Analysis and seasonal variation of conjugated linoleic acid and further *cis-/trans*-isomers of C18:1 and C18:2 in bovine milk fat

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1. Introduction

Conjugated linoleic acids (CLA) are a complex mixture of different positional isomers of linoleic acid (*cis* Δ9,*cis* Δ12-C18:2) such as *cis* Δ8,*trans* Δ10, *cis* Δ9,*trans* Δ11, *cis* Δ10,*trans* Δ12 or *cis* Δ11,*trans* Δ13 and their corresponding geometrical isomers as e.g. *trans* Δ9,*cis* Δ11. Because of their possibly antioxidative, antiatherogenic and in particular anticarcinogenic properties CLA are of great interest (1-6). According to previous investigations considerable amounts of CLA (> 0.1 %) only occur in ruminant milk fats. In bovine milk fat the predominant CLA isomer with a proportion of at least 90 % (7) is *cis* Δ9,*trans* Δ11-C18:2 (c9t11) that at the same time is regarded most effective in cancer prevention (a.o. 2,8). Numerous review articles on CLA have been published particularly in recent years by e.g. Parodi (9,10), Belury (11), Fritsche and Steinhart (12), Jahreis (13) or Martin and Banni (14).

Knowledge of the formation of c9t11 in the rumen of cows as well as of the variation associated with feeding possibly would enable to influence the CLA content in milk fat. On the other hand, it is important to investigate changes associated with an increase of CLA in milk fat concerning the contents of other fatty acids with special physiological properties, such as *trans*-C18:1 fatty acids or non-conjugated linoleic acid isomers as e.g. *trans* Δ9,*trans* Δ12, *cis* Δ9,*trans* Δ13, *cis* Δ9,*trans* Δ12 or *trans* Δ11,*cis* Δ15 (t9t12, c9t13, c9t12, t11c15). These isomers are reported to have atherogenic properties (*trans*-C18:1) or to impair biosynthesis of long-chain polyenoic fatty acids and prostaglandins by inhibition of the Δ6-desaturase (in particular t9t12-C18:2 (15,16)). Thus, advantages of CLA-enriched fats with regard to nutritional physiology should not be compensated by disadvantageous properties of other fatty acids. Moreover, the present study will compare the great changes in the whole fatty acid spectrum asssociated with the relatively small absolute increase of the CLA content being achievable by special feeding. These relations hinder or even prevent the registration of changes in the technological properties of CLA-enriched milk fat or milk products being attributable directly to an increase of the CLA content. In addition to a compilation of CLA contents resulting from special feeding conditions as well as from typical winter and summer feeding in Germany, a particular focus will be put on optimized conditions for the gas chromatographic analysis of *cis-/trans*-C18 isomers.

2. Materials and Methods

2.1 Fat samples

Ca. 1800 bovine milk fats from most different regions of Germany were analysed by gas chromatography to investigate the influence of typical feeding conditions in winter (barn), summer (pasture) as well as in the transition period (barn to pasture in spring or pasture to barn in late autumn) on the content of c9t11.

Moreover, further analyses were performed on milk fats from the following feeding trials that have already been described earlier (17,18):

1) 5 cows (state of lactation ca. in 5th month) at first were kept in the barn for one week. Afterwards, they were driven out to a pasture with young grass and finally kept on a pasture with older grass for 3 weeks, respectively. Barn feeding: 20 kg maize silage, 11 kg grass silage, 7 kg concentrate corresponding to 14.8 % raw protein and 20.6 % raw fibre in the dry matter. Young pasture: particularly young grass; 23.9 % raw protein, 4.8 % raw fat, 19.5 % raw fibre, 41.2 % N-free extract, 11.2 % ash. At night grass silage was fed additionally in barn. Older pasture: After the second movement through all pastures the cows were kept on the last pasture with older grass. 17.4 % raw protein, 4.1 % raw fat, 25.2 % raw fibre, 44.2 % N-free extract, 9.2 % ash. No additional feeding in barn.

2) 5 cows (state of lactation ca. in 4th month) at first were submitted to typical barn feeding. Then they were fed with grass from pasture and finally they received a ration that only covered half their maintenance and led to an energy deficit.

