑C18:1*trans*-9-11 + C18:1*cis*-9 + C18:1*cis*-11 + C22:1 + C24:1 and the sum of PUFA as sum of n-3 fatty acids and the sum of n-6 fatty acids.

Statistical analysis

Data were analysed by the least-squares method using the general linear model procedures of SAS® (2009) with the fixed factor feeding. $P \leq 0.05$ was considered statistically significant.

Results

Fatty acid composition of longissimus muscle and subcutaneous adipose tissue

The fatty acid composition of longissimus muscle and subcutaneous adipose tissue is shown in Tables 3 and 4 and the results are expressed as mg/100 g tissue.

No differences were observed in the content of individual and total SFA and MUFA, except for C18:1 *trans*-10, which was reduced in experimental group compared with control group ($P < 0.001$). In grass silage fed group we have also observed a tendency for reduction in C18:1 *cis*-9 ($P = 0.09$). The sum of n-3 PUFA in the grass silage fed group ($P < 0.001$) was significantly increased (by two-fold) because of an increase in the level of all individual n-3 PUFA, except for C18:4n-3. The sum of total and individual n-6 PUFA was significantly decreased in muscles of the experimental animals ($P = 0.003$). As the result of the opposite changes in n-3 and n-6 PUFA, the muscle n-6/n-3 ratio was significantly lower in experimental group ($P < 0.001$) and the content of total PUFA did not change significantly ($P = 0.534$). As there was no significant effect of experimental diet on total SFA, MUFA and PUFA content, the total fatty acids content in muscle (i.e. sum of fatty acids) was not affected either.

In subcutaneous adipose tissue the sum of fatty acids (mg/100 g) was significantly reduced with the experimental diet. This reduction was mainly caused by a lower content of the SFAs (C14:0, C16:0 and C18:0), C18:1 *cis-9*, C18:1 *cis*11 and C18:1 *trans*-10. The adipose tissue of the bulls from experimental group also had a significantly lower content of n-6 PUFA, in particular C18:2n-6 ($P < 0.001$), C20:3n-6 ($P = 0.002$) and C20:4n-6 ($P = 0.05$). The content of n-3 PUFA in adipose tissue was either not affected by the diet (i.e. C22:5n-3 and C22:6n-3; $P = 0.65$ and $P = 0.99$, respectively), or there was a trend toward the increase in the level of total ($P = 0.07$) and individual n-3 fatty acids in experimental group (C18:3n-3 and C20:5n-3; $P = 0.07$). There was a significant decrease of n-6/n-3 ratio in experimental group ($P < 0.001$).

Lipogenic enzyme expression

Effect of diet on the lipogenic enzymes protein expression. Effects of diet on ACC, SCD and Δ6d protein expression in the longissimus muscle are presented in Figure 1. The expression of ACC protein did not differ significantly between the control and experimental groups ($P = 0.71$). In contrast to ACC, the experimental diet significantly reduced the expression of SCD protein (by 37%, $P = 0.03$) and Δ6d protein (by 33%, $P = 0.03$).

In subcutaneous adipose tissue the expression of SCD in experimental group was decreased by about 29% when compared with control group ($P = 0.02$). The experimental diet did not have significant effects on ACC and Δ6d protein expression ($P = 0.71$ and $P = 0.55$, Figure 2).

Tissue distribution of lipogenic enzymes. Figure 3a–c shows representative blots for ACC, SCD and Δ6d proteins for three randomly selected animals. The blots clearly demonstrate large between-tissue differences. The presence of an ACC immunoreactive protein was observed in both, muscle and subcutaneous adipose tissue (Figure 3a). The size of the band is consistent with the molecular weight of ACC reported for other species (Tanabe *et al.*, 1975). The intensity of ACC signal was much higher in subcutaneous adipose tissue when compared with muscles.

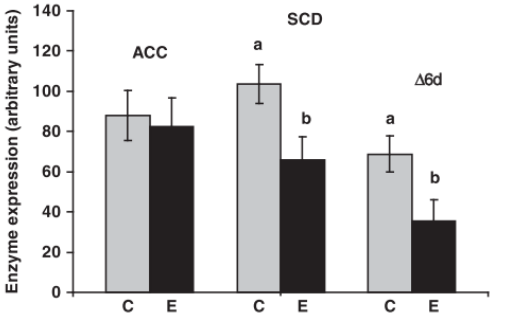

Figure 1 Expression of acetyl-CoA carboxylase (ACC) protein in cytosolic fraction and, stearoyl-CoA desaturase (SCD) and Δ6-desaturase (Δ6d) proteins in microsomal fraction isolated from longissimus muscle. Diets are described in 'Material and Methods' section. Bars represent different least squares means for eight animals in control group and six animals in experimental group. The error bars represent standard error for means. The measurements for each animal were performed in duplicate. Bars with different superscripts differ significantly for a given enzyme ($P < 0.05$). C = control group; E = experimental group.

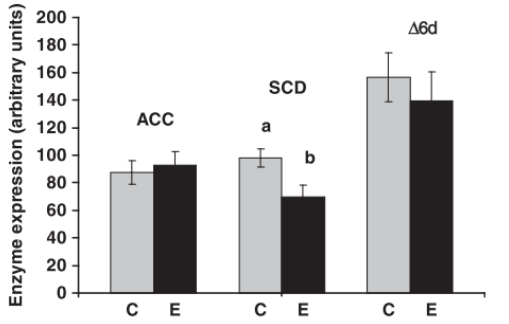

Figure 2 Expression of acetyl-CoA carboxylase (ACC) protein in cytosolic fraction and stearoyl-CoA desaturase (SCD) and Δ6-desaturase (Δ6d) proteins in microsomal fraction isolated from subcutaneous adipose tissue. Diets are described in 'Material and Methods' section. Bars represent different least square means for eight animals in control group and six animals in experimental group. The error bars represent standard error for means. The measurements for each animal were performed in duplicate. Bars with different superscripts differ significantly for a given enzyme ($P < 0.05$). C = control group; E = experimental group.

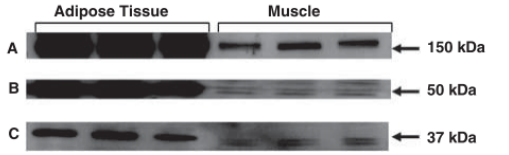

Figure 3 A–C Representative blots of relative expression of lipogenic enzymes in subcutaneous adipose tissue and in longissimus muscle. (A) Acetyl-CoA carboxylase protein expression in isolated cytosolic fraction. (B) Δ6-desaturase protein expression in isolated micosomal fraction. (C) Stearoyl-CoA desaturase protein expression in isolated microsomal fraction. Each signal represents an individual animal.

Similarly to ACC, the presence of Δ6d-immunoreactive proteins was also observed in both tissues (Figure 3b), and the signal was much higher in subcutaneous adipose tissue when compared with the muscle. The bands' size was approximately of 50 kDa, which is consistent with the molecular weight of mouse and human Δ6d (Cho *et al.*, 1999).

Examples of SCD-immunoreactive protein expression are presented in Figure 3c. The size of the immunoreactive proteins was about 37 kDa, which is consistent with the molecular weight of SCD protein reported in rats (Moreau *et al.*, 2006). The intensity of the SCD-immunoreactive band was higher in the adipose tissue when compared with the muscle.

Discussion

It is well known that fatty acid composition of meat, and hence its nutritional value, can be manipulated by dietary means (Enser *et al.*, 1998; Dannenberger *et al.*, 2004). In monogastric animals, the dietary fatty acids can be directly incorporated in animal tissues (Nuernberg *et al.*, 2005). However, in the case of ruminants, direct fatty acid incorporation is restricted because of rumen biohydrogenation. Nevertheless, a number of experiments demonstrated accumulation of n-6 and n-3 fatty acids in cattle tissues in the case of long-term feeding regimes. In this study we have observed significant increase in most of the individual muscle n-3 fatty acids and decrease in n-6 fatty acids in the case of the diet supplemented with linseed oil and rapeseed cake as sources of C18:3n-3, when compared with control group. The above resulted in an improvement (i.e. reduction) of the n-6/n-3 ratio. These results are in agreement with data of the literature (Scollan *et al.*, 2006; Wood *et al.*, 2008). A similar picture was observed for subcutaneous adipose tissue n-3 and n-6 fatty acids and adipose tissue n-6/n-3 ratio. The only difference was that in case of subcutaneous adipose tissue, there was only a trend toward an increase in n-3 fatty acids ($P = 0.07$); whilst in case of muscles such increase was significant. The lack of significance in case of subcutaneous adipose tissue might be related to large between-individual variations.

There might be a number of possible explanations for the above mentioned dietary effects on n-3 and n-6 fatty acids. One possible reason could be dietary modulation of the rate of n-3 and n-6 fatty acids transfer into the tissues. Waters *et al.* (2009) described that the balance between n-6 and n-3 PUFA in the diet is important for the regulation of SCD through SREBP-1c in muscle of beef cattle. However, this explanation could be applied to C18:2n-6 and C18:3n-3 fatty acids, but not to longer-chain fatty acids. Changes in the longer-chain fatty acid profile could be related to dietary effects on activity/expression of Δ5 or Δ6-desaturases and this possibility is discussed below.

Our earlier study on pigs demonstrated that diets, including PUFA-supplemented diets, can trigger changes in the expression of lipogenic enzymes, and these changes are related to variations in fatty acid profiles (Doran *et al.*, 2006; Missotten *et al.*, 2009). Regulatory effects of the dietary fatty acids on enzyme expression have also been demonstrated in experiments on laboratory animals and on human (Jump and Clarke, 1999; Ntambi, 1999). However, there is very limited information regarding lipogenic enzymes distribution and regulation in ruminant tissues.

This study reports the presence of ACC, SCD and Δ6d immunoreactive bands in cattle longisimmus muscle and subcutaneous adipose tissue. ACC is the key enzyme controlling *de novo* biosynthesis of SFAs (Hardie, 1989). SCD is the enzyme catalysing two types of reactions: (i) biosynthesis of MUFA through the insertion of a double bound in SFAs between carbon C9 and C10 and (ii) the tissue biosynthesis of *cis*-9, *trans*-11 CLA from *trans*-vaccenic acid (Enoch *et al.*, 1976). Δ6d is involved in a complex process of biosynthesis of longer chain n-3 and n-6 fatty acids through conversion of linoleic and linolenic acids to their products (Stoffel *et al.*, 2008). The molecular weights of ACC, SCD and Δ6d immunoreactive bands detected in this study were 150, 37 and 50 kDa respectively, which is consistent with the molecular weights of ACC, SCD and Δ6d proteins reported for other species (Tanabe *et al.*, 1975; Cho *et al.*, 1999; Moreau *et al.*, 2006). In a case of the SCD protein expression (in muscle and subcutaneous adipose tissue) and muscle Δ6d, the two closely positioned immunoreactive bands were observed. One possible explanation for the two bands could be existence of more than one SCD (Lengi and Corl, 2007) and Δ6d isoforms in cattle tissues. More than one isoform of Δ6d were found in *Mucor rouxii* by Na-Ranong *et al.* (2006) and in rats by Skrzypski *et al.* (2009). The presence of several SCD isoforms, their tissue-specific distribution and regulation also has been previously reported in mice and rats (Thiede *et al.*, 1986; Miyazaki *et al.*, 2003). The number of Δ6d isoforms in ruminants remains unclear.

The intensity of ACC, SCD and Δ6d immunoreactive bands in this study were relatively higher in subcutaneous adipose tissue when compared with the muscles. These results are in agreement with the data of the literature suggesting that subcutaneous adipose tissue is the primary site of fatty acid biosynthesis in cattle (Smith *et al.*, 2007).

In this study we analysed expression of ACC, SCD and Δ6d proteins in animals fed either with C18:2n-6 or with C18:3 n-3 supplemented diets to evaluate potential contribution of the above enzymes in the variations in fatty acid composition. We have shown that increase in the individual and total n-3 PUFA in muscles of experimental animals was not accompanied by increase in Δ6d protein expression. Moreover, Δ6d protein level was significantly decreased in experimental group. Therefore Δ6d protein expression does not seem to contribute to the n-3 PUFA variations. It is important to admit that in this study we have only analysed the Δ6d protein expression (not the actual Δ6d activity). It is well known that fatty acid composition might be regulated by direct changes in enzyme activity and further study is required to investigate this possibility.

Our data on the cattle Δ6d are consistent with the reports on inhibitory effects of the dietary n-3 PUFA on expression of Δ6d gene in other species (Theil and Lauridsen, 2007).

Interestingly, in our experiment inhibition of Δ6d was observed in cattle muscles but not in subcutaneous adipose tissue. The reason for a tissue-specific response of Δ6d protein expression to the n-3 PUFA-supplemented diet is not clear. It is known that in a number of species the regulation of desaturase gene expression is mediated through the sterol-regulatory element binding protein (SREBP), and that expression of SREPB is tissue specific (Felder *et al.*, 2005). It might be possible that the tissue-specific effect of n-3 PUFA-supplemented diet on Δ6d protein expression in our experiments is related to tissue-specific distribution/regulation of the relevant transcription factors. This suggestion is supported by data of the literature that n-3 PUFA-enriched diet inhibits SREBP-1c in muscle tissue of bulls (Waters *et al.*, 2009).

In respect to the SCD expression, the level of this protein was significantly decreased under the n-3 PUFA-supplemented diet in both, muscle and subcutaneous adipose tissue. This is consistent with the data of the literature regarding inhibitory effects of PUFA on SCD expression in other species (Flowers and Ntambi, 2008). In contrast to Δ6d, we did not observe any tissue-specific effects of diet on the SCD protein expression. This is a particularly interesting observation because according to the literature, the regulation of both, Δ6d and SCD in other species involves the same transcription factors. Different responses of the cattle SCD and Δ6d to the dietary manipulations suggest the existence of tissue-specific mechanisms, and possibly tissue-specific expression of the transcription factors is required for the regulation of these enzymes in ruminants.

Inhibition of SCD protein expression in animals fed with C18:3n-3 supplemented diet was not accompanied by changes in total muscle or subcutaneous adipose tissue MUFA content in this study. However, we observed a significant decrease in one of the products of SCD catalysed reaction, C18:1 *cis*-9 in subcutaneous adipose tissue. We would also like to mention that the average values for total and individual MUFA were lower in C18:3n-3 fed group when compared with the group receiving C18:2n-6. This was observed for both, muscle and subcutaneous adipose tissue. Lack of significant differences might be related to large between-individual variations within the groups and relatively small number of animals per group.

In this study we have also observed some decrease in individual and total SFAs in muscles and subcutaneous adipose tissue of animals fed the experimental diet. However, this decrease was only significant for subcutaneous adipose tissue and only for C16:0 (by 23.4%). Reason for this decrease is not clear. One explanation could be that the tissue-specific dietary inhibition of the enzymes are involved in SFA biosynthesis. That ACC is diverse regulated in different tissues was shown by Xiao *et al.* (2006) in rats. However, the diet supplemented with n-3 PUFA (experimental diet) did not have significant effects on ACC protein expression. This does not rule out a possible direct inhibitory effect of the experimental diet on ACC activity, which could take place without inhibition of ACC protein expression. Further investigation is necessary.

In term of total fatty acids content, this parameter was lower in both muscles and subcutaneous adipose tissue with the experimental diet. However, the differences between the control and experimental diets were statistically significant for subcutaneous adipose tissue only. This might be another indicator of tissue-specific mechanisms regulating fatty acid deposition in bulls.

To summarise, this study established that: (i) a long-term feeding regime with n-3 fatty acids supplementation results in an accumulation of these health-beneficial fatty acids in cattle muscle; (ii) ACC, SCD and Δ6d proteins are expressed in both, cattle muscle and subcutaneous adipose tissue, with relatively higher expression in adipose tissue; (iii) n-3 PUFA-enriched diet tissue-specifically inhibited the expression of bovine SCD and Δ6d proteins and (iv) the lower Δ6d and SCD protein expression in muscles of animals receiving C18:3n-3 supplemented diet was accompanied by a lower n-6 PUFA and C18:1 *cis*-9 content when compared with the animal fed with C18:2n-6 supplemented diet. It is suggested that dietary manipulation of fatty acid composition in ruminants is mediated, at least partially, through the regulation of lipogenic enzymes expression; and that the regulation of the bovine lipogenic enzymes expression is tissue specific.

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Chapter 5

How do *n*-3 fatty acid (short-time restricted *vs.* unrestricted) and *n*-6 fatty acid enriched diets affect the fatty acid profile in different tissues of German Simmental bulls?

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Content

The present study investigated the effect of feeding *n*-3 and *n*-6 PUFA bulls on fatty acid profile of different tissues (MLD, SAT, liver, serum and erythrocytes) and lipid classes of IMF of German Simmental bulls. The *n*-3 feeding was combined with a short-time feed restriction period to increase the IMF resulting in an improved nutritional and sensory quality. *N*-3 PUFAs were enhanced in all tissues and *n*-3 LC PUFA were increased in erythrocytes, MLD and liver. Unfortunately, the feed restriction did not affect the IMF. It has been measured a tissue dependent synthesis and deposition of *n*-3 LC PUFA.

Work	**Contribution**
Study accomplishment	0%
Analyses (fatty acids, diets)	20%
Statistics	20%
Preparation of the manuscript	100%

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How do *n*-3 fatty acid (short-time restricted *vs* unrestricted) and *n*-6 fatty acid enriched diets affect the fatty acid profile in different tissues of German Simmental bulls?

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ABSTRACT

This study investigated the effect of *n*-6 (control group) and *n*-3 polyunsaturated fatty acids (PUFA) supply (treatment group unrestricted) and a short-time feed restriction for *n*-3 PUFA supply (treatment group restricted) on intramuscular fat content and the total fatty acid composition in different tissues (muscle, subcutaneous fat, liver, serum and erythrocytes) and lipid classes of intramuscular fat of German Simmental bulls (n = 25). Exogenous *n*-3 PUFA caused a higher concentration of the sum of all single *n*-3 and *n*-3 long-chain polyunsaturated fatty acids (LC PUFA) in all analysed tissues. Feed restriction compared to control feeding induced a significant decrease of C18:1*cis*-9 in the phospholipid fraction of *longissimus* muscle and in subcutaneous fat. The concentration of C18:3*n*-3 in liver of treatment groups was between 34 and 44% higher compared to control. PUFA in serum and the sum of *n*-3 PUFA in erythrocytes were significantly higher in both treatment groups compared to control. The synthesis and deposition of *n*-3 LC PUFA seems to be tissue dependent according to different relative amounts.

1. Introduction

Lipids have essential effects on palatability, especially on flavour and mouth feel, which are primary components of palatability (Smith, Smith & Lunt, 2004).

Quantity and quality of dietary fat is strongly related to human health (Williams 2000; Rioux & Legrand, 2007). Recommendations for saturated fatty acid (SFA) intake imply not more than 10% of dietary energy (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, und Schweizerische Vereinigung für Ernährung (DACH, 2008)) because an high intake of SFA with more than 15% of daily energy intake is connected with an increased level of blood cholesterol concentrations and a low level of LDL receptor activity which conduct in high coronary heart disease (CHD) mortality rates (Williams 2000; Rioux & Legrand, 2007).

The fact to replace SFA by PUFA or monounsaturated fatty acids (MUFA) in meat is controversially discussed (Lada & Rudel, 2003). An example of a diet high in MUFA and low in SFA is the Mediterranean Diet, where people have the lowest incidences of CHD (Lada & Rudel, 2003). In some studies MUFA showed a reducing effect to LDL-cholesterol but no effects on the HDL-cholesterol fraction, while PUFA reduced both, LDL- and HDL-cholesterol. Other studies did not find any differences (Lada & Rudel, 2003; Harwood & Yaqoob, 2002). Replacing SFA by MUFA and/or PUFA could be done via manipulation of fatty acid content in animal diet.