   Daily barn feeding: 7 kg concentrate, 4.1 kg pasture grass silage, 3 kg green maize and 1.7 kg hay (95 MJ net energy/lactation); daily pasture feeding: 100 kg grass (corresponding to 13.8 kg dry matter) and 1.75 kg concentrate (95 MJ net energy/lactation); daily feeding at energy deficit: 4 kg straw and 2 kg concentrate (20 MJ net energy/lactation). To exclude any influence caused by the weather, all feedings were done in the barn.

3) In a further trial with a two periods cross-over design the influence of quantity and technical treatment of the fed rape-seed (00-sort) on the contents of C18:2 isomers was studied. Numerous other parameters from this study concerning fat composition or milk fat properties have been published earlier by Frede et al. (18). Concentrate variants: a) base fat without rape fat; b) and c) 275 g and 550 g rape oil, respectively; d) and e) 650 g and 1300 g wholemeal from rape-seed, respectively, corresponding to 275 g and 550 g rape oil; f) 1450 g whole rape-seed pellets, corresponding to 550 g rape oil. As basic diet all animals obtained maize silage (4.5 kg per day) and whole plant silage from winter wheat ad libitum. Each of these 6 feeding variants was performed with 6 cows and repeated later with another herd of 6 cows. Thus, the analysed milk fats were based on 12 single cows, respectively.

2.2 Gas chromatography

From 1756 milk fats the contents of the CLA c9t11, of *trans*-C18:1 isomers (sum of *trans* Δ4 to *trans* Δ16 (t4 to t16)) and of *trans*-C18:2 isomers (without CLA; sum of t9t12, c9t13, t8c12, t8c13, c9t12, t9c12, t11c15) were analysed with high precision using triglyceride formulae as described earlier (17,19). Numerous milk fats resulting from several feeding trials as well as from human milk were analysed for total fatty acids and for all *cis*-/*trans*-isomers of the different C18 fatty acids.

Gas chromatographic analyses of the total fatty acid composition were performed using a 25 m - column (CP-Wax 58 CB, corresponding to FFAP, i.d. 0.25, df = 0.20 µm) comprising ca. 70 fatty acids (recorded as methyl esters, FAMEs) in the range of C4 to C24 (19). However, the *trans*-C18:1 and C18:2 isomers (without CLA) after trans-esterification of the total fat into FAMEs and pre-separation by argentation thin-layer chromatography (Ag-TLC) were analysed gas chromatographically using a highly polar 100 m - column (CP-Sil 88, i.d. 0.25 mm, df = 0.20 µm) at 175°C (*trans*-C18:1) or 150°C (C18:2). Further details on Ag-TLC and GC calibration have already been described (19, 20). The analyses of CLA isomers were performed on CP-Sil 88 columns of 100 and 50 m length as well as on a CP-Wax 58 column of 25 m. With each column several oven programs – described in the discussion part – were used to optimize analytical conditions.

Identification of *cis-/trans*-C18:1 and C18:2 isomers was achieved by the FAME standards c6, c7, c9, c11, c12, c13, c15, t6, t7, t9, t11, t12, t13, t15, t9t12, c9t12, t9c12, c9c12 as well as a mixture of CLA isomers and by C20:1 isomers c5, c8 and c11 all obtained from Sigma (St. Louis, Missouri, USA). Single CLA isomers c9t11, c9c11, t9t11 and t10c12 were obtained from Matreya, Inc. (Pleasant Gap, PA, USA). All results are given in g/100 g free fatty acids, as during calibration of FAME the methyl ester contents were converted into free fatty acid contents.

3. Results and discussion

3.1 Analysis of conjugated linoleic and *trans* fatty acids

In contrast to non-conjugated *trans*-C18:2 isomers, that elute before the peak of c9c12-linoleic acid, the group of CLA appears clearly right of linoleic acid (C18:2) and α-linolenic acid (C18:3) in the chromatogram (Fig.1). Among the conjugated linoleic acids particularly the bioactive properties of the c9t11 isomer are emphasized (2,8). Thus, it is important to separate this isomer from further CLAs to enable its quantitation in fats.