Two important fatty acids, which belong to two independent families of PUFA and cannot be synthesized *de novo* by animal cells, are linoleic acid (LA, 18:2*n*-6) and α-linolenic acid (ALA, 18:3*n*-3) (Simopoulos, 2008). Hence it is essential to provide these two fatty acids in the diet, because they are precursors for LC PUFA (Bezard, Blond, Bernard & Clouet, 1994). To ensure balanced eicosanoid production, the ratio of *n*-6 to *n*-3 PUFA should be 4:1 to 6:1 (Gerster, 1998). The DACH-Association (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, und Schweizerische Vereinigung für Ernährung (DACH, 2008)) recommended value for the ratio C18:2*n*-6 to C18:3*n*-3 is at least 5:1 or lower. In the absence of C18:2*n*-6 and C18:3*n*-3 growth, learning and visual activity are negatively influenced (Bezard et al., 1994) and the risk for cardiovascular disease is increased (Williams, 2000).

In addition to previously named, important fatty acids for human's nutrition and health are conjugated linoleic acids (CLA), especially the C18:2*cis*-9,*trans*-11 isomer. It is the main CLA isomer in ruminant tissues and is formed as partial hydrogenation product during ruminal hydrogenation (Williams, 2000; Perfield et al., 2007; Bhattacharya, Banu, Rahman, Causey & Fernandes, 2006). The major part is synthesised in tissues from *trans* vaccenic acid (TVA, C18:1*trans*-11) in the reaction catalysed by stearoyl-CoA-desaturase (SCD). Because of the beneficial properties in animal models, like prevention of carcinogenesis, atherogenesis and obesity, this isomer received much

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attention in human research (Nazare et al., 2007; Watras, Buchholz, Close, Zhang & Schoeller, 2007; Bhattacharya et al., 2006).

The aim of this study was to investigate the effect of diets enriched in *n*-6 PUFA (maize silage/grass silage and commercial concentrate) fed to the control group and *n*-3 PUFA (grass silage, concentrate supplemented with rapeseed cake and oil) fed to two treatment groups on intramuscular fat (IMF) content and the total fatty acid composition in different tissues (muscle, subcutaneous fat, liver, serum and erythrocytes) and lipid classes of intramuscular fat (triacylglycerol, phospholipids and free fatty acids). One of the treatment groups received a short-time feed restriction to enhance the IMF by compensatory growth in the following finishing period and to improve the marbling of German Simmental bull beef.

2. Materials and methods

2.1. Animals and experimental design

Details and design of the study are described by Mahecha et al. (2009). A short summary is given in Table 1. Animals were randomly distributed to three feeding groups. Control group was fed with maize silage/grass silage (70%/30%, *ad libitum*) and a commercial concentrate (63% triticale, 10% barley, 20% soybean meal, 5% minerals and 2% soybean oil). The main fatty acid in the fat of the total mixed ration (TMR) was C18:2*n*-6 (46.6%), the concentration of C18:3*n*-3 was 7.3%. The concentration of C16:0 and C16:1*cis*-9 was 12.1% and 0.4%, respectively. Both treatment groups were fed with grass silage (*ad libitum)* and a concentrate (39% triticale, 22% barley, 32% rape cake, 5% minerals and 2% rapeseed oil). The concentration of C18:3*n*-3 and C18:2*n*-6 in TMR was 8.1% and 27.0% and the concentration of C16:0 and C16:1*cis*-9 was 8.0% and 1.4%. Additionally all groups received 1 kg hay over the whole fattening period. During the restriction phase of 112 days animals of restricted group were fed with 1 kg of concentrate whereas the unrestricted and control group were fed with 2 kg of concentrate. In the following treatment period of 110 d the amount of concentrate was 2.5 kg for all groups. The last part of the finishing period bulls were fed 3 kg of concentrate. All groups have got the same amount of hay and straw. The energy level of diets was twofold higher than the maintenance requirement. After slaughtering tissue samples were collected from the right side of carcass immediately after slaughter. *Longissimus* muscle (MLD) for analysing lipid classes were taken at 13th/14th rib, erythrocytes and serum were separated from blood and stored at −80 °C. Liver samples and subcutaneous adipose tissue (SAT) for analysing the total fatty acid composition were frozen and stored at −20 °C. MLD for total fatty acid composition was collected at 9th/10th rib of carcasses after 24 h cooling.

2.2. Fatty acid composition

Intramuscular fat (IMF) of *longissimus* muscle (2 g sample), liver (2 g), erythrocytes (2 g), serum (1.5 g) and subcutaneous adipose tissue (1 g) was extracted with chloroform/methanol (2:1, v/v) according to Folch, Lees and Stanley (1957) by homogenisation at room temperature. The fatty acid composition for all tissues mentioned before was determined by the methodology described by Nuernberg et al. (2002).

2.3. Analysis of lipid classes from longissimus muscle

Using the thin layer chromatography described by Dannenberger, Nuernberg, Scollan, Ender and Nuernberg (2007) triglycerides, phospholipids and free fatty acids of the extracted IMF of MLD were separated, scrapped off and analysed. Fatty acid composition was determined as described in Section 2.2.

2.4. Image analysis of marbling and adipocyte size

Histological parameters were analysed using the image analysis technique with the methodology described by Albrecht, Wegner and Ender (1996) and Albrecht, Teuscher, Ender and Wegner (2006). After measuring the area of muscle cross-section the size, position and distribution of marbling flecks were analysed. Based on these results the quantitative marbling feature average number of intramuscular adipocytes was calculated.

Marbling, measured 24 h after slaughter, was scored using a 6-point scale, where one means extremely low marbling and six indicates an extremely high marbling.

2.5. Statistical analysis

Data were analysed by the least-squares method using the generalized linear model (GLM) procedures with the fixed factor feeding (SAS)®. $P \leq 0.05$ was considered as statistically significant.

In all Tables only the main fatty acids are shown whereas 33–38 individual fatty acids (depending on tissue) were identified and all are used for calculation of proportion in % and the concentration in mg/100 g or μg/g.

The Pearson correlation coefficient has been used for the calculation of the relation between selected fatty acids and IMF.

3. Results

3.1. Fatty acid composition in different tissues

3.1.1. MLD

The fatty acid composition (mg/100 g) of total intramuscular fat in *longissimus* muscle is given in Table 2. Significant differences between control and grass fed groups were measured for several fatty acids. The amount of C18:3*n*-3, C22:6*n*-3 and the sum of *n*-3 LC PUFA were significantly higher in both groups fed grass silage compared to control group whereas *n*-6 fatty acids did not differ between groups. This results in a lower *n*-6/*n*-3 ratio in grass silage fed groups. Interestingly, the amount of *n*-3 LC PUFA was the highest in the restricted group and the differences to both other groups were significant. The same case was validated for C20:5*n*-3 and C22:5*n*-3. A significant difference was observed for the unrestricted group compared to the control group for

Table 1
Experimental design.

	Control Group (n = 9)	Restricted (n = 9)	Unrestricted (n = 7)
Age at the beginning of the experiment		3–4 months	
Duration of restriction	–	112 d (50% of concentrate)	–
Diet	Maize silage/Grass silage (70%/30%, *ad libitum*), sugar beet pulp, concentrate supplemented with 20% soybean meal and 2% soybean oil	Grass silage (*ad libitum)*, sugar beet pulp, concentrate supplemented with 32% rapeseed cake and 2% rapeseed oil	
Feeding		Indoor, single fixed kept	
Live weight at slaughter (kg) (LSM_{SEM})	$636.4_{4.3}$	$637.2_{4.3}$	$631.7_{4.8}$
Breed and Gender		German Simmental bulls	

Table 2
Fatty acid composition (mg/100 g) in *longissimus* muscle of German Simmental bulls.

	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
C12:0	$2.1_{0.2}$	$2.0_{0.2}$	$1.6_{0.2}$
C14:0	$63.9_{10.1}$	$40.5_{10.1}$	$31.6_{11.4}$
C16:0	$652.5_{91.4}$	$422.2_{91.4}$	$380.2_{103.7}$
C16:1*cis*-9	$103.1^{a}_{14.5}$	$60.7^{a,b}_{14.5}$	$46.4^{b}_{16.4}$
C18:0	$362.8_{48.6}$	$251.0_{48.6}$	$232.5_{55.1}$
C18:1*cis*-9	$902.9_{120.8}$	$534.9_{120.8}$	$496.1_{137.0}$
C18:1*trans*-11	$28.3_{5.3}$	$28.1_{5.3}$	$22.2_{6.0}$
C18:2*n*-6	$95.4_{4.0}$	$96.9_{4.0}$	$92.1_{4.6}$
C18:3*n*-3	$14.6^{a}_{1.6}$	$22.8^{b}_{1.6}$	$21.0^{b}_{1.8}$
C20:4*n*-6	$26.9_{1.2}$	$30.5_{1.2}$	$29.0_{1.4}$
C20:5*n*-3	$3.9^{a}_{0.3}$	$8.2^{b}_{0.3}$	$6.5^{b}_{0.4}$
C22:5*n*-3	$9.18^{a}_{0.41}$	$13.35^{b}_{0.41}$	$11.60^{c}_{0.46}$
C22:6*n*-3	$1.35^{a}_{0.16}$	$2.27^{b}_{0.16}$	$2.11^{b}_{0.18}$
CLA*cis*-9,*trans*-11*	$7.7_{1.3}$	$6.7_{1.3}$	$5.6_{1.4}$
Σ SFA[a]	$1121.6_{154.2}$	$747.4_{154.2}$	$673.4_{174.9}$
Σ PUFA[c]	$167.4^{a}_{6.2}$	$188.6^{b\ (0.06)}_{6.2}$	$175.3^{a,b}_{7.0}$
Σ *n*-3 fatty acids[d]	$29.1^{a}_{1.6}$	$46.6^{b}_{1.6}$	$41.2^{b}_{1.8}$
Σ *n*-6 fatty acids[e]	$133.2_{4.6}$	$138.4_{4.6}$	$131.3_{5.2}$
Ratio *n*-6/*n*-3	$4.6^{a}_{0.1}$	$3.0^{b}_{0.1}$	$3.2^{b}_{0.1}$
Σ *n*-3 LC PUFA[f]	$14.5^{a}_{0.7}$	$23.8^{b}_{0.7}$	$20.2^{c}_{0.8}$
Σ *n*-6 LC PUFA[g]	$31.8_{1.2}$	$33.8_{1.2}$	$32.1_{1.3}$
Δ9-desaturase Index	$58.8_{0.7}$	$57.5_{0.7}$	$57.8_{0.8}$
Σ *trans* FA[h]	$73.4_{12.4}$	$70.2_{12.4}$	$56.8_{14.1}$
Σ FA[i]	$2450.7_{311.8}$	$1660.9_{311.8}$	$1497.3_{353.58}$

a,b – Significant effect of the diet at $P \leq 0.05$.
[a] Σ SFA = 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.
[c] Σ PUFA = Σ *n*-3 fatty acids (FA) + Σ *n*-6 FA.
[d] 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.
[e] 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.
[f] Σ *n*-3 LC FA = C20:3*n*-3 + C22:6*n*-3 + C22:5*n*-3 + C20:5*n*-3.
[g] 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.
[h] 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.
[i] Σ FA = sum of all 33–38 identified fatty acids.
* - coelution with C18:2*trans*-7, *cis*-9 and C18:2*trans*-8, *cis*-10.
Δ-9 desaturase index = 100 × [C14:1 + C16:1 + C18:1*cis*-9 + C18:1*trans*-11/C14:1 + C16:1 + C18:1*cis*-9 + C18:1*trans*-11 + C14:0 + C16:0 + C18:0 + CLA].

Table 3
Relative fatty acid composition (g/100 g) of TG and PL in *longissimus* muscle.

	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
Triglycerides			
C10:0	$0.4_{0.1}$	$0.3_{0.1}$	$0.4_{0.1}$
C12:0	$0.1_{0.0}$	$0.1_{0.0}$	$0.1_{0.0}$
C14:0	$0.6_{0.1}$	$0.7_{0.1}$	$0.7_{0.1}$
C16:0	$28.0^{a}_{0.7}$	$24.9^{b}_{0.6}$	$24.8^{b}_{0.7}$
C16:1*cis*-9	$3.9_{0.3}$	$3.1_{0.3}$	$3.1_{0.3}$
C18:0	$16.0_{0.8}$	$18.4_{0.8}$	$17.7_{0.9}$
C18:1*cis*-9	$40.1_{0.9}$	$38.3_{0.8}$	$38.4_{1.00}$
C18:1*trans*-11	$1.5^{a}_{0.2}$	$2.0^{b}_{0.1}$	$2.4^{b}_{0.2}$
C18:2*n*-6	$1.8_{0.2}$	$2.4_{0.2}$	$2.3_{0.2}$
C18:3*n*-3	$0.5^{a}_{0.1}$	$0.8^{b}_{0.1}$	$0.9^{b}_{0.1}$
C20:4*n*-6	$0.2_{0.1}$	$0.2_{0.1}$	$0.1_{0.1}$
C20:5*n*-3	$0.01_{0.0}$	$0.03_{0.0}$	$0.01_{0.0}$
CLA*cis*-9,*trans*-11*	$0.3^{a}_{0.0}$	$0.5^{b}_{0.0}$	$0.6^{b}_{0.0}$
Σ SFA[a]	$47.0_{0.8}$	$46.5_{0.7}$	$45.9_{0.8}$
Σ PUFA[c]	$3.0^{a}_{0.3}$	$4.4^{b}_{0.3}$	$4.2^{b}_{0.4}$
Σ *n*-3 fatty acids[d]	$0.6^{a}_{0.1}$	$1.1^{b}_{0.1}$	$1.0^{b}_{0.1}$
Σ *n*-6 fatty acids[e]	$2.0_{0.2}$	$2.7_{0.2}$	$2.6_{0.3}$
Ratio *n*-6/*n*-3	$3.6^{a}_{0.2}$	$2.6^{b}_{0.2}$	$2.5^{b}_{0.2}$
Σ *n*-3 LC PUFA[f]	$0.1_{0.0}$	$0.2_{0.0}$	$0.2_{0.0}$
Σ *n*-6 LC PUFA[g]	$0.2_{0.1}$	$0.2_{0.1}$	$0.1_{0.1}$
Phospholipids			
C10:0	$1.1_{0.4}$	$1.0_{0.4}$	$0.9_{0.4}$
C12:0	$0.1_{0.0}$	$0.1_{0.0}$	$0.1_{0.0}$
C14:0	$0.3_{0.0}$	$0.3_{0.0}$	$0.3_{0.0}$
C16:0	$10.0^{a}_{0.2}$	$8.9^{b}_{0.2}$	$9.2^{b}_{0.2}$
C16:1*cis*-9	$1.0_{0.1}$	$0.9_{0.1}$	$0.8_{0.1}$
C18:0	$16.1_{0.8}$	$15.2_{0.8}$	$15.4_{0.8}$
C18:1*cis*-9	$18.9^{a}_{0.8}$	$15.5^{b}_{0.8}$	$16.2^{a,b}_{0.9}$
C18:1*trans*-11	$0.4_{0.0}$	$0.4_{0.0}$	$0.5_{0.0}$
C18:2*n*-6	$22.6_{1.1}$	$23.5_{1.1}$	$23.7_{1.2}$
C18:3*n*-3	$2.5^{a}_{0.2}$	$4.4^{b}_{0.2}$	$4.3^{b}_{0.2}$
C20:4*n*-6	$11.7_{0.5}$	$12.4_{0.5}$	$12.3_{0.5}$
C20:5*n*-3	$1.6^{a}_{0.1}$	$2.7^{b}_{0.1}$	$2.4^{b}_{0.1}$
C22:5*n*-3	$3.5^{a}_{0.2}$	$4.8^{b}_{0.2}$	$4.5^{b}_{0.2}$
C22:6*n*-3	$0.5^{a}_{0.1}$	$0.7^{b}_{0.1}$	$0.6^{a,b}_{0.1}$
CLA*cis*-9,*trans*-11*	$0.1^{a}_{0.0}$	$0.2^{b}_{0.0}$	$0.2^{b}_{0.0}$
Σ SFA [a]	$28.9_{1.00}$	$26.8_{1.00}$	$27.1_{1.0}$
Σ PUFA[c]	$46.8^{a}_{1.6}$	$52.4^{b}_{1.6}$	$51.5^{a,b}_{1.7}$
Σ *n*-3 fatty acids[d]	$8.3^{a}_{0.3}$	$12.7^{b}_{0.3}$	$12.1^{b}_{0.3}$
Σ *n*-6 fatty acids[e]	$38.3_{1.4}$	$39.5_{1.4}$	$39.2_{1.5}$
Ratio *n*-6/*n*-3	$4.6^{a}_{0.1}$	$3.1^{b}_{0.1}$	$3.3^{b}_{0.1}$
Σ *n*-3 LC PUFA[f]	$5.6^{a}_{0.3}$	$8.1^{b}_{0.3}$	$7.5^{b}_{0.3}$
Σ *n*-6 LC PUFA[g]	$13.5_{0.4}$	$13.5_{0.4}$	$13.4_{0.5}$

See Table 2 for explanations.

C16:1*cis*-9. In the restricted group C16:1*cis*-9 tended to be lower in muscle compared to the control group but no significances are calculated. C18:1*cis*-9 and the sum of fatty acids were not affected by diet but apparently there was a declining tendency from control group to the restricted and unrestricted group.

CLA*cis*-9,*trans*-11 isomer of muscle fat was analysed by GC (Tables 2–7). No significant differences in CLA concentration among treatment groups and treatment and control groups were received.

The fatty acid composition of triglycerides and phospholipids is shown in Table 3. In the triglyceride fraction the relative proportion of C16:0 was significant lower in treatment groups than in control. Grass silage fed bulls deposited a significantly higher concentration of C18:1*trans*-11, C18:3*n*-3, CLA*cis*-9,*trans*-11, *n*-3 and total PUFA in the triglyceride fraction. This resulted in a significantly lower *n*-6/*n*-3 ratio in treatment groups. No significant differences were found between restricted and unrestricted group in the phospholipids fraction.

The proportion of C18:1*cis*-9 in phospholipids fraction was significantly lower in the restricted compared to the control group whereas the amount in the unrestricted group was localised between these two groups. The concentration of PUFA was significantly higher in the restricted group than in the control group whereas C18:3*n*-3, *n*-3 PUFA and *n*-3 LC PUFA, especially C20:5*n*-3, were significantly increased in treatment groups compared to the control group.

3.1.2. SAT

As well as in muscle diet had also significant effects on numerous fatty acids in SAT. The main fatty acids are shown in Table 4. The concentrations of C18:1*trans*-11, C18:3*n*-3 and the sum of *n*-3 fatty acids demonstrated a higher amount in restricted and unrestricted group compared to the control group. On the other hand the concentration of C18:1*cis*-9 and the sum of *n*-6 LC PUFA decreased significantly in restricted and unrestricted group. As in muscle the concentration of C16:1*cis*-9 was significantly decreased in the unrestricted group compared to the control group. The sum of fatty acids in SAT was not significantly lower in both experimental groups compared to the control group but there were approximately 7000 mg fatty acids/100 g SAT less in experimental groups.

3.1.3. Liver

Most of the identified fatty acids in liver tissue varied significantly between control and experimental groups (Table 5). The concentration of C16:1*cis*-9 and *n*-6 LC PUFA was significantly lower in the restricted and unrestricted group than in the control group whereas the concentrations for the unrestricted group were ranged between the restricted and control group. Both experimental groups had a significantly higher C18:3*n*-3 (approximately 51%), *n*-3 LC PUFA (approximately 30%) and C22:5*n*-3 concentration compared to the control group. Several fatty acids like C16:0, C20:5*n*-3, C22:6*n*-3, SFA, sum of *n*-6 fatty acids and the sum of total fatty acids in the liver were significantly different between the control group and the restricted group whereas the unrestricted

Table 4
Fatty acid composition (mg/100 g) in subcutaneous adipose tissue of German Simmental bulls.