Analysing fatty acid isopropyl ester (FAIPE) and fatty acid methyl ester (FAME) on a 50 m-BPX 70 column Lavillonnière et al. (21) besides c9t11 identified further 7 CLA isomers as e.g. t9c11, c10t12 or t10c12 as well as the isomers Δ8Δ10 and Δ11Δ13 with all possible *cis-/trans*-configurations in the fat from French cheese. However, several isomers could not be separated on this column. In human adipose Fritsche et al. (22) characterized the CLA isomers c9t11, t9t11, c9c11 and t9c11 by their 4,4-dimethyloxazoline derivatives (DMOX) using a 50 m - CP-Sil 88 column. Moreover, S. Fritsche and J. Fritsche (23) identified the same isomers in bull and steer fats with c9t11 being the main component (50 m - CP-Sil 88, FAME). Adlof and Lamm (24) described the resolution of c9c11, t9c11, c9c11 and c10c12 in synthetic CLA mixtures applying FAME and a 30 m - SP2380 column. In a recent publcation Ramamurthi et al. (25) established that using a 50 m - CP-Sil 88 column does not enable to separate the isomers c9t11 and t9c11 as well as t9t11 and t10t12 in a mixture of CLA standards, whereas the isomer c10t12 lying on the flank of t10c12 could partly be resolved. In further recent publications Lavillonnière et al. (21) confirmed the identity of the five minor CLA isomers c8t10, c8c10, t8t10, t11t13 and ?11?13 in cheese and Sehat et al. (26) described 19 CLA isomers in commercial cheese products.

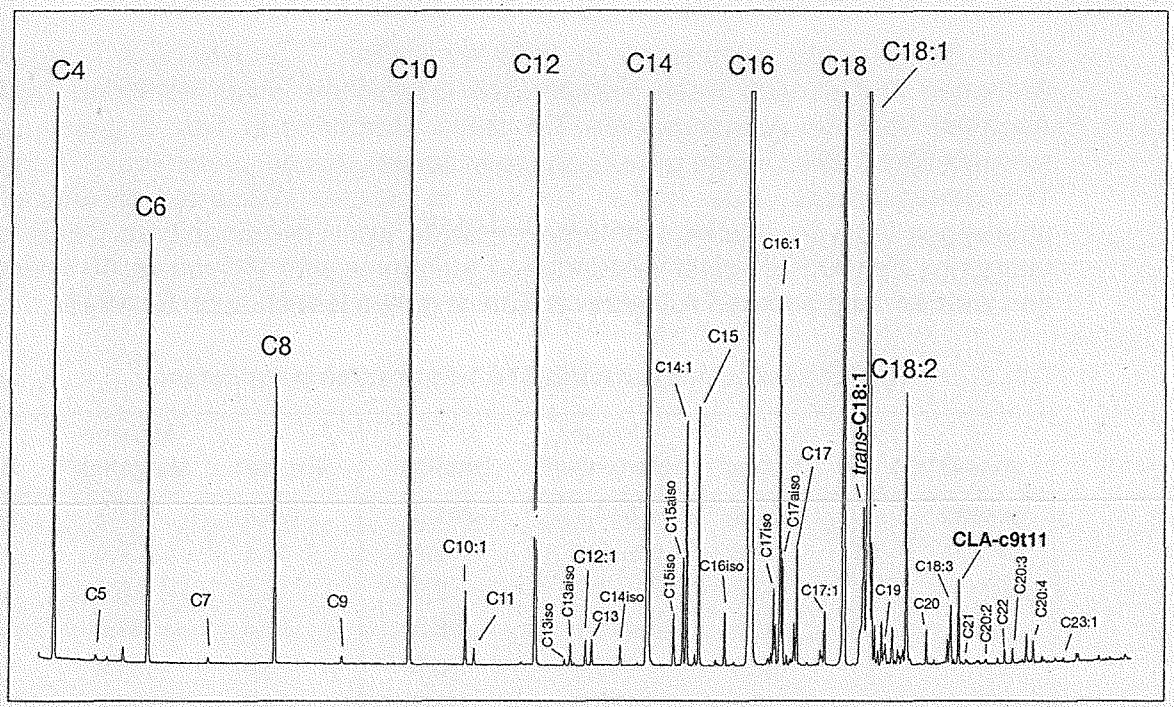

Fig. 1: Gas chromatogramm of FAME from milk fat; analysis by a CP-Sil 88 capillary column of 50 m; oven: 50°C (1 min isothermal) – 5°C/min – 240°C