	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
C10:0	$43.4_{3.6}$	$44.6_{3.6}$	$42.0_{4.0}$
C12:0	$39.9_{3.0}$	$39.4_{3.0}$	$33.8_{3.4}$
C14:0	$2045.9_{132.1}$	$1861.8_{132.1}$	$1719.4_{149.7}$
C16:0	$15,849.1_{696.9}$	$13,832.5_{696.9}$	$14,083.7_{790.3}$
C16:1*cis*-9	$36,54.9^{a}_{247.5}$	$3134.6^{a,b}_{247.5}$	$2628.9^{b}_{280.7}$
C18:0	$6917.1_{537.0}$	$6385.9_{537.0}$	$7463.3_{608.9}$
C18:1*cis*-9	$23,880.0^{a}_{1020.1}$	$19,557.1^{b}_{1020.1}$	$19,687.9^{b}_{1156.7}$
C18:1*trans*-11	$577.0^{a}_{44.7}$	$725.3^{b}_{44.7}$	$758.5^{b}_{50.7}$
C18:2*n*-6	$1127.7_{56.1}$	$1080.7_{56.1}$	$1044.5_{63.6}$
C18:3*n*-3	$244.1^{a}_{16.4}$	$364.0^{b}_{16.4}$	$326.2^{b}_{18.5}$
C20:4*n*-6	$35.7^{a}_{1.9}$	$31.2^{a,b}_{1.9}$	$26.0^{b}_{2.1}$
C22:5*n*-3	$26.70^{ab}_{2.60}$	$30.92^{a}_{2.60}$	$18.76^{b}_{2.94}$
CLA*cis*-9,*trans*-11*	$386.7_{27.4}$	$461.9_{27.4}$	$390.5_{31.1}$
Σ SFA [a]	$25,979.5_{1219.3}$	$23,388.7_{1219.3}$	$24,521.9_{1382.5}$
Σ PUFA[c]	$1544.4_{74.2}$	$1597.4_{74.2}$	$1488.1_{84.2}$
Σ *n*-3 fatty acids[d]	$288.0^{a}_{17.7}$	$413.6^{b}_{17.7}$	$361.6^{b}_{20.1}$
Σ *n*-6 fatty acids[e]	$1236.7_{57.6}$	$1164.8_{57.6}$	$1108.8_{65.3}$
Ratio *n*-6/*n*-3	$4.4^{a}_{0.1}$	$2.8^{b}_{0.1}$	$3.1^{b}_{0.1}$
Σ *n*-3 LC PUFA[f]	$26.7^{a,b}_{2.6}$	$30.9^{a}_{2.6}$	$18.8^{b}_{2.9}$
Σ *n*-6 LC PUFA[g]	$60.9^{a}_{3.4}$	$49.1^{b(0.054)}_{3.4}$	$39.9^{b}_{3.8}$
Δ9-desaturase Index	$53.0_{1.2}$	$51.7_{1.2}$	$49.6_{1.4}$
Σ *trans* FA[h]	$1363.1_{95.9}$	$1638.3_{95.9}$	$1671.4_{108.7}$
Σ FA[i]	$59,952.2_{2361.1}$	$52,493.3_{2361.1}$	$52,942.8_{2677.2}$

See Table 2 for explanations.

Table 6
Fatty acid composition (μg/g) in erythrocytes of German Simmental bulls.

	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
C12:0	$1.23_{0.18}$	$1.72_{0.16}$	$1.37_{0.23}$
C14:0	$3.78_{0.43}$	$3.58_{0.38}$	$3.74_{0.54}$
C16:0	$50.35_{2.53}$	$51.48_{2.26}$	$46.93_{3.20}$
C16:1*cis*-9	$26.04_{1.37}$	$24.85_{1.23}$	$22.99_{1.74}$
C18:0	$109.41^{a}_{6.54}$	$100.40^{a,b}_{5.85}$	$79.54^{b}_{8.27}$
C18:1*cis*-9	$253.12^{a}_{10.98}$	$240.57^{a,b}_{9.82}$	$211.03^{b(0.07)}_{13.89}$
C18:1*trans*-11	$3.35_{0.29}$	$3.80_{0.26}$	$3.52_{0.37}$
C18:2*n*-6	$152.15_{7.95}$	$139.56_{7.11}$	$126.10_{10.06}$
C18:3*n*-3	$7.80^{a}_{0.95}$	$13.60^{b}_{0.85}$	$13.29^{b}_{1.20}$
C20:4*n*-6	$60.3_{3.4}$	$50.5_{3.1}$	$51.5_{4.4}$
C20:5*n*-3	$8.91^{a}_{0.96}$	$12.71^{b}_{0.86}$	$12.21^{a,b}_{1.22}$
C22:5*n*-3	$4.14_{0.42}$	$4.59_{0.38}$	$4.90_{0.54}$
C22:6*n*-3	$1.46_{0.17}$	$1.48_{0.15}$	$1.68_{0.21}$
CLA*cis*-9,*trans*-11*	$4.00^{a}_{0.43}$	$5.76^{b}_{0.38}$	$5.24^{a,b}_{0.54}$
Σ SFA [a]	$265.99_{10.01}$	$249.09_{8.96}$	$241.14_{12.67}$
Σ PUFA[c]	$243.93_{12.45}$	$229.87_{11.14}$	$216.42_{15.75}$
Σ *n*-3 fatty acids[d]	$22.30^{a}_{2.00}$	$32.38^{b}_{1.79}$	$32.09^{b}_{2.53}$
Σ *n*-6 fatty acids[e]	$220.54_{10.86}$	$196.50_{9.71}$	$183.28_{13.73}$
Ratio *n*-6/*n*-3	$9.94^{a}_{0.31}$	$6.24^{b}_{0.28}$	$5.73^{b}_{0.40}$
Σ *n*-3 LC PUFA[f]	$14.51_{1.40}$	$18.78_{1.25}$	$18.80_{1.77}$
Σ *n*-6 LC PUFA[g]	$62.84^{a}_{3.46}$	$52.05^{b(0.08)}_{3.09}$	$53.34^{a,b}_{4.37}$
Δ9-desaturase Index	$62.48_{0.77}$	$62.41_{0.69}$	$63.52_{0.98}$
Σ FA	$823.8_{31.0}$	$780.7_{27.7}$	$725.3_{39.2}$

See Table 2 for explanations.

group showed no differences to the both other groups. The sum of total fatty acids, the sum of *n*-6 fatty acids, SFA and C16:0 were lower in the restricted grass silage fed group compared to the control group. The concentration of C20:5*n*-3 and C22:6*n*-3 was increased in the restricted group.

3.1.4. Erythrocytes

In erythrocytes the sum of total fatty acids was similar between control and experimental groups (Table 6). There were no effects of restriction. Some significant differences among control and unrestricted group were measured whereas the concentration was decreased in second mentioned. This was the case for C18:0 and C18:1*cis*-9 (P = 0.07). The grass silage diet resulted in a significantly higher concentration of C18:3*n*-3 and the sum of *n*-3 fatty acids in the restricted and unrestricted group. The control group tended (P = 0.08) to have a higher sum of *n*-6 LC PUFA than in the restricted group.

Table 5
Fatty acid composition (mg/100 g) in liver of German Simmental bulls.

	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
C10:0	$10.72_{0.55}$	$10.31_{0.55}$	$12.08_{0.62}$
C12:0	$0.81_{0.11}$	$1.00_{0.11}$	$0.73_{0.12}$
C14:0	$8.94_{0.72}$	$7.21_{0.72}$	$7.46_{0.82}$
C16:0	$239.26^{a}_{8.86}$	$184.98^{b}_{8.86}$	$209.30^{a,b}_{10.05}$
C16:1*cis*-9	$25.86^{a}_{1.40}$	$17.63^{b}_{1.40}$	$19.19^{b}_{1.59}$
C18:0	$787.35_{15.72}$	$748.24_{15.72}$	$787.49_{17.83}$
C18:1*cis*-9	$297.75^{a}_{13.00}$	$225.06^{b}_{13.00}$	$251.16^{b(0.07)}_{14.74}$
C18:1*trans*-11	$24.94^{a}_{1.28}$	$29.95^{b}_{1.28}$	$33.41^{b}_{1.45}$
C18:2*n*-6	$322.82_{11.68}$	$293.35_{11.68}$	$333.13_{13.24}$
C18:3*n*-3	$37.07^{a}_{4.44}$	$56.22^{b}_{4.44}$	$66.53^{b}_{5.03}$
C20:4*n*-6	$264.1^{a}_{7.3}$	$231.8^{b}_{7.3}$	$244.0^{a,b}_{8.2}$
C20:5*n*-3	$26.35^{a}_{1.86}$	$38.20^{b}_{1.86}$	$32.71^{a,b}_{2.11}$
C22:5*n*-3	$112.89^{a}_{5.02}$	$151.57^{b}_{5.02}$	$150.84^{b}_{5.69}$
C22:6*n*-3	$39.43^{a}_{3.97}$	$52.82^{b}_{2.97}$	$49.05^{a,b}_{3.37}$
CLA*cis*-9,*trans*-11*	$8.48^{a}_{0.60}$	$8.97^{a}_{0.60}$	$11.05^{b}_{0.68}$
Σ SFA [a]	$1091.69^{a}_{21.43}$	$998.16^{b}_{21.43}$	$1062.00^{a,b}_{24.29}$
Σ PUFA[c]	$955.46_{20.16}$	$947.64_{20.16}$	$992.72_{22.86}$
Σ *n*-3 fatty acids[d]	$219.20^{a}_{8.63}$	$301.33^{b}_{8.63}$	$301.31^{b}_{9.78}$
Σ *n*-6 fatty acids[e]	$733.80^{a}_{14.84}$	$643.06^{b}_{14.84}$	$688.88^{a,b}_{16.83}$
Ratio *n*-6/*n*-3	$3.40^{a}_{0.10}$	$2.14^{b}_{0.10}$	$2.29^{b}_{0.11}$
Σ *n*-3 LC PUFA[f]	$178.67^{a}_{8.78}$	$242.59^{b}_{8.78}$	$232.60^{b}_{8.96}$
Σ *n*-6 LC PUFA[g]	$316.88^{a}_{8.25}$	$266.71^{b}_{8.35}$	$282.26^{b}_{9.47}$
Δ9-desaturase Index	$55.75_{0.69}$	$54.96_{0.69}$	$55.10_{0.78}$
Σ *trans* FA[h]	$75.53^{a}_{3.31}$	$90.98^{b}_{3.31}$	$98.24^{b}_{3.76}$
Σ FA[i]	$2495.4^{a}_{45.2}$	$2323.1^{b}_{45.2}$	$2470.5^{a,b}_{51.3}$

See Table 2 for explanations.

3.1.5. Serum

Contrary to the fatty acid composition of erythrocytes PUFA, *n*-3 PUFA concentration and especially C20:5*n*-3 was significantly higher in both experimental groups compared to control group in serum (Table 7). The concentration of C18:3*n*-3 in both experimental groups was approximately 2.2 times higher compared to control group.

Table 7
Fatty acid composition (μg/g) in serum of German Simmental bulls.

	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
C12:0	$0.96_{0.20}$	$0.47_{0.19}$	$.61_{0.23}$
C14:0	$8.47^{a}_{0.54}$	$6.75^{b(0.07)}_{0.51}$	$7.20^{a,b}_{0.62}$
C16:0	$136.38_{6.31}$	$119.86_{5.95}$	$130.15_{7.29}$
C16:1*cis*-9	$28.35_{2.96}$	$18.53_{2.79}$	$22.93_{3.41}$
C18:0	$191.72_{8.50}$	$210.24_{8.02}$	$215.50_{9.82}$
C18:1*cis*-9	$126.89_{7.53}$	$118.28_{7.10}$	$128.97_{8.69}$
C18:1*trans*-11	$7.19^{a}_{0.83}$	$9.93^{b(0.06)}_{0.78}$	$11.79^{b}_{0.96}$
C18:2*n*-6	$449.49^{a}_{25.03}$	$539.56^{b}_{23.60}$	$536.14^{a,b}_{28.91}$
C18:3*n*-3	$56.61^{a}_{7.58}$	$127.34^{b}_{7.14}$	$123.91^{b}_{8.75}$
C20:4*n*-6	$31.5_{1.5}$	$32.7_{1.4}$	$31.4_{1.7}$
C20:5*n*-3	$4.24^{a}_{0.73}$	$8.80^{b}_{0.68}$	$7.20^{b}_{0.84}$
C22:5*n*-3	$14.75_{2.62}$	$14.90_{2.47}$	$20.48_{3.02}$
C22:6*n*-3	$5.35_{1.52}$	$4.17_{1.43}$	$6.04_{1.75}$
CLA*cis*-9,*trans*-11*	$2.68_{0.23}$	$2.72_{0.22}$	$3.27_{0.26}$
Σ SFA [a]	$434.80_{22.94}$	$395.27_{21.63}$	$429.19_{26.49}$
Σ PUFA[c]	$590.93^{a}_{32.69}$	$750.99^{b}_{30.82}$	$748.08^{b}_{37.75}$
Σ *n*-3 fatty acids[d]	$83.17^{a}_{8.95}$	$155.59^{b}_{8.44}$	$158.74^{b}_{10.34}$
Σ *n*-6 fatty acids[e]	$504.20^{a}_{25.80}$	$591.91^{b(0.06)}_{24.92}$	$585.28^{a,b}_{29.79}$
Ratio *n*-6/*n*-3	$6.21_{0.25}$	$3.85_{0.24}$	$3.77_{0.29}$
Σ *n*-3 LC PUFA[f]	$24.34_{4.52}$	$27.87_{4.27}$	$33.71_{5.22}$
Σ *n*-6 LC PUFA[g]	$37.83_{1.77}$	$35.96_{1.67}$	$35.60_{2.04}$
Δ9-desaturase Index	$69.58^{a}_{2.71}$	$57.46^{b}_{2.56}$	$61.63^{a,b}_{3.13}$
Σ *trans* FA[h]	$23.91^{a}_{2.13}$	$27.67^{a,b}_{2.00}$	$33.40^{b}_{2.45}$
Σ FA[i]	$1223.0_{57.2}$	$1323.3_{54.0}$	$1378.8_{66.1}$

See Table 2 for explanations.

Table 8
Histological parameters of *longissimus* muscle in German Simmental bulls.

Histological parameters	Control group LSM_{SEM}	Restricted group LSM_{SEM}	Unrestricted group LSM_{SEM}
Marbling	$1.9_{0.2}$	$1.7_{0.2}$	$1.9_{0.2}$
Intramuscular adipocyte area, μm^2	$5190.1^{a}_{256.8}$	$3872.9^{b}_{256.8}$	$4028.7^{b}_{291.1}$
Number of intramuscular adipocytes	$158.7^{a}_{12.7}$	$125.9^{a,b}_{12.7}$	$112.4^{b\ (0.06)}_{14.3}$
MLD cross-sectional area, cm^2	$80.7_{3.0}$	$82.0_{3.0}$	$82.7_{3.4}$
Intramuscular fat area, cm^2	$4.6_{0.7}$	$3.6_{0.7}$	$3.4_{0.7}$

Interestingly, the concentration of C18:2*n*-6 was significantly higher in the restricted compared to the control group.

3.2. Histological parameters

Selected histological parameters are shown in Table 8. Marbling was relatively low for all three groups, which is related to a low intramuscular fat content. The area of adipocytes was significantly smaller in experimental groups compared to the control group whereas the number of intramuscular adipocytes was significantly different between control and unrestricted group with a decrease of approximately 46 adipocytes in the unrestricted group. No differences were measured in the MLD cross-sectional and the intramuscular fat area.

3.3. Relative proportion of selected FA in different tissues

Fig. 1 visualise the proportions of PUFA, *n*-3 PUFA and *n*-3 LC PUFA in serum, erythrocytes, *longissimus* muscle, liver and subcutaneous adipose tissue. In general, the relative amount of *n*-6 PUFA was higher than *n*-3 PUFA in all tissues. The concentration of *n*-6 LC PUFA in the groups was constant across each tissue. The liver had the highest amount of *n*-3 LC PUFA and liver and serum the highest sum of *n*-3 PUFA, relative and quantitative, whereas serum had the highest relative amount of *n*-6 fatty acids. Thirty percent more *n*-3 LC PUFA was contained in the liver compared to total MLD fat. The proportion of the sum of *n*-3 and *n*-6 PUFA was nearly similar for MLD and SAT but the subcutaneous adipose tissue contained much less *n*-3 (40%) and *n*-6 (20%) LC PUFA compared to MLD.

3.4. Correlations in longissimus muscle

The correlation coefficient of r = 0.93 indicated a strong relationship between the concentrations of C16:1*cis*-9 and the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0 and C16:0) in total intramuscular fat of *longissimus* muscle (Fig. 2). The same strong correlation between both traits with a coefficient of r = 0.9 was given for triglycerides extracted from *longissimus* muscle (Fig. 2b) and for liver fat (r = 0.75, Figure is not shown). The correlation coefficient calculated for phospholipids was r = 0.54. No relationship was found in subcutaneous adipose tissue.

4. Discussion

The aim of this study was to investigate the effects of different dietary fats on fatty acid profile and additionally feed restriction on fat content and composition in German Simmental bulls.

There have been no effects on performance and meat quality between unrestricted and restricted fed bulls (Mahecha et al., 2009. In the present study the IMF content did not differ between all feeding groups. It can be concluded that short-time restriction induced no compensatory growth for fat deposition in muscle. Total daily gain was nearly the same for restricted and unrestricted animals over the whole feeding period (Mahecha et al., 2009).

The composition of diet in beef cattle production can affect the composition of fatty acids, and the content of essential fatty acids in

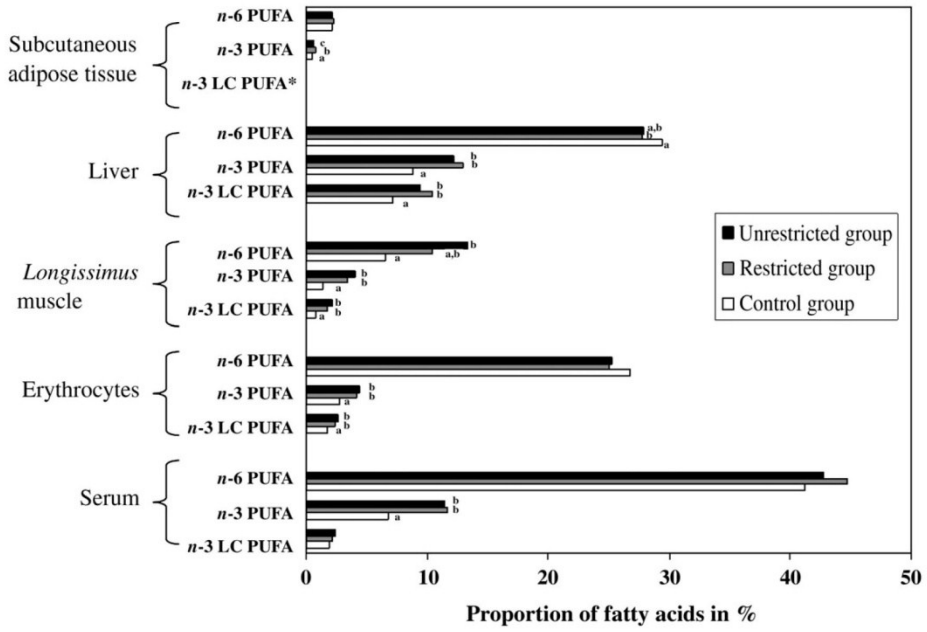

Fig. 1. Proportion of *n*-6, *n*-3 and *n*-3 LC PUFA for subcutaneous adipose tissue, liver, *longissimus* muscle, erythrocytes and serum in German Simmental bulls (*n*-3 LC PUFA* proportion for this tissue is too low to represent it in this figure).

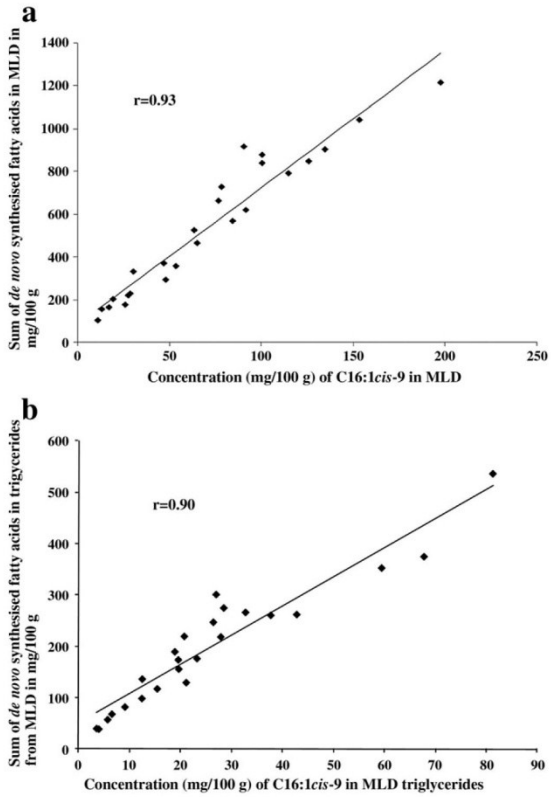

Fig. 2. (a) Correlation between the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0 and C16:0) and C16:1*cis*-9 as a signalling molecule in *longissimus* muscle. (b) Correlation between the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0 and C16:0) and C16:1*cis*-9 as a signalling molecule in triglycerides extracted from *longissimus* muscle.

red meat, due to variations of fatty acid composition in diets (Wachira et al., 2002). Ponnampalam, Mann and Sinclair (2006) revealed an alteration of essential lipid components in beef by feeding grass and grain. Especially feeding grass silage and concentrates supplemented with *n*-3 FA enriched components (rapeseed, linseed and algae) enhances the content of essential fatty acids like *n*-6 and *n*-3 PUFA and LC PUFA. This feeding regime demonstrated good results in enhancing these beneficial fatty acids for human health in beef (Nuernberg et al., 2005; Scollan et a., 2006; Warren et al., 2008a,b).

Based on the present study feeding grass silage and concentrate supplemented with rapeseed cake was successful in enhancing *n*-3 fatty acids in different tissues. The fat composition of *longissimus* muscle in experimental groups is more beneficial for human nutrition because of a lower content of saturated fatty acids and a higher content of PUFA, especially *n*-3 PUFA. The ratio of *n*-6/*n*-3 is with 3.0 ± 0.1 and 3.2 ± 0.1 in the restricted and unrestricted group low, corresponds to the recommendation $\leq 5{:}1$ (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, und Schweizerische Vereinigung für Ernährung (DACH, 2008)). The composition of intramuscular fat concerning SFA and PUFA are similar between restricted and unrestricted groups as shown in pigs (Dunker, Rey, Lopez-Bote & Daza, 2007), but the concentration of *n*-3 LC PUFA, and especially C20:5*n*-3 and C22:5*n*-3 in *longissimus* muscle and C22:5*n*-3 in subcutaneous tissue are significantly increased in the restricted compared to the unrestricted group although the concentration of the precursor ALA and the product/precursor ratio was similar in both experimental groups. It can be supposed that the short-time restriction have provoked differences in PUFA elongation and desaturation and in absolute fat deposition.

The fraction of triglycerides was determined as well as phospholipids and free fatty acids. Analysis of triglycerides and phospholipids afford a more exact answer to how muscle fatty acid composition responds to diet of ruminant animals (Aurousseau et al., 2007). Aurousseau et al. (2007) have found an influence of grass diet on PUFA and especially on *n*-3 PUFA in TG fraction in muscle of lambs. The present experiment with German Simmental bulls confirms the results of Aurousseau et al. (2004) because the relative proportion of PUFA was significantly increased in both treatment groups compared to the control group. This increase of PUFA is realised by a higher deposition of *n*-3 PUFA in muscle.

PUFA are predominantly deposited in phospholipids where they maintain the permeability of cell membranes (Martonosi, 1975). The fatty acid composition of phospholipids in muscle is affected by diet as shown by Lorenz et al. (2002) and Dannenberger et al. (2004). In line with Dannenberger et al. (2004) we did not find significant differences in C18:2*n*-6 in the PL fraction whereas the sum of PUFA is significant increased in restricted compared to control group and the relative concentration of PUFA in unrestricted group tend to be higher compared to control group. Lorenz et al. (2002) found an increase of C18:3*n*-3 in PL fraction of pasture fed bulls compared to bulls finished on grain. Feeding grass silage supplemented with rapeseed cake and oil resulted also in a higher proportion of C18:3*n*-3 in PL fraction compared to a diet with maize silage. Despite the biohydrogenation of *n*-3 PUFA in the rumen, the higher concentration in grass silage is obviously absorbed and deposited into the muscle lipid fraction of phospholipids.

Marbling is a subjective trait for description of the amount and distribution of fat in muscle. Marbling values are between 1.7 and 1.9 and did not differ among the groups although the IMF tended to be higher in control group (2.8%) compared to both experimental groups (1.8 and 2.1%). This result is in line with image analysis results where the intramuscular adipocyte area is significant higher in control group. The difference in IMF could be attributed to the different digestibility of maize silage and grass silage. The content of starch is clearly higher in maize silage and provides rapid available energy due to easily digestive starch to accumulate IMF in control group animals. Furthermore, exogenous *n*-3 PUFA inhibits SCD protein expression in muscle and subcutaneous adipose tissue resulting in a decrease of C18:1*cis*-9 and ended up with lower IMF deposition (Herdmann, Nuernberg, Martin, Nuernberg & Doran, 2010).

The direct comparison of relative composition of *n*-6, *n*-3 and *n*-3 LC PUFA in subcutaneous tissue, liver, MLD, erythrocytes and serum is demonstrated in Fig. 1. Serum as a transport medium for lipids contains the highest relative content in PUFA and especially of *n*-6 fatty acids compared to all other tissues. The relative proportion of PUFA and *n*-3 PUFA in restricted and unrestricted group is significant higher compared to the control group. These increases in *n*-3 PUFA is caused by grass silage and concentrate enriched with rapeseed in both treatment groups. Yeom, Schonewille and Beynen (2005) demonstrated the alteration of fatty acid composition of plasma and erythrocytes in ruminating goats due to feeding a corn oil or olive oil diet. In human it is possible to increase the serum *n*-3 PUFA of overweight and obese men with a diet high in fish oil (Hanwell, Kay, Lampe, Holub & Duncan, 2009). Erythrocyte phospholipids composition is a marker for long-term fat intake because of a lifespan of 120 days (Hodson, Skeaff & Fielding, 2008). The enrichment of diet with C18:2*n*-6 increased deposition of this fatty acid in erythrocytes, which demonstrate that the fatty acid composition of erythrocytes is dynamic and exchange occurs with other lipid pools (Hodson et al., 2008). The amount of *n*-6 and *n*-3 PUFA is lower in erythrocytes than in serum. Serum reflects the current status in blood transported lipids

whereas the membrane fluidity of erythrocytes must be maintained (Smith, 1987).

SAT represents the main tissue for *de novo* fatty acid and triacylglycerol synthesis. Short time restriction of concentrate feeding did not affect the lipid profile but it was affected by diet.

The liver in ruminants has less effect on *de novo* synthesis (Bauchart, Gruffat & Durand, 1996) and disposal of lipids than adipose tissue or lactating mammary gland (Bell, 1980). Nevertheless, there is a low rate of *de novo* biosynthesis where acetate is the main carbon source. Availability of acetate partially depends on the uptake of LC PUFA in the liver and is used for synthesis of glycerides, complex lipids, oxidation and a relative high rate of ketogenesis (Bell, 1980). The relative *n*-3 PUFA content in liver of control group is obviously lower than in both treatment groups. PUFA concentration is 10–18% lower than in serum but *n*-3 LC PUFA are between 5.18 and 8.31% higher in liver. Two main usages are given for metabolic products of LC PUFA in form of LCFA-CoA: the esterification into triacylglycerols, whereas the secretion of these as a part of VLDL by the liver is low, or, to a lower extent, the esterification into phospholipids and cholesteryl esters. The second usage is the complete oxidation to CO_2 or incomplete oxidation to generate acetate and ketone bodies (Bauchart et al., 1996). A third possibility could be the competition of *n*-3 and *n*-6 PUFA in using a common elongation-desaturation system with catalyst Δ6-desaturase. This enzyme catalyses the desaturation of the 18-carbon and 24-carbon substrates and it prefers *n*-3 rather than *n*-6 PUFA (Simopoulos, 2008, Contreras & Rapoport, 2002). The intake of *n*-3 PUFA in experimental groups was higher than in control group and supported in this way the preferred synthesis of *n*-3 LC PUFA in liver. The amount of *n*-3 LC PUFA in muscle is nearly the same like in serum. The higher concentration of *n*-3 LC PUFA in the liver seems to be generated by a higher *de novo* synthesis compared to muscle.

Palmitoleate is a fatty acid which is transported by fatty acid binding proteins (FABP) and is postulated to be the main signalling hormone that controls the metabolism in muscle and liver in mice and contributes to the regulation (suppression) of SCD-1 expression (Cao et al., 2008). Palmitoleate also acts as an insulin-sensitizing hormone improving glucose metabolism in mice and it is a unique fatty acid as a marker for *de novo* lipogenesis (Cao et al., 2008). The authors reported a strong correlation between C16:1*cis*-9 in plasma and muscle and liver tissue of mice. In German Simmental bulls a strong correlation ($r = 0.93$) between the sum of *de novo* synthesised fatty acid concentration (C10:0, C12:0, C14:0 and C16:0) and palmitoleate in *longissimus* muscle and a correlation of $r = 0.90$ in triglycerides of *longissimus* muscle has been calculated (Fig 2 and 2b). Interestingly, the correlation coefficient between SFA and C18:1*cis*-9 in *longissimus* muscle is 0.95. It seems that oleic acid as the most abundant fatty acid also acts as a signal molecule in muscle of bulls. It is well known that the main tissue in ruminants to synthesise fatty acids is the adipose tissue but we did not find a correlation for C16:1*cis*-9 in subcutaneous adipose tissue whereas the correlation between C16:1*cis*-9 and *de novo* fatty acids in liver is 0.75. Additionally, we did not find a relationship between palmitoleate in serum and *de novo* synthesised fatty acids in *longissimus* muscle and subcutaneous fat. Alterations in lipid metabolism are often reflected in blood non-esterified fatty acids (NEFA) because adipose tissue is the major source of FFA mobilisation (Cao et al., 2008). For this experiment NEFA fraction of blood was not separated. Therefore, no information about NEFA composition in blood is available. This is an approach for further research in this field.

5. Conclusion

Feeding *n*-3 PUFA enriched experimental diets (grass silage and concentrate with rapeseed cake and rapeseed oil; short-time restricted or unrestricted) to cattle did not affect the fat content in *longissimus* muscle and subcutaneous fat compared to control group (*n*-6 enriched diet, maize silage and concentrate with soybean meal). Muscle lipid composition of bulls fed the experimental diets was more beneficial for human nutrition due to a ratio *n*-6/*n*-3 of 3.0–3.2. Exogenous *n*-3 supply leads to an enrichment of *n*-3 and *n*-3 LC PUFA in MLD, triglycerides and phospholipids in *longissimus* muscle, subcutaneous adipose tissue, liver, erythrocytes and serum. The deposition of these PUFA was tissue dependent.

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Chapter 6

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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Content

The present study investigated the effect of LA and ALA feeding on meat quality, fatty acid composition and the SCD activity in MLD and SAT of German Holstein bulls. The diet increased the LA and the ALA content in the tissues. Feeding the diet supplemented with ALA resulted in a decreased SCD activity in MLD and SAT with a reduced relative concentration of oleic acid in muscle. According to the interest of increasing the dietary intake of *n*-3 PUFAs in the human diet the transfer of beneficial *n*-3 fatty acids from fresh muscle to the product German Corned Beef sausage was effective with no significant losses of beneficial fatty acids during the production process.

Work	**Contribution**
Study accomplishment	90%
Analyses (fatty acids, diets)	50%
Statistics	20%
Preparation of the manuscript	100%

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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.

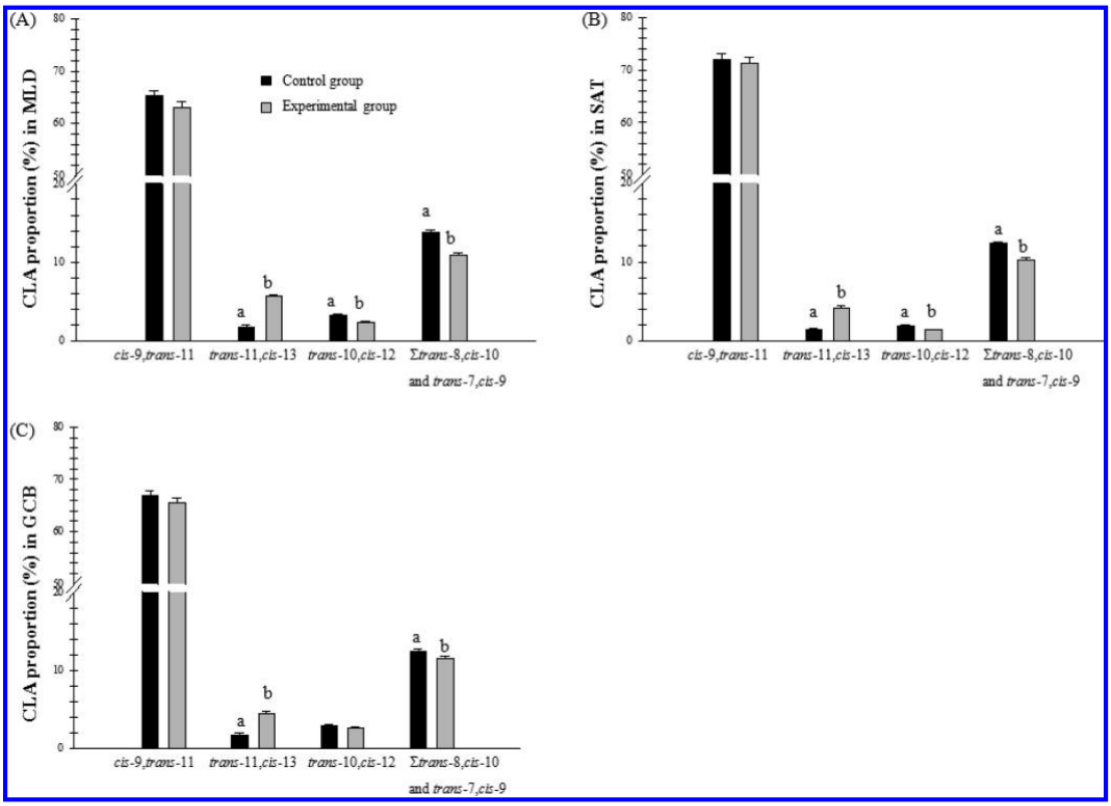

Figure 1. Relative composition of selected CLA isomers (% of total CLA isomers measured by HPLC) in longissimus muscle (**A**), subcutaneous adipose tissue (**B**), and German corned beef sausages (**C**) of German Holstein bulls (a and b indicate significant differences between groups within muscle,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

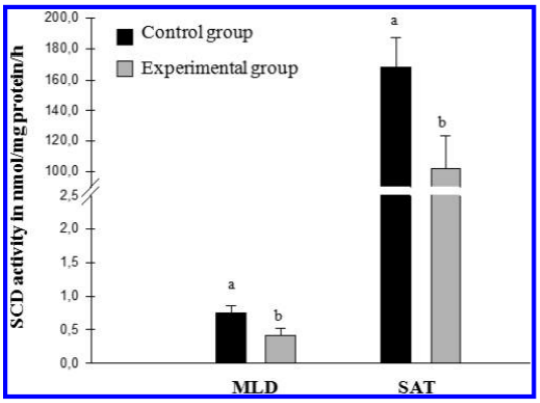

Figure 2. Specific SCD enzyme activity in longissimus muscle and subcutaneous adipose tissue of German Holstein bulls (a and b indicate significant differences between groups within muscle and within subcutaneous fat at $P \leq 0.05$).

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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Chapter 7

General Discussion

7. General Discussion

Two different animal experiments (described in chapter 3) were conducted to find answers to the questions listed in chapter 2 considering the fatty acid composition of several tissues, the protein expression and activity of lipogenic enzymes, and the fatty acid composition of beef products.

German Holstein (GH) and German Simmental (GS) bulls are mostly used for beef production in Germany. Therefore, these breeds are used for the animal experiments. The beef production in Germany is based on fattening bulls instead of steers due to economic reasons and animal welfare arguments against castration. Based on the sum of cattle in Germany, (2008) approximately 41% were from German Holstein breed and 29% from German Simmental breed (Zentrale Datenbank für Rinder, 2008). The breed German Holstein is mainly known as a dairy cattle but the male calves of this breed were used for fattening. German Simmental are bred to use the male animals for fattening and the females to produce milk, or keeping them as mother cow keeping for the meat producing calves.

Based on the interest to enhance the *n*-3 fatty acids in meat and meat products the animals in the treatment groups of both experiments received diets enriched in ALA. Especially in the second trial the German Holstein bulls (Table 7) of the treatment group received approximately a 4-times higher concentration of ALA by the diet compared to the control group. The total PUFA concentration was 1.5-times higher. The diet of the control group was 1.4-time higher in LA compared to the experimental group. The relative proportion of *n*-3 fatty acids and *n*-6 fatty acids resulted in a decreased ratio of *n*-6/*n*-3 to approximately 3 in the diets of all experimental groups of both experiments, but the ratio of the control group in the experiment with German Holstein bulls was with 3.68 approximately 2.7 lower than in the control group of the experiment with German Simmental bulls (Table 4).

The animals of both experiments were slaughtered at normal slaughter weight ranges for Germany [German Holstein bulls (ProSafeBeef) at approximately 625 kg live weight and German Simmental bulls (MPA) at 630 kg live weight]. However, the fattening period was different for both experiments because of the breed differences (dairy cattle and dual-purpose cattle). The German Holstein bulls received the diet for approximately 241-244 days, and the German Simmental bulls 274 days in the control and 334-368 days in the treatment groups. This distinction is attributed to the different total daily gains. The German Simmental bulls in the control group had nearly the same total daily gain (1.26 kg±0.03) as the control group of

the German Holstein bulls (1.23 kg±0.03). By contrast, the treatment groups of the MPA trial had only a total daily gain of 1.03 kg±0.03 for the unrestricted and 0.97 kg±0.03 for the feed restricted group, compared to a total daily gain of 1.16 kg±0.03 for the treatment group of German Holstein bulls. Reasons for these differences could be the breed, the lower feed intake of the German Simmental bulls in the treatment groups, the lower digestibility of the offered feed, or some combination of the three.

In summary, the two animal experiments take the same basic approach, which is modified for different breeds.

Fatty acid composition, including *trans*-fatty acids and CLA, of muscle and SAT under LA and ALA feeding

The influence of the tissue fatty acid composition in monogastric animals is easier to quantify due to the more direct incorporation into tissues (Nuernberg *et al.*, 2005a) than in ruminants because of their reliance on bacterial biohydrogenation. Nevertheless, a number of studies demonstrated the successful accumulation of *n*-6 and *n*-3 fatty acids in ruminants (Scollan *et al.*, 2006; Wood *et al.*, 2008). Notably, the feeding of grass silage in combination with concentrate supplemented with linseed, rapeseed, and algae or the natural grazing resulted in an accumulation of for human health beneficial *n*-3 PUFA and *n*-3 LC PUFA in ruminant's tissues (Nuernberg *et al.*, 2005b; Kraft *et al.*, 2008; Wood *et al.*, 2008; Warren *et al.*, 2008).

Considering the expression of the data it is of relevance, which, absolute (mg/100 g) or relative (g/100 g) data, were used to represent the results of a study. In recent studies the most common method of data description for fatty acid composition is the use of normalized percentage of total fatty acids (relative proportion) to determine whether a quantitative change in IMF content would cause a qualitative change (Nuernberg *et al.,* 1999; Chung *et al.*, 2006; Padre *et al.*, 2006). In the study of Hoehne *et al.* (2012), there were different relationships of several parameters to the IMF identified depending on the use of the absolute or relative fatty acid composition. Negative correlations between C18:0 concentration and IMF were found and are in contrast to the positive correlations for the proportion of FA to IMF.