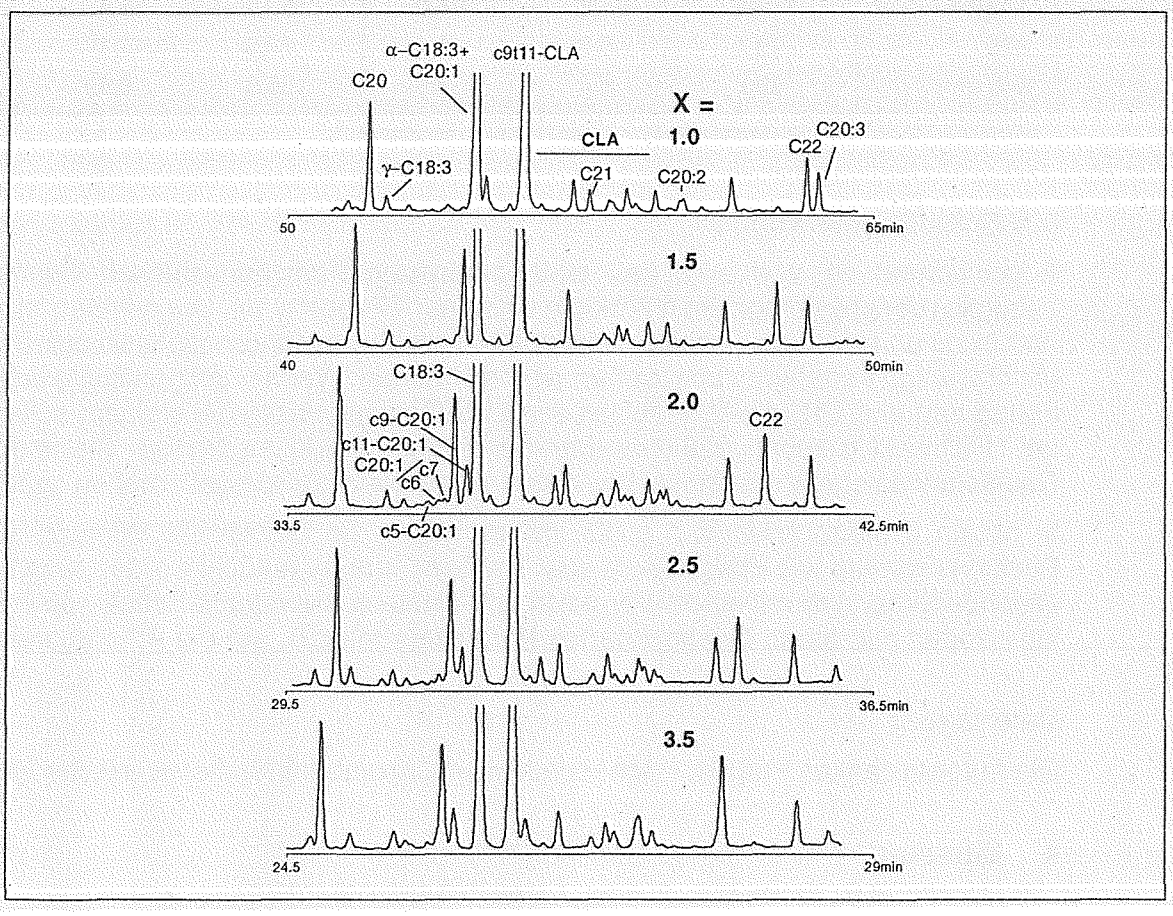

Fig. 2: Partial gas chromatograms of FAME in the range of C20 to C20:3 obtained on a 100 m - CP-Sil 88 column; X indicates the respective oven heating rates between 1.0 and 3.5°C/min starting from 125°C and going up to 240°C