In the present studies differences were also found between the relative and absolute concentration of fatty acids. In general, the supplementation of diets enriched with ALA induced an accumulation of ALA, *n*-3 PUFA and *n*-3 LC PUFA in tissues. The following

section addresses the fatty acid composition of muscle including discussion of the differences between the relative and absolute concentrations of *n*-3 and *n*-6 fatty acids.

Longissimus muscle (Table 9):

The absolute concentration of ALA, the sum of *n*-3 PUFA and the sum of *n*-3 LC PUFA are significantly increased in the *longissimus* muscle of both experiments. By contrast the unrestricted group of the MPA study had a higher concentration than the restricted group because of the EPA content. Contrary to these results the relative concentration of *n*-3 LC PUFA in the MLD of MPA is equal between both treatment groups. This contrary result has also been found for the sum of *n*-6 fatty acids (%, mg/100 g).

The relative concentrations for ALA and *n*-3 PUFA of PSB are consistent according to the absolute concentration, but differences were revealed for *n*-6 fatty acids. The relative concentration of *n*-6 PUFA and *n*-6 LC PUFA are similar between control and treatment group but the absolute concentration is significantly higher in the control group. This is explained by the significantly lower total sum of fatty acids in the treatment group. The ratio of *n*-6/*n*-3 is significantly lower in the treatment groups of both experiments (MPA: 3.0±0.1 for the restricted and 3.2±0.1 in the unrestricted group; PSB: 2.3±0.1). These ratios correspond to the recommendation of the German Nutrition Society of a ratio *n*-6/*n*-3 of ≤5:1in human nutrition (DGE, 2008).

Despite the biohydrogenation of PUFA in the rumen (Wood *et al.*, 2008) the *n*-3 LC PUFA EPA and DHA are significantly increased under ALA feeding in the absolute and relative concentration of the muscle from German Holstein and Simmental bulls (except EPA in % and DHA in % for restricted group). It seems that the concentration of *n*-3 LC PUFA in muscle is dependent upon the concentration of ALA in the diet. The higher the concentration of ALA in the diet the higher ALA amount bypasses the biohydrogenation, and it will be absorbed at a higher level in the digestive tract. Another fact is that there is a competition between *n*-3 and *n*-6 fatty acids for the desaturation system, especially the Δ6d. This desaturase prefers the fatty acids of the *n*-3 series but a high LA intake interferes with the ALA metabolism (Simopoulos, 2008) and this effect seems to be dose-dependent (Holman, 1986).

In summary, a high ALA concentration and a low *n*-6/*n*-3 ratio (in the diet of the experimental group: 0.6:1) in the diet of bulls induced a significant increase in ALA, DHA, and EPA in absolute concentration (Exp. 1 and 2) and relative proportion (Exp. 1 except EPA

and Exp. 2) of muscle tissue. The high amount of LA in the diet of Exp. 2 control group resulted in a significantly increased absolute concentration in muscle tissue of this group but the relative proportion was similar between both groups. This gives evidence that fatty acids of the *n*-3 series are predominantly synthesised and incorporated into tissues. Interestingly, the relative concentration of LA is increased in the restricted and unrestricted compared to the control group despite the low LA amount in the diet. This could be attributed to the longer fattening period of the bulls fed the mix of grass/maize silage.

A diet generally high in ALA and PUFA is linked to high concentrations of CLA and C18:1*trans*-isomers in tissues (Givens, 2006). *Trans* fatty acids (TFA) are known as critical in human nutrition and the dietary intake is directly related to the TFA content in human adipose tissue (Chardigny *et al.*, 2007; Kuhnt *et al.*, 2007).

Two main sources, industrially produced by partial hydrogenation of vegetable oils and as products of the bacterial biohydrogenation in ruminants (Gebauer *et al.*, 2007) generate the same TFA isomers, but in different amounts (Brouwer *et al.*, 2010). Effects induced by TFA are the rise of LDL and plasma total cholesterol and reduction of HDL cholesterol (Sanders, 2009). The main TFA isomer in ruminant tissue is the vaccenic acid (VA). It is a biohydrogenation product of LA and ALA and the precursor for the formation of CLA*cis*-9,*trans*-11 in tissues (Scollan *et al.*, 2006; Griinari *et al.*, 2000). The proportion of VA (Table 9) is increased in MLD of the experimental group in Exp. 2 but not the absolute concentration. This is related to the lower amount of fatty acids in this group. The absolute and relative concentrations in MLD of bulls from Exp. 1 are not different between groups. C18:2*n*-6 rich concentrate diets are known to have a small particle size, resulting in a shorter rumen transit time than fibrous forage diets (Wood *et al.*, 2008). The treatment groups received also 30% maize silage with the diet. This proportion seems to be sufficient to prevent a higher VA amount in the treatment groups. Comparing the VA amounts of both experiments, it is nearly twice as high in MLD of Exp. 1 (1.16-1.63%) as in MLD from PSB bulls (0.6-0.8%). The expectation would be that the amount in tissues of PSB bulls is higher because of the greater amount of LA and ALA in the diet. The longer feeding durations of the German Simmental bulls (control: appr. 274 d; unrestricted: appr. 334 d; restricted: appr. 368 d *versus* 241-244 d for German Holstein bulls) could be the reason for a higher accumulation of VA. Especially for VA further research is needed to assess the risk outgoing from this fatty acid for human health (Smith *et al.*, 2009; Field *et al.*, 2009).

Table 9: Selected fatty acid composition of *longissimus* muscle of German Holstein and Simmental bulls

	Experiment 2 – German Holstein bulls				**Experiment 1 – German Simmental bulls**					
	Relative proportion (%)		**Absolute concentration (mg/100 g)**		**Relative proportion (%)**			**Absolute concentration (mg/100 g)**		
	CG LSM$_{SEM}$	**TG LSM$_{SEM}$**	**CG LSM$_{SEM}$**	**TG LSM$_{SEM}$**	**CG LSM$_{SEM}$**	**RG LSM$_{SEM}$**	**UG LSM$_{SEM}$**	**CG LSM$_{SEM}$**	**RG LSM$_{SEM}$**	**UG LSM$_{SEM}$**
C18:2*n*-6	$5.3_{0.4}$	$5.6_{0.4}$	$112.9_{3.3}^{a}$	$95.2_{3.4}^{b}$	$4.5_{1.2}^{a}$	$7.2_{1.2}^{a,b}$	$9.2_{1.3}^{b}$	$95.4_{4.0}$	$96.9_{4.0}$	$92.1_{4.6}$
C18:3*n*-3	$0.6_{0.1}^{a}$	$2.0_{0.1}^{b}$	$13.0_{1.1}^{a}$	$33.4_{1.1}^{b}$	$0.7_{0.2}^{a}$	$1.6_{0.2}^{b}$	$1.9_{0.2}^{b}$	$14.6_{1.6}^{a}$	$22.8_{1.6}^{b}$	$21.0_{1.8}^{b}$
C20:5*n*-3 (EPA)	$0.2_{0.03}^{a}$	$0.5_{0.03}^{b}$	$3.9_{0.3}^{a}$	$8.8_{0.3}^{b}$	$0.2_{0.1}$	$0.6_{0.1}$	$0.7_{0.1}$	$3.9_{0.3}^{a}$	$8.2_{0.3}^{b}$	$6.5_{0.4}^{c}$
C22:6*n*-3 (DHA)	$0.05_{0.0}^{a}$	$0.08_{0.01}^{b}$	$1.0_{0.05}^{a}$	$1.4_{0.05}^{b}$	$0.1_{0.04}^{a}$	$0.17_{0.04}^{a,b}$	$0.23_{0.04}^{b}$	$1.4_{0.2}^{a}$	$2.3_{0.2}^{b}$	$2.1_{0.2}^{b}$
CLA*cis*-9,*trans*-11	$0.26_{0.01}^{a}$	$0.31_{0.01}^{b}$	$6.31_{0.65}$	$5.41_{0.68}$	$0.31_{0.02}^{a}$	$0.39_{0.02}^{b}$	$0.35_{0.02}^{a,b}$	$7.67_{1.25}$	$6.74_{1.25}$	$5.62_{1.42}$
C18:1*trans*-11 (TVA)	$0.60_{0.03}^{a}$	$0.79_{0.04}^{b}$	$13.63_{1.18}$	$13.94_{1.22}$	$1.16_{0.13}$	$1.63_{0.13}$	$1.45_{0.15}$	$28.27_{5.28}$	$28.14_{5.28}$	$22.21_{5.98}$
Σ*n*-3 FA	$1.3_{0.1}^{a}$	$3.3_{0.1}^{b}$	$27.5_{1.4}^{a}$	$56.5_{1.4}^{b}$	$1.4_{0.5}^{a}$	$3.4_{0.5}^{b}$	$4.1_{0.6}^{b}$	$29.1_{1.6}^{a}$	$46.6_{1.6}^{b}$	$41.2_{1.8}^{b}$
Σ*n*-6 FA	$7.5_{0.6}$	$7.7_{0.6}$	$157.6_{4.3}^{a}$	$131.5_{4.4}^{b}$	$6.5_{1.8}^{a}$	$10.4_{1.8}^{a,b}$	$13.4_{2.1}^{b}$	$133.2_{4.6}$	$138.4_{4.6}$	$131.3_{5.2}$
Σ*n*-3 LC PUFA	$0.6_{0.1}^{a}$	$1.3_{0.1}^{b}$	$13.2_{0.6}^{a}$	$22.2_{0.6}^{b}$	$0.7_{0.3}^{a}$	$1.8_{0.3}^{a,b}$	$2.2_{0.4}^{b}$	$14.5_{0.7}^{a}$	$23.8_{0.7}^{b}$	$20.2_{0.8}^{c}$
Σ*n*-6 LC PUFA	$1.7_{0.1}$	$1.7_{0.1}$	$34.6_{1.0}^{a}$	$29.2_{1.1}^{b}$	$1.6_{0.5}$	$2.6_{0.5}$	$3.4_{0.6}$	$31.8_{1.2}$	$33.8_{1.2}$	$32.1_{1.3}$
***n*-6/*n*-3**	$5.8_{0.1}^{a}$	$2.3_{0.1}^{b}$	$5.8_{0.1}^{a}$	$2.3_{0.1}^{b}$	$4.6_{0.1}^{a}$	$3.0_{0.1}^{b}$	$3.2_{0.1}^{b}$	$4.6_{0.1}^{a}$	$3.0_{0.1}^{b}$	$3.2_{0.1}^{b}$
Sum of FA	$2366.9_{182.2}^{a}$	$1764.5_{188.6}^{b}$			$2450.7_{311.8}$	$1660.9_{311.8}$	$1497.3_{353.6}$			

CG-Control group TG-Treatment group RG-Restricted group UG-Unrestricted group

LSM- least-square means, SEM-standard error of the mean a

a, b- significant differences between groups at P≤0.05

Σ SFA = C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C21:0+C22:0+C23:0+C24:0

Σ MUFA = C14:1+C15:1+C16:1+C17:1+C18:1trans-9+C18:1trans-10+C18:1trans-11+C18:1cis-9+C18:1cis-11+C22:1+C24:1

Σ n-3 FA= C20:3n-3+C22:6n-3+C22:5n-3+C20:5n-3+C18:4n-3+C18:3n-3

Σ n-6 FA= C22:2n-6+ C20:2n-6+ C18:3n-6+ C22:4n-6+ C20:3n-6+ C18:2n-6+ C20:4n-6

Σ n-3 LC FA= C20:3n-3+C22:6n-3+C22:5n-3+C20:5n-3

Σ n-6 LC FA= C22:2n-6+C20:2n-6+C22:4n-6+C20:3n-6+C20:4n-6

Σ trans FA= C18:1trans-6+ C18:1trans-7+ C18:1trans-8+ C18:1trans-9+ C18:1trans-10+ C18:1trans-11

Σ FA= sum of all identified fatty acids

Trans fatty acids with beneficial effects are conjugated linoleic acid isomers. They are known to have anti-inflammatory, anti-carcinogenic, anti-atherogenic, and anti-diabetogenic properties among animal models (Bhattacharya *et al.*, 2006; Kuhnt *et al.*, 2007). Two different methods were used to detect CLA isomers in tissues, the gas chromatography (GC) method and silver ion high performance liquid chromatography (Ag^+ HPLC). The analysis with GC is limited on the detection of the main CLA isomer in ruminants, CLA*cis*-9,*trans*-11, but mostly with coelution of CLA*trans*-7,*cis*-9 and CLA*trans*-8,*cis*-10. With the Ag^+ HPLC method the detection of 17 CLA isomers (*trans*,*trans*; *cis*,*trans*; *trans*,*cis*; and *cis*,*cis*) is possible. For both experiments the CLA isomers were detected and analysed with both methods. Diets enriched in PUFA have been shown to increase the level of CLA*cis*-9,*trans*-11 in tissues (Griinari *et al.*, 2000; Mosley *et al.*, 2006; Scollan *et al.*, 2006). In both experiments no differences of the absolute concentration were found between groups for the main CLA isomer (Table 9). The relative proportion of CLA*cis*-9,*trans*-11 is higher in treatment groups fed the grass silage diets. As in the case of TVA the amount of CLA*cis*-9,*trans*-11 is nearly twice as high in the muscle of German Simmental bulls compared to German Holstein bulls. This difference could be explained by the longer feeding duration of the German Simmental bulls or by the genetic difference in the SCD activity which is responsible for the formation of CLA*cis*-9,*trans*-11 from the precursor TVA in tissues. Breed differences were found for SCD activity and gene expression in Angus and Wagyu steers fed corn- or hay-based diets (Chung *et al.*, 2007). The second important CLA isomer in ruminants is CLA*trans*-10,*cis*-12. It is known to have a particular influence on fat metabolism. It prevents the development of adiposity (Park *et al.*, 1997; Park *et al.*, 1999; Ostrowska *et al.*, 1999), inhibits the differentiation of human adipocytes and reduces the TG content of mature or newly differentiated human adipocytes (Brown *et al.*, 2001, 2003, and 2004). CLA*trans*-10,*cis*-12 has been shown to block the conversion of TVA to CLA*cis*-9,*trans*-11 *via* inhibition of SCD gene expression (Smith *et al.*, 2009). While no significant differences for this CLA isomer in MLD of German Simmental bulls were found, the muscle of German Holstein bulls and the control group that were fed the maize silage contained a significantly higher amount in both absolute (0.25±0.02 *vs.* 0.17±0.02 mg/100 g), and relative (3.21±0.18 *vs.* 2.36±0.19 %) terms. Feeding maize silage to goats (Bernard *et al.,* 2009) and late lactating British Holstein Friesian (Shingfield *et al.,* 2005) provided similar results to those of the Exp. 2, in an increased amount of CLA*trans*-10,*cis*-12 in muscle and in milk compared to feeding grassland hay or grass silage.

Analysis of the triglyceride, free fatty acid (FFA) and phospholipid fraction of muscle gives a more exact answer how the dietary fatty acid composition influences the fatty acid composition in animal muscle (Aurousseau *et al.*, 2007). All animals of Exp. 1 were fasted one day before slaughter resulting in an increased lipolysis and an increase of FFA concentration in the serum to act as energy source for most tissues including skeletal muscle (Coppack *et al*., 1994). The FFA composition has not shown any differences between groups of Exp. 1. However, PUFA are predominantly located in the phospholipids where they maintain the permeability of cell membranes (Martonosi, 1975). Many studies have shown that the phospholipid fraction is affected by the diet. The PL fraction of MLD in pasture-fed bulls in the study of Lorenz *et al.* (2002) presented increased amounts of ALA. This result is confirmed by the higher concentration of ALA in the MLD PL fraction of German Simmental bulls fed with grass silage and concentrate supplemented with rapeseed. The accumulation of all single *n*-3 FA by pasture feeding was also observed in all single PL classes (Dannenberger *et al.,* 2007). Additionally, in Exp. 1 the relative concentration of *n*-3 LC PUFA was significantly increased in the restricted and unrestricted group. The concentration of LA in the PL fraction of MLD was not different between groups and is in line with results found by Dannenberger *et al.* (2004). In conclusion, despite the biohydrogenation of *n*-3 PUFA in the rumen by bacteria, the transfer of these FA contained in grass silage and concentrates supplemented with rapeseed cake and oil is sufficient to result in a greater absorption and deposition of ALA into PL. Also the *de novo* synthesis and deposition of *n*-3 LC PUFA into the PL fraction of MLD in German Simmental bulls was higher in treatment groups.

The TG fraction is also affected by the grass silage diet, because the relative proportion of ALA and the sum of *n*-3 PUFA is significantly increased, whereas LA and the sum of *n*-6 fatty acids is unchanged between groups. These results are in line with a feeding experiment made with lambs by Aurousseau *et al.* (2007).

<u>*Subcutaneous adipose tissue:*</u>

In ruminants, SAT represents the main tissue for *de novo* fatty acid and TG synthesis but the fatty acid composition is influenced by the diet. In both experiments the grass silage feeding enhanced the amount of ALA and the sum of *n*-3 PUFA resulting in a lower *n*-6/*n*-3 ratio. Considering the *n*-6 fatty acids, the sum of *n*-6 and LC *n*-6 PUFA and the LA concentration was greater in SAT of the Exp. 2 control group. However, no differences were shown for n-6 fatty acids, except the *n*-6 LC PUFA, between animal groups in Exp. 1. It

seems that a particular ratio of LA and ALA in the diet is needed to receive a result as in Exp. 2. The *n*-3 fatty acids EPA and DHA were not detectable in SAT samples from German Simmental, but in SAT samples from German Holstein bulls. In ruminants the long-chain C_{20} and C_{22} are mainly deposited in the phospholipid fraction of muscle and SAT but not in triacylglycerols and neutral lipids (Ashes *et al.*, 1992). The phospholipid fraction in SAT is very low. Therefore, a low amount of LC PUFA is difficult to detect.

Fincham *et al.* (2009) observed an increase of TVA and ALA and a decrease of LA in the ruminal fluid and SAT of pasture-finished cattle compared to feedlot-finished. Scollan *et al.* (2001) found out that a diet with whole linseeds fed to steers increased the TVA and ALA concentration in SAT. In the unrestricted and restricted group of the Exp. 1 the concentration of LA was not lower in SAT of these groups as well as in muscle. Barton *et al.* (2007) investigated the effect of a diet supplemented with extruded linseed, and an *n*-6/*n*-3 ratio in the animal diet of 1.9:1, to heifers. The feeding resulted in an increased amount of LA and CLA. Barton *et al.* (2007) did not find an explanation for this result; but in the studies of Maddock *et al.* (2006) and Kim *et al.* (2004) the tissue concentrations of LA were also higher despite the feeding of linseed. It seems, as in muscle, that the amount of ALA should be higher than the amount of LA to achieve a reduction of LA in tissue. A high intake of corn and a consequent high LA and starch concentration lowers the pH in the rumen and this leads to a lower biohydrogenation of fatty acids by microbial organisms. Subsequently, the concentration of LA and CLA increases in the duodenal flow and results in an increased incorporation into tissues (Smith, 2009). Additionally, the particle size of LA enriched concentrate diets is small compared to fibrous diets, and this leads to a shorter rumen transit time with a limiting effect on ruminal biohydrogenation (Wood *et al.*, 2008).

Short-time feeding restriction

One of the two experimental groups of Exp. 1 (grass silage/rapeseed cake and oil) had been exposed for a short time feeding restriction at the beginning of the feeding trial. To improve the meat tenderness in gilts it is useful to induce compensatory growth (Kristensen *et al.*, 2004). Compensatory growth causes high plasma levels of growth hormone (GH) which could be responsible for deposition of lean tissue in this period of growth (Hornick *et al.*, 2000). In the recent Exp. 1 the short time restriction period induced a reduced daily gain (1.26 kg/day in control; 0.97 kg/day in restricted fed animals; Mahecha *et al.,* 2009) but not a significantly higher intramuscular fat deposition as expected. It also seems that in general no

compensatory growth occurred because the total daily gain was nearly the same for restricted and unrestricted animals (1.03 kg/day *vs* 0.97 kg/d) over the whole feeding period (Mahecha *et al.*, 2009). It is expected, that the feeding restriction was, with 1 kg concentrate per day instead of 2 kg, not far reaching enough.

Comparison of the incorporation of *n*-6 and *n*-3 fatty acids in several tissues

Based on the two animal experiments one question should be answered: Is there a tissue specific effect of LA and ALA in the diet of these animals? Figure 7 shows the comparison of the proportion of *n*-6, *n*-3, and *n*-3 LC PUFA for subcutaneous adipose tissue and *longissimus* muscle of German Holstein bulls and for subcutaneous adipose tissue, liver, *longissimus* muscle, erythrocytes, and serum of German Simmental bulls.

Serum as a transport medium for lipids had the highest proportion of PUFA, and especially of *n*-6 PUFA, compared to all other tissues. The relative concentration of *n*-3 PUFA is comparable with that of the liver. Grass silage/rapeseed supplemented concentrate feeding (Experiment 1) resulted in a significant increase of LA in both tissues (serum and liver). While the subcutaneous adipose tissue represents the main source in ruminants for *de novo* fatty acid and triacylglycerol synthesis, the liver has less effect on *de novo* synthesis (Bauchart *et al.*, 1996) and disposal of lipids in ruminants (Bell, 1980). The higher intake of *n*-3 PUFA in the experimental groups was associated with a higher accumulation of these fatty acids and also led to a higher *de novo* synthesis of *n*-3 LC PUFA in the liver.

The fatty acid distribution of serum and erythrocytes of German Simmental bulls has shown differences. The proportion of *n*-6 and *n*-3 PUFA is lower in erythrocytes than in serum. Erythrocyte phospholipid composition helps maintain membrane fluidity and is a marker for the long-term fat intake because of its 120 day lifespan (Hodson *et al.*, 2008). Meanwhile, serum reflects the current status of lipids transported with the blood (Smith, 1987) and this difference explains the differences in fatty acid composition.

The fatty acid proportions in SAT and MLD of the PSB experiment are in the same range as in the same tissues in Exp. 1 but the proportions for *n*-6 in SAT and *n*-6, *n*-3 and *n*-3 LC PUFA in MLD of Exp. 2 are lower compared to the corresponding tissues from animals of the MPA experiment. This is caused by the shorter feeding duration of the German Holstein bulls. Interestingly, the relative proportion of *n*-3 and *n*-3 LC PUFA in SAT is nearly the same. This supports the finding from Wood *et al.* (2008) that ruminants, especially bulls,

preferentially incorporate essential fatty acids in muscle rather than in SAT. This is consistent with the relative proportions but not for the absolute concentration.

In summary, there is a tissue specific incorporation and deposition depending on the function of the respective tissue. Despite the low contribution of liver to fatty acid synthesis in ruminants, the relative concentration of *n*-3 LC PUFA is the highest compared to MLD, SAT, serum and erythrocytes.

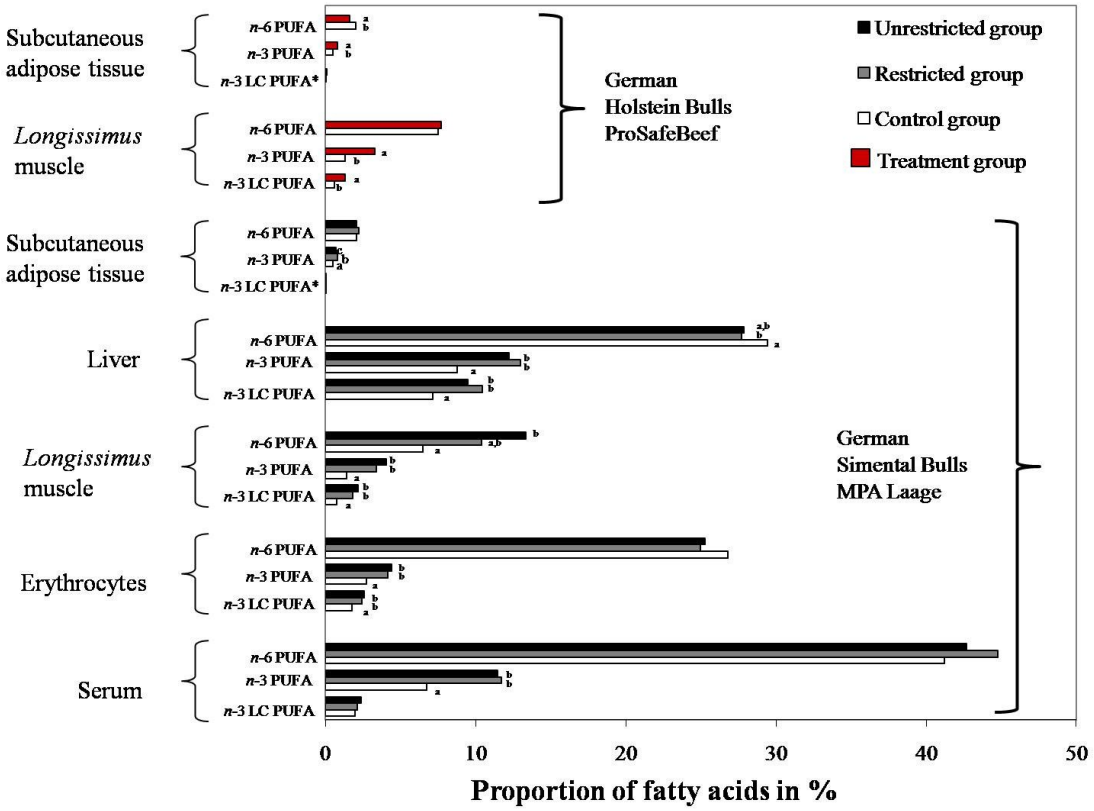

Figure 7: Proportion of *n*-6, *n*-3 and *n*-3 LC PUFA for subcutaneous adipose tissue and *longissimus* muscle of German Holstein bulls and for subcutaneous adipose tissue, liver, *longissimus* muscle, erythrocytes, and serum of German Simmental bulls

C16:1*cis*-9 as a signalling molecule in *longissimus* muscle

Figure 8 shows correlations between palmitoleate (C16:1*cis*-9) and *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) in the *longissimus* muscle and the phospholipid fraction of the *longissimus* muscle in German Simmental (black box) and German Holstein bulls (red box). Palmitoleate is postulated to be a signalling hormone, produced by adipose tissue, which acts as mediator between adipose and muscle and liver tissues in mice. Additionally, palmitoleate contributes to the regulation (suppression) of SCD-1 expression and it is a unique fatty acid as a marker for *de novo* lipogenesis with a strong correlation

between C16:1*cis*-9 in plasma, muscle and liver tissue of mice. Lipid chaperones, like FABPs, regulate the lipokine palmitoleate in a negative way. In absence of FABPs the flux of palmitoleate from adipose tissue to muscle and liver increases severely and this cause an improved metabolic response, which is of interest for further research of the metabolic syndrome (Cao *et al.*, 2008).

In the present animal experiments strong correlations were found for palmitoleate and *de novo* synthesised fatty acids in MLD of German Simmental bulls (r^2=0.93) and in MLD and in the phospholipid fraction of MLD of German Holstein bulls (r^2=0.92). Why the correlation is with r^2=0.54 low between the phospholipid fraction of MLD and palmitoleate in German Simmental bulls is unclear. Nonetheless, the within variation in German Simmental bulls is higher than that in German Holstein bulls.

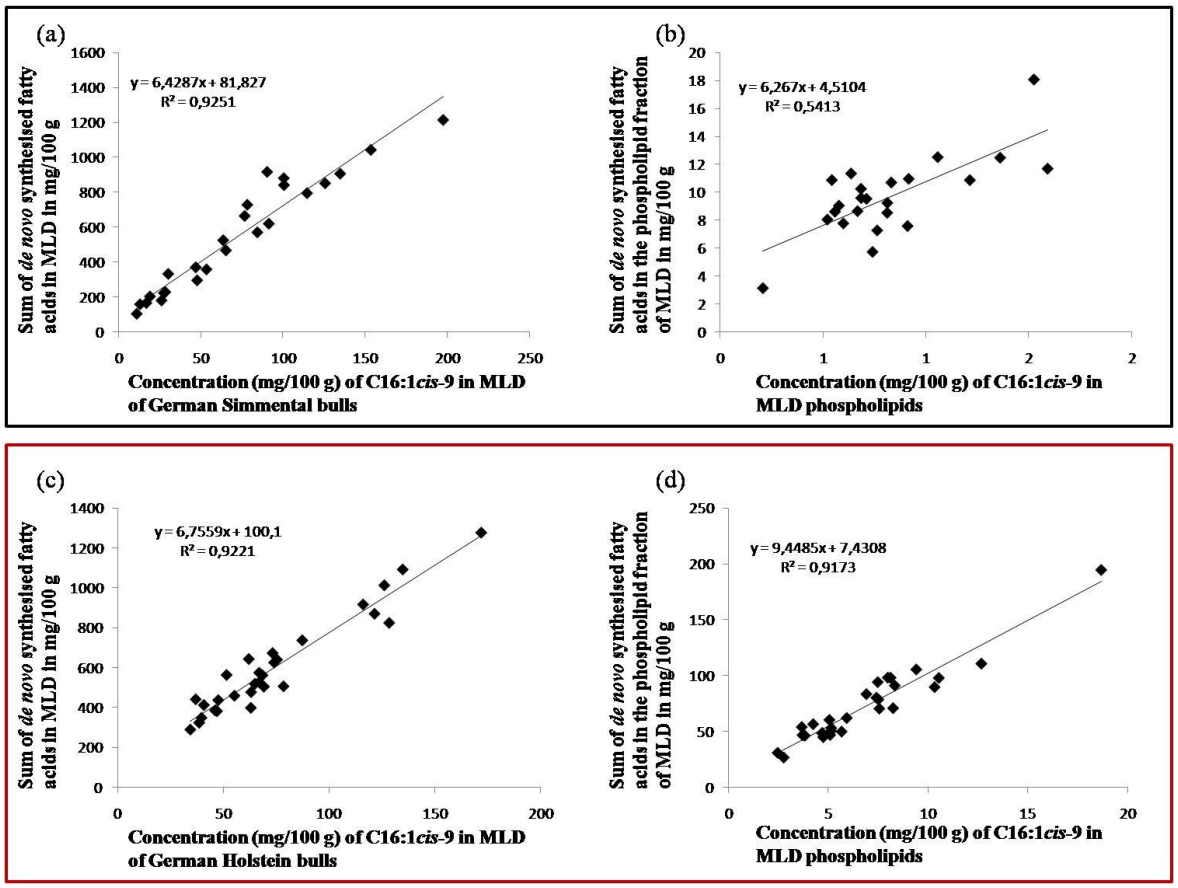

Figure 8: **(a)** Correlation between the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) and C16:1cis-9 as a signalling molecule in MLD of German Simmental bulls. **(b)** Correlation between the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) and C16:1*cis*-9 as a signalling molecule in PL fraction of MLD of German Simmental bulls. **(c)** Correlation between the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) and C16:1*cis*-9 as a signalling molecule in MLD of German Holstein bulls. **(d)** Correlation between the sum of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) and C16:1*cis*-9 as a signalling molecule in PL fraction of MLD of German Holstein bulls.

Interestingly, a strong correlation in muscle of German Holstein (r=0.94) and Simmental bulls (r=0.95) between the sum of saturated fatty acids and oleic acid was found as shown in Figure 9. It seems that oleic acid as the predominant fatty acid in cattle tissue alyo has a signalling function on *de novo* synthesis of fatty acids in muscle tissue, such as palmitoleate.

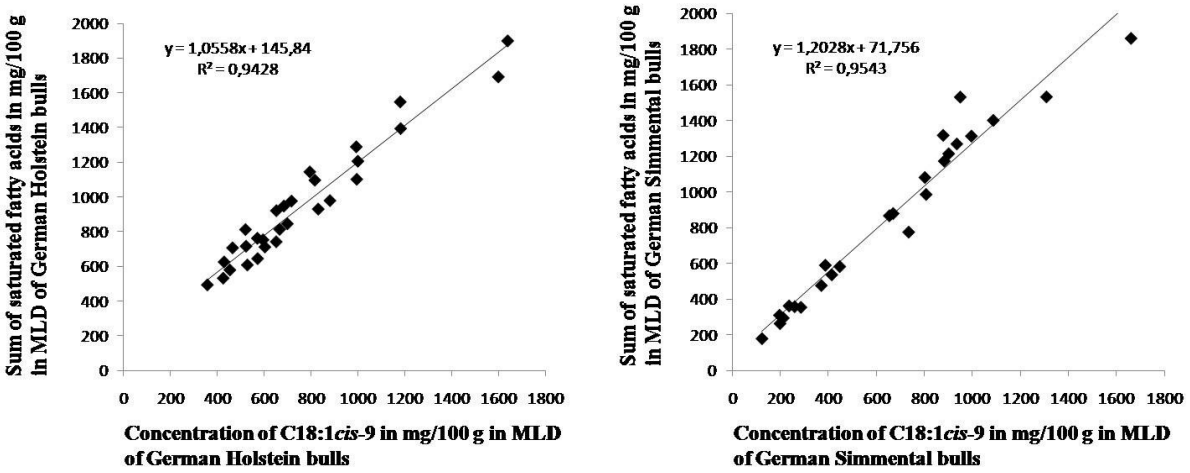

Figure 9: Correlation between the sum of saturated fatty acids and oleic acid (C18:1*cis*-9) in MLD of German Holstein and Simmental bulls

Figure 10 displays the correlations between the intramuscular fat content and the concentration of C16:1*cis*-9 on the left and C18:1*cis*-9 on the right side of the figure of the *longissimus* muscle of German Holstein bulls. The absolute concentrations of both fatty acids are highly correlated to the IMF in MLD. This result confirms that C18:1*cis*-9 is the predominant fatty acid in beef muscle. The relation between C16:1cis-9 and IMF supports the result of a signalling connection between this fatty acid and the fat content of beef muscle.

In SAT, which is the main tissue for synthesis of fatty acids, no relationship was found for C16:1*cis*-9 and *de novo* synthesised fatty acids nor between serum, plasma, *de novo* synthesised fatty acids in muscle tissue, or the muscle fatty acid fractions (PL, TG).

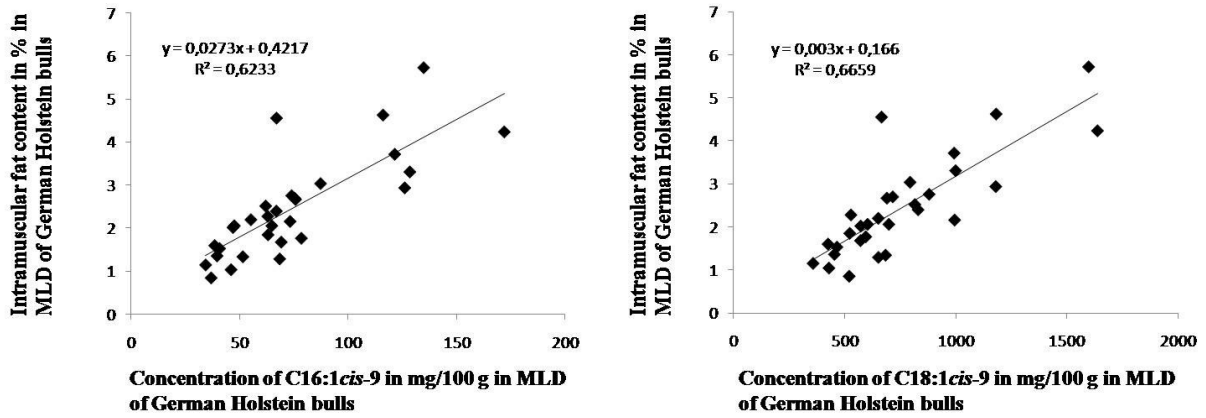

Figure 10: Correlation between the intramuscular fat content and C16:1*cis*-9 or C18:1*cis*-9 in MLD of German Holstein bulls

Influence of LA and ALA supplemented diet on lipogenic enzyme protein expression and activity

The protein expression of ACC, SCD and Δ6D was measured in a subgroup of 14 German Holstein bulls (control group: n=8; experimental group: n=6) from the PSB experiment. The results are shown in Figure 10 and 11(a).

Studies in pigs have shown that PUFA supplemented diets can trigger changes in the expression of lipogenic enzymes in muscle and SAT, and these changes are related to variations in the fatty acid profile (Doran *et al.*, 2006; Misotten *et al.*, 2009). That dietary fatty acids have regulatory effects on enzyme expression was shown in laboratory animals and human (Jump and Clarke, 1999; Ntambi, 1999), but less information is available about the distribution and regulation of lipogenic enzymes in ruminant tissues.

The protein expression of ACC as the key enzyme in the control and regulation of *de novo* synthesised SFA (Hardie, 1989) was not affected by dietary ALA despite the lower amount of saturated fatty acids in the experimental group. ACC is diversely regulated in different tissues as shown in rats (Xiao *et al.*, 2006). Therefore, despite no obvious alterations of the protein expression of ALA, it cannot be ruled out that the ACC activity may be altered by dietary PUFA.

The study of Theil and Lauridsen (2007) has shown inhibitory effects of dietary *n*-3 PUFA on the gene expression of Δ6D, involved in the biosynthesis of longer chain *n*-3 and *n*-6 PUFA (Stoffel *et al.*, 2008), in weaning pigs. Inhibitory effects of the ALA supplemented diet of the PSB experiment on the protein expression of Δ6D were shown only for MLD of German Holstein bulls. A number of lipogenic enzymes are regulated *via* SREBP and the

expression of SREBP is tissue specific (Felder *et al.*, 2005). In bulls an inhibitory effect of *n*-3 PUFA on SREBP-1c was found (Waters *et al.*, 2009); therefore, a relationship between the tissue specific effect, the tissue specific distribution and regulation of the relevant transcription factor is possible. Two closely positioned immunoreactive bands were detected for Δ6D in SAT and MLD. This gives evidence for more than one isoform in cattle tissues as shown in *Mucor rouxii* (Na-Ranong *et al.*, 2006) and rats (Skrzypski *et al.*, 2009).