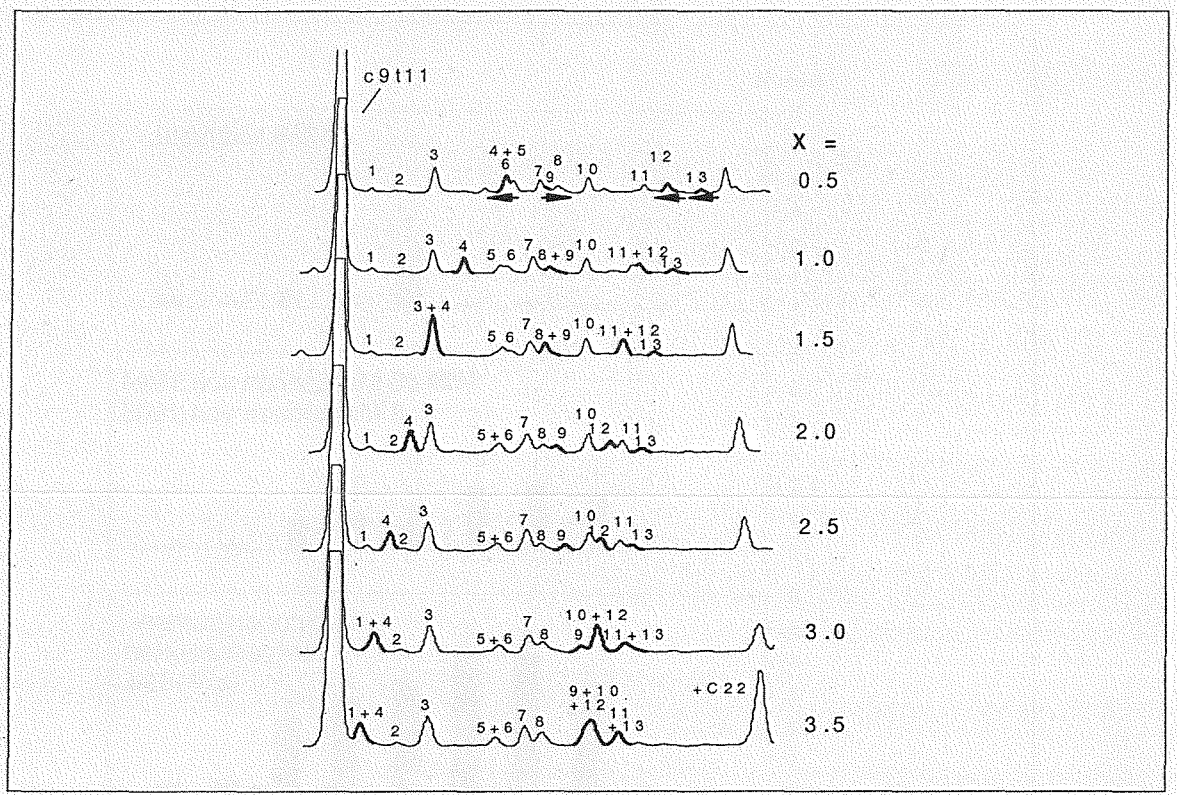

Fig. 3: Partial gas chromatograms of FAME in the CLA region obtained on a 100 m - CP-Sil 88 column; X indicates the respective oven heating rates between 0.5 and 3.5°C/min starting from 125°C and going up to 240°C; the numbers are assigned to individual CLA isomers and some further unknown fatty acids; identification: 1. t9c11, 2. t10c12, 3. c9c11, 4. C21 and 8. t9t11