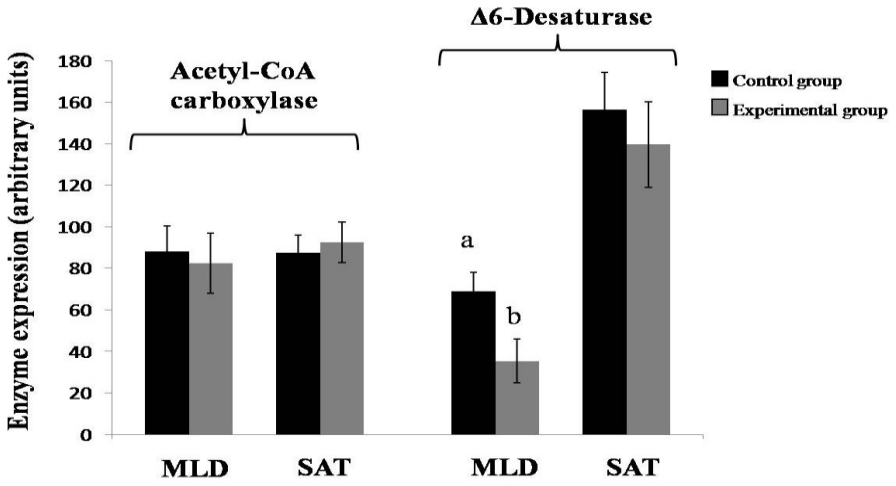

Figure 11: Lipogenic protein enzyme expression of acetyl-CoA carboxylase (ACC) and Δ-6desaturase (Δ6D) in *longissimus* muscle (MLD) and subcutaneous adipose tissue (SAT) of German Holstein bulls (control group: n=8; experimental group: n=6; a,b-significant different between groups at P≤0.05)

The SCD is responsible for the biosynthesis of MUFA with an insertion of a double bond in a SFA between carbon 9 and 10. This enzyme also catalyses the conversion of TVA to CLA*cis*-9,*trans*-11 in ruminant tissues (Enoch *et al.*, 1976). As for the Δ6D two immunoreactive bands for SCD were found in muscle and SAT which gives evidence that in ruminant tissues exists more than one isoform (Lengi and Corl, 2007). Thiede *et al.* (1986) and Miyazaki *et al.* (2003) found a tissue-specific distribution and regulation of SCD in mice and rats. Feeding grass silage with concentrate supplemented with linseed oil and rapeseed cake resulted in a significant decrease of SCD protein expression and SCD activity (Figure 11b) in MLD and SAT of German Holstein bulls. Feeding PUFA to other species resulted in an inhibition of SCD expression (Flowers and Ntambi, 2008). The balance between *n*-6 and *n*-3 fatty acids was shown to be important for the regulation of SCD *via* the transcription factor SREBP in muscle of cattle (Waters *et al.*, 2009). The regulation of Δ6D is also controlled by SREBP but the tissue-specific effect of the Δ6D protein expression was not confirmed for SCD protein expression and activity. These results imply that the mechanisms and the

expression of the transcription factor are possibly tissue-specific. The analysis of the fatty acid composition of the 14 animals (subgroup) resulted in a significantly decreased concentration of oleic acid, a product of SCD, in SAT of the grass silage fed group and in MLD a tendency for a decrease was revealed, as well as the MUFA concentration in both tissues. Reasons for no significant differences in the subgroup could be the large individual variations and the small number of animals. Using all German Holstein bulls (n=29) the absolute concentration of oleic acid and the sum of MUFA in MLD and SAT of the grass silage fed group is significantly lower compared to control animals (Mahecha *et al.,* 2010). These results are consistent with the significantly decreased SCD activity as well as the protein expression in the experimental group. Furthermore, the SCD activity is lower in muscle compared to SAT. Archibeque *et al.* (2005) found out that the catalytic activity of SCD in SAT is twice that of interfascicular adipose tissue with a higher proportion of MUFA in SAT. The activity in MLD tissue is in the same range as in pigs, demonstrated by Doran *et al.* (2006). The CLA*cis*-9,*trans*-11 concentration is similar between both feeding groups, despite the higher relative proportion of TVA in the experimental group. This is caused by the inhibition of the SCD protein expression and activity in tissues of this group.

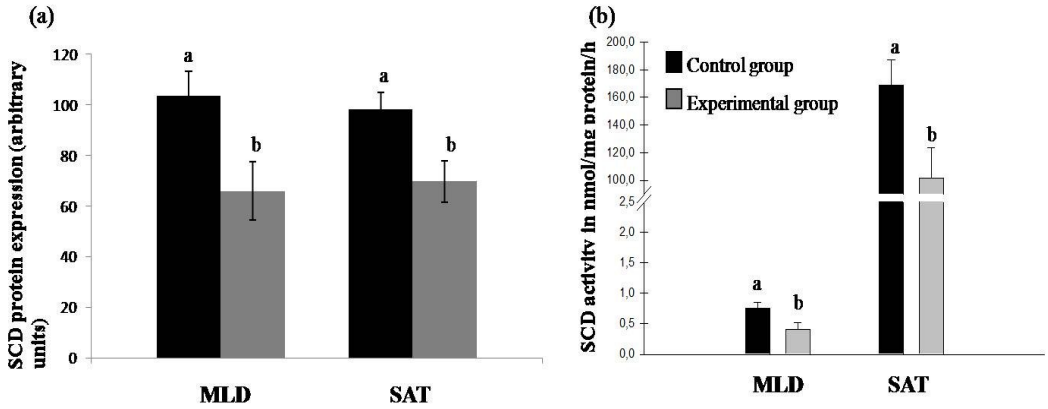

Figure 12: **(a)** SCD protein expression in *longissimus* muscle (MLD) and subcutaneous adipose tissue (SAT) of German Holstein bulls (control group: n=8; experimental group: n=6). **(b)** SCD activity (nmol/mg Protein/h) in *longissimus* muscle (MLD) and subcutaneous adipose tissue (SAT) of German Holstein bulls (control group: n=15; experimental group: n=14; a,b-significant different between groups with $P \leq 0.05$).

Enrichment of processed meat with good fatty acids and the consumers benefit

The awareness of consumers for more nutritious and value-added meat products has increased, and the interest in pasture-based beef production systems is growing (Verbeke *et*

al., 2010). The consumption of processed meat is about 61 g/day for men and 30 g/day for women (Max Rubner-Institut, Bundesforschungsinstitut für Ernährung und Lebensmittel, 2008). Therefore it is of interest to consider the fatty acid composition of sausages made from the carcasses of the animal experiment. There are few investigations showing the fate of single fatty acids starting in meat, progressing through processing, and ending up in products. From each carcass of the PSB experiment sausages were produced as the following: German corned beef sausages (GCB), Tea sausage spread (TSS), and Feuerli (a kind of wiener). For the present work GCB and TSS are of interest. Both sausages have different recipes and were produced with different methods (Table 10).

Table 10: Recipes and production methods of German corned beef sausages (GCB) and Tea sausage spreads (TSS)

	German corned beef sausages	**Tea sausage spreads**
Recipe	- 58% lean beef - 5% beef rind - Drinking water - Gelatin - Pickling salt - Spices - Yeast extracts - Celeriac - Corn, soy, and plant proteins	- 94% beef and pork meat - Pickling salt - Spices - Sugar, Lactose, Dextrose - Antioxidant (E301, E300) - Flavour enhancer (E621) - Rum - Beech wood smoke
Preparation	1) Lean meat injected with spice brine 2) Scalded until an internal temperature of 68°C 3) Cool down 4) Minced 5) Mixed with aspic 6) Filling the mass in cleaned guts 7) Scalded until an internal temperature of 78°C 8) Cool down	1) Mincing the lean beef and pork meat 2) Adding salt and spices while mincing 3) Filling the mass in artificial sausage skin 4) Maturation 5) Smoking with 25°C 6) Postmaturation until a pH<5.6 is reached

While the GCB was scalded by 68°C and 78°C, the TSS was treated with only 25°C. This difference in the temperature treatment could influence the fatty acid composition because of an intense lipolysis (Gandemer, 2002). *N*-3 PUFA added to raw meat before heat

processing resulted in a loss of these fatty acids due to the cooking process to produce ham (Lee *et al.*, 2006). Moreover, it was found that heat catalyses the initiation of lipid peroxidation and the formation of oxidation products (Bilek and Turhan, 2009).

Table 11 represents the relative concentration of fatty acids in GCB and TSS. The relative concentration of the *n*-3 LC PUFA (EPA, DHA, and DPA) is approximately the same in MLD and GCB. For these fatty acids a short-time heating did not cause losses. Only the relative concentration of ALA in GCB is compared to the fresh muscle reduced. In fact, a study with pork showed an increase of PUFA after grilling due to water loss (Nuernberg *et al.*, 2006). Frozen meatballs made from beef heated for 10 minutes at 230°C and then at 203°C for the last 10 minutes showed no loss of LA and ALA compared to the fresh meat (Baggio and Bragagnolo, 2006). The conclusion for the production of GCB is as follows: No appreciable losses of *n*-3 fatty acids in GCB compared to the fresh meat were detected. The proportion of SFA is lower than that of MUFA. The ratio of *n*-6/*n*-3 fatty acids corresponds to the recommendations of the DGE with ≤5:1 (DGE, 2008). Considering the predominance of CLA*cis*-9,*trans*-11 in ruminant tissues (65-66%), no decline was observed. Therefore, a transfer of beneficial fatty acids from fresh muscle into the product GCB is possible without appreciable losses of these fatty acids.

To compare the TSS fatty acid composition with that of the fresh beef is not very useful because a part of the mixture contains pork meat with an unknown fatty acid composition. Nonetheless, the relative concentration of ALA in TSS from the experimental group is significantly higher compared to that of the control group with similar *n*-3 LC PUFA, but less when compared to GCB. The lower proportion of SFA compared to MUFA in TSS of both groups is particularly advantageous. Due to the significantly greater proportion of *n*-3 fatty acids in the experimental TSS, the ratio of *n*-6/*n*-3 is lower in this group but is higher than the recommendation of the DGE by 6.4±0.3. Moreover, the fat concentration in TSS is ten times higher compared to GCB associated with a clearly higher absolute amount of ALA, DPA, DHA, and AA. However, the absolute amount of EPA is only 1 mg/100 g more in TSS of the control and 1 mg/100 g less of TSS in the experimental group, compared to the corresponding GCB. In summary, despite the high amount of fat in TSS and the additional supplies of ALA and *n*-3 LC PUFA, the inclusion of TSS made from the meat of the experimental group into the human diet is supported.

Table 11: Relative fatty acid composition (%) of German Corned Beef and Tea Sausage Spread made from German Holstein bulls

	Corned beef					**Tea sausage spread**				
	CG		TG			CG		TG		
	LSM	SEM	LSM	SEM	P	LSM	SEM	LSM	SEM	P
C16:0	22.6	0.3	22.1	0.3	0.17	24.7	0.2	24.2	0.2	0.06
C16:1*cis*-9	3.8	0.1	3.4	0.1	0.01	3.6	0.1	3.3	0.1	**0.01**
C18:0	13.8	0.3	14.8	0.3	**0.04**	15.4	0.4	16.1	0.4	0.25
C18:1*cis*-9	38.7	0.5	37.3	0.5	0.07	38.1	0.3	38.2	0.3	0.83
C18:1*trans*-1	0.7	0.1	0.8	0.1	0.50	0.5	0.0	0.5	0.0	0.34
C18:2*n*-6	7.7	0.5	7.8	0.5	0.94	7.5	0.3	7.6	0.3	0.83
C18:3*n*-3	1.0	0.1	1.5	0.1	**0.01**	0.8	0.1	1.0	0.1	**0.00**
C20:4*n*-6	1.3	0.1	1.5	0.1	0.29	0.2	0.0	0.2	0.0	0.99
C20:5*n*-3	0.2	0.0	0.4	0.0	**0.00**	0.02	0.0	0.03	0.0	0.13
C22:4*n*-3	0.2	0.0	0.2	0.0	0.09	0.1	0.0	0.1	0.0	0.33
C22:5*n*-3	0.4	0.0	0.6	0.1	**0.00**	0.1	0.0	0.1	0.0	0.11
C22:6*n*-3	0.05	0.0	0.08	0.0	**0.00**	0.0	0.0	0.0	0.0	0.92
Σ SFA	40.1	0.4	40.7	0.4	0.32	43.8	0.5	43.9	0.5	0.86
Σ MUFA	47.2	0.6	45.4	0.6	**0.04**	46.7	0.3	46.2	0.3	0.27
Σ *n*-3 fatty acids	1.8	0.2	2.7	0.2	**0.00**	1.1	0.1	1.3	0.1	**0.00**
Σ *n*-6 fatty acids	9.9	0.5	10.1	0.5	0.79	9.2	0.4	8.3	0.4	0.83
Ratio *n*-6/*n*-3 FA	5.9	0.4	4.0	0.4	**0.00**	8.0	0.3	6.4	0.3	**0.00**
Σ *n*-3 LC PUFA	0.7	0.1	1.1	0.1	**0.00**	0.1	0.0	0.2	0.0	0.19
Σ *n*-6 LC PUFA	1.6	0.1	1.7	0.1	0.44	0.3	0.0	0.3	0.0	0.88
Σ *trans* FA	1.3	0.1	1.5	0.1	0.29	1.2	0.1	1.1	0.1	0.46
Σ FA	2165.7	118.0	1966.3	122.1	0.25	21776.9	599.4	21836.4	620.4	0.95

For explanation see Table 9

Conclusion

Two feeding experiments were conducted to investigate the effects of *n*-3 and *n*-6 fatty acids on the regulation of metabolism in ruminants and the fatty acid composition of cattle tissues. The current results validate, that the animal feeding experiments were successful and suited for the research.

A diet with ingredients high in *n*-3 fatty acids, like linseed oil, rapeseed and grass silage, results in an increase of *n*-3 fatty acids in cattle tissues despite the biohydrogenation in the rumen. The concentration of EPA and DHA (for human health important n-3 long-chain products) in cattle tissues depends upon the ALA concentration in the animal diet. Considerably more ALA than LA in the animal diet results in a clearly greater concentration of EPA, DHA and ALA, and a reduced LA concentration in cattle tissues. With two servings of meat (200g per serving) per week, a person could ingest up to 40 mg EPA+DHA and 130 mg ALA. The intake of GCB (2 servings per week with 50 g per serving) could provide up to 20 mg EPA+DHA and 65 mg ALA to the nutrition. Unfortunately, it is just a small contribution to the human nutrition but it should be kept in mind especially for persons who eat no fish. Feeding linseed oil to cattle is not exactly cheap. Therefore, it could be difficult to realize in the conventional agriculture but should however be an option for organic farming. Another option for the conventional farming is the application of linseed pellets as supplementation to the concentrate in combination with grass silage.

In the future, research should be geared to find alternatives instead of fish, meat or algae for the sustenance of humans with *n*-3 fatty acids and the so important long chain products like EPA and DHA. It should be of interest to find sustainable sources, like plants, with a sufficient concentration of *n*-3 fatty acids to provide humans according to the current recommendations.

Chapter 8

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Chapter 9

Summary

9. Summary

Background:

Animal-derived foods contribute a substantial part of dietary fat. In recent years the consumer interests have focused not only on the amount of fat but also on the fatty acid composition. An adequate intake of *n*-3 fatty acids is recommended because of several health benefits in human, for example the treatment of rheumatoid arthritis, coronary heart disease, in general inflammatory diseases and various cancers (especially in the case of gastrointestinal disorders). In this context, options to replace saturated and enhance the beneficial *n*-3 fatty acids in milk, meat and products by different feeding systems are important. Furthermore, the clarification of the exogenous *n*-6 and *n*-3 PUFA effect on lipid metabolism, especially the lipogenic enzymes, and in consequence on the fatty acid composition of tissues in cattle is required.

Objectives:

The decrease of SFA and the increase of essential fatty acids in meat are desirable from nutritional point of view. The aim of the two experiments with bulls was to clarify the effect of different exogenous PUFA (*n*-6 *vs.* *n*-3 FA) on the regulation of lipid metabolism and the final fatty acid synthesis and deposition in different tissues. There are only very few studies investigating the effect of LA and ALA enriched diets on the protein expression of lipogenic enzymes in cattle. Furthermore, there is a need for research of tissue specific differences and the relationship between protein expression of lipogenic enzymes, enzyme activities and the products of these enzymes as well as the absorption and deposition of *n*-3 and *n*-6 fatty acids in ruminants.

Consumers are becoming more aware of the relationships between diet and health and this has increased consumer interest in the nutritional value of foods. In Germany the consumption of processed meat is higher than fresh meat. There is little information about the fate of PUFA in fresh beef during processing to products. Therefore, the influence of the processing method on beneficial *n*-3 fatty acid content during production of different beef sausages by using the fresh beef was investigated.

Design:

Two animal experiments were conducted to investigate the effect of diets enriched in

ALA or LA in different cattle breeds.

The first experiment was realised with German Simmental bulls. Nine animals were fed a control diet with maize silage and a concentrate supplemented with soybean meal (20%) and soybean oil (2%) and was rich in LA. Seven animals received the experimental diet with a 70/30 grass and maize silage mixture and the concentrate was supplemented with rapeseed cake (32%) and rapeseed oil (2%). This diet was rich in ALA. Additionally, a third group of nine animals was fed the experimental diet but at the beginning of the feeding experiment with a short-time feeding restriction of 112 days.

The second animal experiment was realised with German Holstein bulls. The control group with 15 animals received a diet with maize silage and a concentrate supplemented with soybean meal (41%) resulting in a diet high in LA. The experimental group with 14 animals received grass silage and a concentrate supplemented with rapeseed cake (12%) and linseed oil (3%) resulting in a diet high in ALA.

<u>*Results:*</u>

Fatty acid composition in longissimus muscle and subcutaneous adipose tissue of German Holstein bulls

The sum of fatty acids was about > 25% significantly decreased in MLD of the experimental group. A significant increase was observed in the experimental compared to the control group for the relative concentration of ALA (> 3-times), EPA, *n*-3 DPA, and DHA. The sum of *n*-3 LC PUFA was 2-times higher in experimental compared to control group. The absolute concentration of CLA*cis*-9,*trans*-11 was not affected. The ratio of *n*-6/*n*-3 was with 2.3±0.1 significantly lower than in the control group with 5.8±0.1.

As in muscle the sum of fatty acids was significantly decreased in experimental SAT. While LA, the sum of *n*-6 fatty acids, and the ratio of *n*-6/*n*-3 was significantly increased in the control group, C18:1*trans*-11, ALA (1.75-times), and EPA were significantly increased in SAT of the experimental group. CLA*cis*-9,*trans*-11 was not affected and the concentration was 6-7% higher compared to MLD.

Fatty acid composition of German Corned beef and Tea sausage spread made from meat of German Holstein bulls

The LA concentration in German Corned beef was similar between groups. Significantly higher concentrations were revealed for ALA (1.5-times) and the sum of *n*-3 LC

PUFA (1.6-times) in experimental group. The ratio of *n*-6/*n*-3 was with 4.0±0.4 significantly lower in GCB of experimental group than in control group (5.9±0.4). No differences were revealed for the CLA concentration.

The relative concentration of ALA in TSS from the experimental group is significantly higher compared to that of the control group with similar *n*-3 LC PUFA concentrations. The ratio *n*-6/*n*-3 is with 6.4±0.3 lower in the experimental compared to the control group with 8.0±0.3.

Fatty acid composition in tissues of German Simmental bulls

The absolute concentration of ALA, the sum of *n*-3 fatty acids and the sum of *n*-3 LC PUFA were significantly increased in MLD of experimental groups, whereas *n*-3 LC PUFA is highest in the unrestricted group. The CLA and LA concentration was similar between groups and the ratio *n*-6/*n*-3 was significantly lower in experimental groups (3.0±0.1 and 3.2±0.1). C18:1*trans*-11, ALA, CLA*cis*-9,*trans*-11 and the sum of PUFA was significantly increased in the triglyceride fraction of MLD of the experimental groups and in the phospholipid fraction a significant increase in experimental groups was observed for ALA, EPA, and the *n*-3 DPA. DHA was only in the restricted group significantly increased.

The sum of fatty acids of SAT was about 7000 mg/100 g and the sum of *n*-6 LC PUFA (significantly) lower in the experimental groups. The experimental diet resulted in an increased absolute concentration of ALA, the sum of *n*-3 fatty acids and the C18:1*trans*-11.
The relative concentration of *n*-3 PUFA and *n*-3 LC PUFA was significantly increased in the liver of experimental groups and the control diet resulted in an increased *n*-6 fatty acid concentration in the liver of the control compared to the restricted group.

Considering the relative fatty acid concentration of erythrocytes and serum no differences were revealed for the sum of *n*-6 fatty acids between feeding groups. In both tissues were the sum of *n*-3 fatty acids significantly increased in the experimental groups but only in erythrocytes the *n*-3 LC PUFA concentration.

Short-time feeding restriction and compensatory growth

A short-time feeding restriction of 112 days did not result in an increase of IMF in muscle of German Simmental bulls. The deposition of beneficial *n*-3 fatty acids is not negatively influenced.

C16:1cis-9 as a signalling molecule

The correlations in MLD of German Holstein and German Simmental bulls between the concentration of *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) in mg/100 g and the concentration of C16:1*cis*-9 in mg/100 g are very strong with r^2=0.92 for German Holstein and 0.93 for German Simmental bulls. Additionally, a strong correlation in MLD was found between *de novo* synthesised fatty acids (C10:0, C12:0, C14:0, C16:0) and C18:1*cis*-9 with r^2=0.94 for German Holstein bulls and r^2=0.95 for German Simmental bulls.