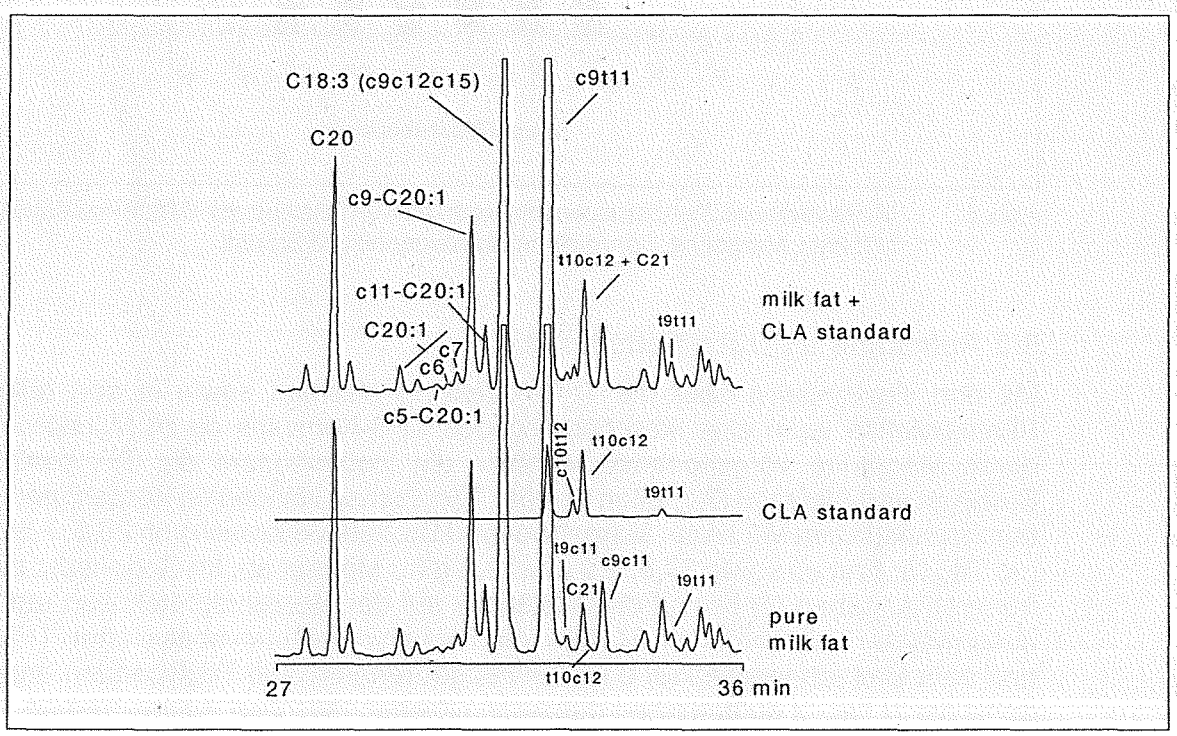

Fig. 4: Partial chromatograms (FAME) of CLA isomers in a CLA standard, a pure milk fat and a mixture of the standard with the milk fat obtained using a CP-Sil 88 column of 100 m; oven: 125°C – 2.5°C/min – 240°C

Applying a highly polar 100 m - column (CP-Sil 88, 100 % cyanopropyl polysiloxane) we tried several different oven programs to separate the CLA isomers in bovine milk fat. As can be seen from Fig. 2 depending on the oven program the peak of C20:1 may elute either before C18:3 and CLA, together with C18:3 or after this peak (not shown). This may lead to overlaps with the CLA isomers.

Fig. 3 shows partial chromatograms of the CLA region obtained at 7 different oven heating rates. First of all, it is striking that the peaks assigned with 4, 8, 12 and 13 (marked with arrows in the first chromatogram) with increasing heating rate considerably move through parts of the chromatogram, whereas other fatty acids, in particular the 4 CLA isomers identified by standards, do not change their relative position. Fig. 3 demonstrates that depending on the analytical conditions chosen the overlap of several peaks may cause wrong results. Particularly striking are the overlaps of the peaks 1+4 in the last chromatogram or of peak 3+4 in the third chromatogram.

The oven program used for the analyses shown in Fig. 4 (125°C – 2.5°C/min – 240°C) causes the C20:1 peak to elute before C18:3 and thus already allows a good separation of CLA isomers all eluting right of the main isomer c9t11. This way C20:1 elutes directly in a region exhibiting the isomers of α-linolenic acid occurring e.g. in human milk lipids (27). However, according to own analyses these C18:3 isomers do not occur in bovine milk fat (27). A comparable dependence of C20:1 elution from the operating temperature of a CP-Sil 88 column (50 m) has already in 1994 been described by Wolff (28) in connection with studies on rape-seed and soy bean oils. Under the analytical conditions (50 m - Sil 88, FAME) chosen by Fritsche and Steinhart (12) C20:1 together with its isomers elutes between the close peaks of C18:3 and CLA. Thus, as has been published for steer fat (23) a baseline resolution between C20:1 and c9t11 is not always achieved and isomers of C20:1 overlap with c9t11. The identification of CLA isomers given in Figs. 3 and 4 should be regarded as definite though some small amounts of other CLA isomers may overlap with the identified peaks shown here. The identification of c10t12 was adopted from Ramamurthi et al. (25) who identified this peak from their standard mixture by GC-MS. We could not confirm the high concentration of the CLA isomer t11c13 found by Sehat et al. (26) though we investigated various bovine milk fats with a concentration range of c9t11 between 0.29 to 1.93 %. This isomer may be formed during processing or ripening of cheese.

Fig. 5 shows chromatograms of a milk fat blended with a CLA standard as well as the pure milk fat and CLA standard obtained with a heating rate of 1°C/min. Although in this case C20:1 and C18:3 coelute, the individual CLA isomers are quite well resolved and separated to a greater extent.

Fig. 6 exhibits partial chromatograms of bovine and human milk fat obtained with a medium polar 25 m - column (CP-Wax 58; ester of nitroterephthalic acid and polyethylen glycol) in the range of C18:3 and C20:1. Also with this column a separation of c9t11 and t9c11 could be achieved, provided a new column and suitable conditons including a precise evaluation are used. On this column C20 and C20:1 are found right of the CLA group. Many measurements of c9t11 contents in the present study are based upon this relatively cheap and durable column.

Whereas Lavillonnière et al. (21), due to their analytical conditions, identified the two peaks after the main component c9t11 in milk fat from cheese as combinations of CLAs t9c11+c10t12+t10c12 and c8c10+c9c11+c10c12+c11c13, our chromatograms exhibit a partial further resolution of these isomers confirmed by the applied standards. In accordance to Adlof and Lamm (24) we as well assigned peak 1 of Fig. 3 as t9c11 (cp.

Fig. 5). Fritsche et al. (22) also identified the corresponding peak in fat from human tissue as t9c11 and our examination of human milk lipids led to the same result. Apparently the gas chromatographic conditions used by us at least allow to resolve t9c11 from c9t11.