Lipogenic enzyme protein expression and activity

The protein expression of ACC is not affected by diet. However, the Δ6D protein expression is significantly 33% lower in muscle of experimental group compared to the control German Holstein bulls. Additionally, there might be a tissue specific difference because there have been no effects of the diet on Δ6D protein expression in SAT. The SCD protein expression was significantly lower in muscle (37%) and SAT (29%) of experimental group as well as the enzyme activity in muscle (50%) and SAT (39%). These results are in line with the reduced MUFA concentration in tissues. It is the first functional proteomics approach showing a causal relationship between SCD protein expression, enzyme activity and the final product of this SCD (MUFA) in different tissues of bulls.

Conclusion:

Feeding a diet enriched in LA or ALA results in an increased deposition of these fatty acids, induced chain elongation and desaturation and deposition of the long-chain products in tissues of ruminants despite the high bacterial biohydrogenation in the rumen. The supplementation of the concentrate with rapeseed cake and linseed oil in combination with feeding grass silage is efficient, at least for German Holstein bulls. The diet fed to German Simmental bulls resulted in approximately 3-times higher ALA content and 0.5% more *n*-3 LC PUFA in MLD. Despite the higher concentration of LA in the diet the relative LA concentration in MLD of German Holstein bulls were similar between groups. This gives evidence that the metabolic important *n*-3 fatty acids are preferred deposited in MLD and it seems that LA is more hydrogenated in the rumen and it can be speculated that LA is intensively used for oxidation. Essential fatty acids are preferentially deposited in muscle compared to subcutaneous adipose tissue and also preferred in erythrocytes; conclusively they

are good long-time marker for the fatty acid intake. Despite the low proportion of the *de novo* fatty acid synthesis in the liver of ruminants the concentration of *n*-3 fatty acids and *de novo* synthesised *n*-3 LC PUFA is much higher compared to MLD. Summarizing, there is a tissue specific incorporation and deposition of *n*-6 and *n*-3 fatty acids depending on the function of the respective tissue.

The production conditions of German Corned beef and tea sausage spread made from the meat of the German Holstein bulls did not lead to a loss of beneficial *n*-3 fatty acids. Consequently, the ratio *n*-6/*n*-3 for German Corned beef is quite low and is in the range recommended from the German Society for Nutrition.

Further research is necessary considering the signalling function of C16:1*cis*-9 and C18:1*cis*-9 in ruminant tissues. The questions should be answered whether there is a relationship between the concentration of *de novo* synthesised fatty acids in tissues, as muscle, liver and subcutaneous adipose tissue, and C16:1*cis*-9 and C18:1*cis*-9 in plasma and how this mechanism works and how these fatty acids interfere.

Additionally to the ACC protein expression the ACC activity should be considered under LA and ALA feeding to explain the lower sum of fatty acids under ALA feeding. It is of interest to figure out the mechanisms how the tissue-specific effect of Δ6D works on protein expression level and on the level of activity. The SCD protein expression, the enzyme activity and the MUFA concentration are more inhibited by *n*-3 fatty acids than by *n*-6 fatty acids in different tissues of bulls.

Chapter 10

Zusammenfassung

10. Zusammenfassung

Hintergrund:

Lebensmittel tierischer Herkunft liefern einen bedeutenden Teil des Nahrungsfettes in der menschlichen Ernährung. In den letzten Jahren hat sich das Interesse der Verbraucher nicht nur auf den Fettanteil sondern auch auf die Fettsäurezusammensetzung der Lebensmittel fokussiert. Die Empfehlung verschiedener Gremien sieht vor, täglich eine ausreichende Menge an *n*-3 Fettsäuren aufzunehmen, da diese positiven Einfluss auf verschiedene Erkrankungen haben. *N*-3 Fettsäuren werden in der Behandlung von rheumatischer Arthritis, koronarer Herzkrankheit, generell bei entzündlichen Erkrankungen und bei verschiedenen Krebsarten (vor allem im gastrointestinalen Bereich) eingesetzt. In diesem Zusammenhang ist es wichtig, Möglichkeiten zu haben, die gesättigten Fettsäuren im Fleisch zu reduzieren und die gewünschten *n*-3 Fettsäuren in Milch, Fleisch und Fleischprodukten anzureichern.

In diesem Zusammenhang ist die Aufklärung der Wirkung von differenten exogenen (Futter) *n*-6 und *n*-3 Fettsäuren auf den Lipidstoffwechsel, und hier besonders der lipogenen Enzyme, und in der Konsequenz auf die Fettsäurezusammensetzung in den Geweben von Nutztieren relevant.

Ziele:

Aus ernährungsbedingter Sicht ist es wünschenswert, den Gehalt an gesättigten Fettsäuren im Fleisch zu reduzieren und den der essentiellen Fettsäuren zu erhöhen. Das Ziel der beiden Fütterungsversuche mit Bullen lag darin, den Einfluss exogen zugeführter mehrfach ungesättigter Fettsäuren (*n*-6 *vs.* *n*-3 Fettsäuren) auf die Regulation des Lipidmetabolismus und die Fettsäuresynthese sowie die Einlagerung der Fettsäuren in die Gewebe zu untersuchen. Bisher gibt es nur wenige wissenschaftliche Untersuchungen, die den Einfluss von Linol- und Linolensäure angereicherten Diäten auf die Proteinexpression lipogener Enzyme beim Rind. Außerdem ist es notwendig, gewebespezifische Differenzen der Proteinexpression lipogener Enzyme beim Wiederkäuer und vor allem den Zusammenhang zwischen der Proteinexpression, der spezifischen Aktivität und der synthetisierten Fettsäureprodukte lipogener Enzyme sowie die Aufnahme und Einlagerung von *n*-3 und *n*-6 Fettsäuren aufzuklären.

Die Nachfrage nach qualitativ hochwertigen Lebensmitteln durch den Verbraucher ist stetig gestiegen, da der Zusammenhang von Ernährung und Gesundheit immer bewusster

wahrgenommen wird. Der Verzehr von verarbeitetem Fleisch ist in Deutschland höher als der Verzehr von reinem Fleisch. Bis heute gibt es allerdings nur wenige Informationen über das Verhalten von mehrfach ungesättigten Fettsäuren während des Verarbeitungsprozesses zu Fleischprodukten. Deshalb wurde im Rahmen dieser Arbeit auch der Einfluss der Verarbeitungsmethoden auf den Gehalt der *n*-3 Fettsäuren in verschiedenen Wurstprodukten, die unter Verwendung des frischen Fleisches der Bullen aus einem Fütterungsversuch, geprüft.

Design:

Es wurden zwei Fütterungsversuche durchgeführt, um den Einfluss einer Linolsäure- bzw. Linolensäure angereicherten Diät mit zwei verschiedenen Rinderrassen zu erforschen.

Der erste Versuch wurde mit Deutschen Fleckvieh Bullen durchgeführt. Die Kontrolldiät, reich an Linolsäure, war aus Maissilage und einem Konzentrat, angereichert mit Sojaextraktionsschrot (20 %) und Sojabohnenöl (2 %), zusammengesetzt, und wurde an neun Bullen verfüttert. Das Versuchsfutter, reich an Linolensäure, bestand aus einer Mischung aus Gras- und Maissilage im Verhältnis 70/30 und einem Konzentrat, angereichert mit Rapskuchen (32 %) und Rapsöl (2 %). Diese Futtermischung wurde an sieben Tiere verfüttert. Zusätzlich gab es eine zweite Versuchsgruppe mit neun Bullen. Diese erhielt ebenso das Versuchsfutter, aber zu Beginn der Fütterung wurde eine Futterrestriktion von 112 Tagen durchgeführt.

Das zweite Fütterungsexperiment wurde mit Deutschen Holstein Bullen durchgeführt. Die Kontrolldiät, ebenfalls reich an Linolsäure, wurde an 15 Bullen verfüttert und bestand aus Maissilage und einem Konzentrat, angereichert mit Sojaextraktionsschrot (41 %). Die Versuchsdiät, reich an Linolensäure, wurde an 14 Bullen verfüttert und bestand aus Grassilage und einem Konzentrat, angereichert mit Rapskuchen (12 %) und Leinöl (3 %).

Ergebnisse:

Fettsäurezusammensetzung in Muskel und subkutanem Fettgewebe von Deutschen Holstein Bullen

Die Summe der gesamten Fettsäuren war im MLD der Versuchsgruppe um mehr als 25 % signifikant verringert. Die relative Konzentration von ALA (> 3-fach), EPA, *n*-3 DPA, und DHA im Muskel der Versuchsgruppe ist im Vergleich zur Kontrollgruppe signifikant erhöht. Die Summe der *n*-3 LC PUFA war zweifach höher im MLD der Versuchsgruppe verglichen

mit der Kontrollgruppe. Die Konzentration der CLA*cis*-9,*trans*-11 war nicht beeinflusst. Das Verhältnis *n*-6/*n*-3 war mit 2,3±0,1 signifikant niedriger in der Versuchsgruppe verglichen zur Kontrollgruppe mit 5,8±0,1.

Wie im Muskel ist auch im Fettgewebe (SAT) der Versuchsgruppe die Summe der Fettsäuren signifikant niedriger. Während die Konzentration der Linolsäure, die Summe der *n*-6 Fettsäuren und das Verhältnis von *n*-6/*n*-3 signifikant höher waren in der Kontrollgruppe, sind C18:1*trans*-11, ALA (1,75-fach), und EPA signifikant in der Versuchsgruppe erhöht. Die Konzentration der CLA*cis*-9,*trans*-11 war 6-7 % höher im Fettgewebe verglichen mit dem Muskel und war gleich zwischen den Gruppen.

Fettsäurezusammensetzung von Deutschem Corned Beef und der Teewurst, hergestellt aus dem Fleisch der Deutschen Holstein Bullen

Die Konzentration der Linolsäure für das Deutsche Cornedbeef war gleich zwischen den Gruppen, die Konzentration von Linolensäure (1,5-fach) und die Summe der langkettigen *n*-3 Fettsäuren (1,6-fach) war signifikant höher im Corned Beef der Versuchsgruppe. Das Verhältnis von *n*-6/*n*-3 war mit 4,0±0,4 signifikant niedriger im Corned Beef, hergestellt aus dem Fleisch der Versuchstiere, verglichen mit dem Cornedbeef der Kontrollgruppe (5,9±0,4). Die CLA-Konzentration in den Corned Beefs war gleich zwischen den Gruppen.

Die Teewurst, hergestellt aus dem Fleisch der Versuchstiere, enthielt eine signifikant höhere Konzentration an Linolensäure verglichen zur Teewurst aus dem Fleisch der Kontrolltiere. Das Verhältnis *n*-6/*n*-3 ist mit 6,4±0,3 niedriger in der Teewurst mit dem Versuchstierfleisch verglichen zur Teewurst der Kontrolltiere mit 8,0±0,3.

Fettsäurezusammensetzung der Gewebe der Deutschen Fleckvieh Bullen

Die absolute Konzentration der Linolensäure, die Summe der *n*-3 Fettsäuren und die Summe der langkettigen *n*-3 Fettsäuren waren signifikant erhöht im MLD der Tiere aus den Versuchsgruppen, wobei die Konzentration der langkettigen *n*-3 Fettsäuren in der Versuchsgruppe ohne Futterrestriktion am höchsten war. Die Konzentration der CLA und der Linolsäure war gleich zwischen den Gruppen. Das Verhältnis *n*-6/*n*-3 war signifikant niedriger in den Versuchsgruppen (3,0±0,1 und 3,2±0,1) verglichen zur Kontrolle. Die Konzentration von C18:1*trans*-11, der Linolensäure, der CLA*cis*-9,*trans*-11 und der Summe der mehrfach ungesättigten Fettsäuren war signifikant erhöht in der Triglyzeridfraktion des MLD der Versuchsgruppen. In der Phospholipidfraktion des MLD der Versuchsgruppen

wurde eine signifikant erhöhte Konzentration von Linolensäure, EPA und der *n*-3 DPA detektiert. Die Konzentration der DHA war lediglich in der futterrestriktierten Gruppe signifikant erhöht.

Die Summe der Fettsäuren im subkutanen Fettgewebe war rund 7000 mg/100 g niedriger in den Versuchsgruppen. Die Summe der langkettigen *n*-6 Fettsäuren war ebenfalls niedriger im subkutanen Fettgewebe der Versuchsgruppen. Die absolute Konzentration der Linolensäure, die Summe der *n*-3 Fettsäuren und der C18:1*trans*-11 war im Gewebe der Versuchstiere erhöht.

Die relative Konzentration der Summe der *n*-3 Fettsäuren und der langkettigen *n*-3 Fettsäuren war signifikant erhöht in der Leber der Versuchstiere verglichen mit dem Lebergewebe der Kontrolltiere. Die Kontrolldiät resultierte in einer höheren *n*-6 Fettsäurekonzentration in der Leber der Bullen verglichen mit den Versuchstieren.

Die Summe der *n*-6 Fettsäuren ergab bei der Analyse der Erythrozyten und des Serums keine Unterschiede bezüglich der relativen Konzentration zwischen den Gruppen. Die Summe der *n*-3 Fettsäuren hingegen war in Erythrozyten und Serum der Versuchsgruppen signifikant erhöht, die relative Konzentration der langkettigen *n*-3 Fettsäuren allerdings nur in den Erythrozyten.

Kurzzeit-Futterrestriktion und kompensatorisches Wachstum

Die Futterrestriktion von 112 Tagen hatte keine Erhöhung des intramuskulären Fettgehaltes im Muskel der Deutschen Fleckvieh Bullen zur Folge gehabt. Die Einlagerung von *n*-3 Fett-säuren im Muskel ist nicht negativ beeinflusst.

C16:1cis-9 als Signalmolekül

Die Korrelationen im Muskel von Deutschen Holstein und Fleckvieh Bullen zwischen der Konzentration der *de novo* synthetisierten Fettsäuren (C10:0, C12:0, C14:0, C16:0) in mg/100 g und der Konzentration der C16:1*cis*-9 in mg/100 g sind sehr eng mit r^2=0,92 im Muskel der Deutschen Holstein Bullen und r^2=0,93 für Deutsche Fleckvieh Bullen. Außerdem wurde eine enge Korrelation zwischen den *de novo* synthetisierten Fettsäuren (C10:0, C12:0, C14:0, C16:0) und C18:1*cis*-9 mit einem Korrelationsfaktor von r^2=0,94 für Deutsche Holstein und r^2=0,95 für Deutsche Fleckvieh Bullen gefunden.

Proteinexpression lipogener Enzyme und Enzymaktivität

Die Proteinexpression der ACC ist nicht beeinflusst durch die Futterzusammensetzung. Die Δ6D-Proteinexpression hingegen ist im Muskel der Versuchsgruppe um 33 % reduziert im Vergleich zur Kontrollgruppe. Zusätzlich scheint es einen gewebespezifischen Unterschied zu geben, da im subkutanen Fettgewebe kein Effekt auf die Proteinexpression der Δ6D beobachtet wurde. Die SCD-Proteinexpression war im Muskel und im subkutanen Fettgewebe der Versuchstiere um 37 % bzw. 29 % reduziert. Ebenfalls war die Aktivität im Muskel und Fettgewebe der Versuchstiere reduziert, um 50 % und 39 %. Diese Ergebnisse werden durch die reduzierte Konzentration der einfach ungesättigten Fettsäuren in den Geweben der Versuchstiere bestätigt. Dies ist der erste funktionelle Proteomics-Ansatz der zeigt, dass ein kausaler Zusammenhang zwischen der SCD-Proteinexpression, Aktivität und dem Endprodukt, den einfach ungesättigten Fettsäuren, in verschiedenen Geweben von Bullen besteht.

Schlussfolgerung:

Das Füttern einer Diät, die reich an Linol- und Linolensäure ist, führt zu einer Einlagerung dieser Fettsäuren, zur Kettenverlängerung und Desaturierung sowie zur Einlagerung der langkettigen Fettsäureprodukte im Wiederkäuergewebe trotz der Biohydrogenierung durch Mikroorganismen im Pansen. Die Supplementierung des Konzentrates mit Rapskuchen und Leinöl in Kombination mit dem Füttern von Grassilage ist erfolgreich in Deutschen Holstein Bullen einzusetzen, wenn die Konzentration der *n*-3 Fettsäuren im Gewebe erhöht werden soll. Die Diät, welche den Deutschen Fleckvieh Bullen gefüttert wurde, führte zu einem ca. 3-fach höheren Gehalt an Linolensäure und einer 0,5%igen höheren Einlagerung von langkettigen *n*-3 Fettsäuren im MLD. Trotz der höheren Konzentration der Linolsäure im Futter der Kontrollgruppe war die relative Konzentration der Linolsäure im Muskel gleich zwischen den Gruppen der Deutschen Holstein Bullen. Dies ist ein Hinweis darauf, dass die metabolisch wichtigen *n*-3 Fettsäuren bevorzugt im Muskel eingelagert werden. Außerdem scheint es, dass die Linolsäure stärker im Pansen hydrogeniert wird und zudem vorwiegend zur Oxidation genutzt wird. Essentielle Fettsäuren werden bevorzugt im Muskel eingelagert im Vergleich zum subkutanen Fettgewebe und ebenso bevorzugt in Erythrozyten. Diese sind also gute Langzeitmarker für die Fettsäureaufnahme. Trotz des geringen Anteils der Leber an der *de novo* Fettsäuresynthese in Wiederkäuern ist die relative Konzentration der *n*-3 Fettsäuren und der langkettigen *de novo* synthetisierten *n*-3

Fettsäuren in der Leber höher als im Muskel. Zusammenfassend ist zu sagen, dass eine gewebespezifische Einlagerung und Deponierung der *n*-6 und *n*-3 Fettsäuren festgestellt wurde, die abhängig von der Funktion der jeweiligen Gewebe zu sein scheint.

Die Herstellung von Deutschem Corned Beef und Teewurst aus dem Fleisch der Deutschen Holstein Bullen aus dem Fütterungsversuch hat durch den Herstellungsprozess nicht zum Verlust der wertvollen *n*-3 Fettsäuren geführt. Dies hat zur Konsequenz, dass das Verhältnis *n*-6/*n*-3 Fettsäuren im Fett von Deutschem Corned Beef im empfohlenen Bereich durch die DGE liegt.

Weiterführende Untersuchungen sind notwendig, um Informationen zur Signalfunktion der C16:1*cis*-9 und C18:1*cis*-9 im Wiederkäuergewebe zu erhalten. Es sollten folgende Fragen beantwortet werden: Gibt es einen Zusammenhang zwischen der Konzentration der *de novo* synthetisierten Fettsäuren im Muskel und subkutanem Fettgewebe und der Konzentration der C16:1*cis*-9 beziehungsweise C18:1*cis*-9 im Serum und wenn ja, wie funktionieren die Mechanismen und wo greifen diese beiden Fettsäuren ein?

Zusätzlich zur ACC-Proteinexpression sollte die Aktivität der ACC im Wiederkäuergewebe unter exogener Linol- und Linolensäuresupplementierung untersucht werden, um eine Erklärung für die niedrigere Summe der Fettsäuren in Muskel und subkutanem Fettgewebe der Deutschen Holstein Bullen zu finden. Außerdem ist es von Interesse, die Mechanismen der gewebespezifischen Wirkung der Δ6D auf dem Level der Proteinexpression und der Aktivität herauszufinden. Aus den Ergebnissen kann geschlussfolgert werden, dass die SCD-Proteinexpression, die SCD-Aktivität und die Konzentration der einfach ungesättigten Fettsäuren im Gewebe von Bullen stärker durch exogene *n*-3 Fettsäuren inhibiert werden als durch *n*-6 Fettsäuren.

Appendix

Selbständigkeitserklärung

Hiermit erkläre ich, die vorliegende Arbeit selbstständig verfasst zu haben. Alle dafür genutzten Quellen und Hilfsmittel sind angegeben.

Diese Dissertation, sowie Ausschnitte daraus, wurden keiner anderen Prüfungsbehörde vorgelegt.

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Andrea Herdmann Langen, den 12.12.2010

Curriculum Vitae

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Andrea Herdmann

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