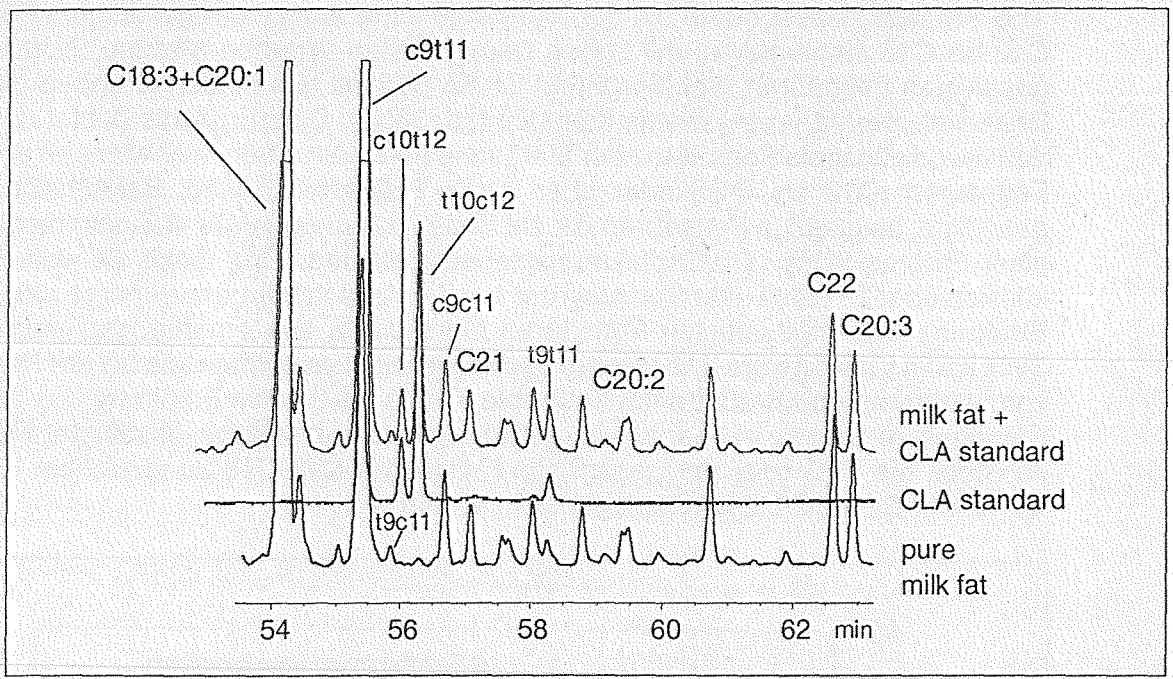

Fig. 5: Gas chromatographic separation of CLA isomers in milk fat (100 m - CP-Sil 88) with indication of CLA standards; oven: 125°C – 1°C/min – 240°C

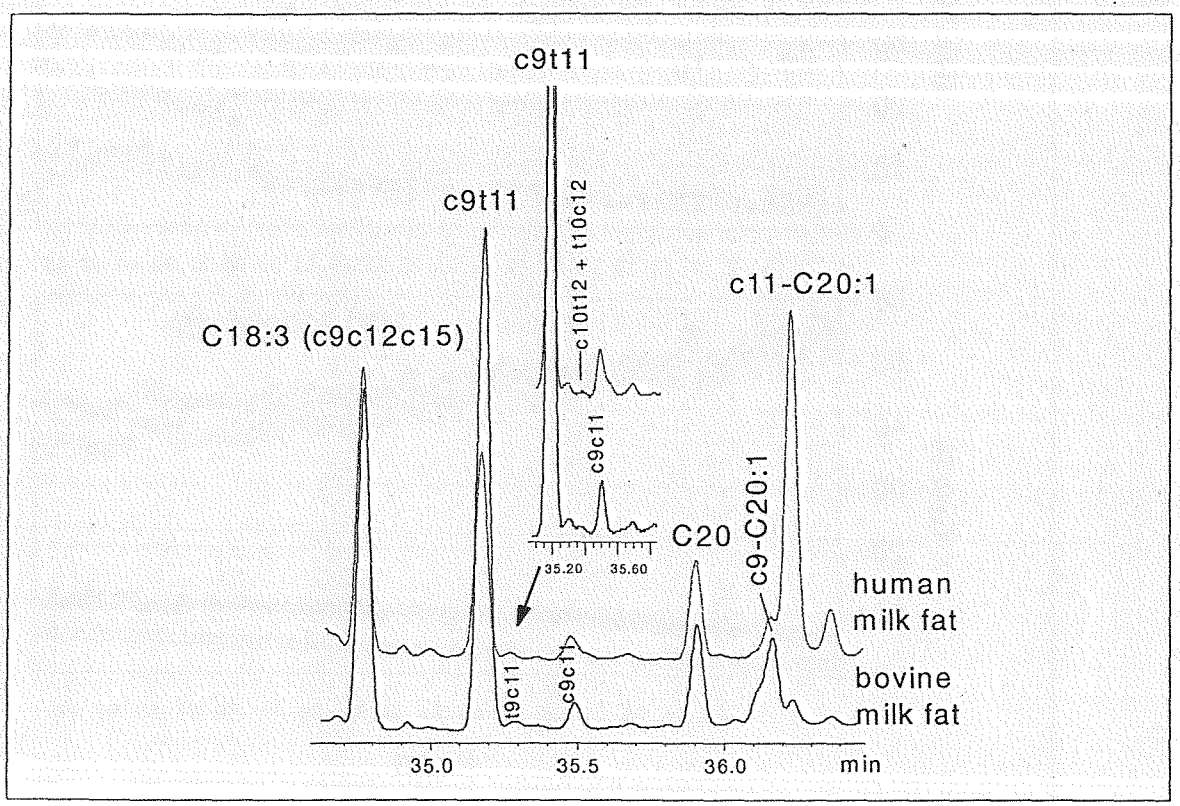

Fig. 6: Partial chromatograms in the range of C18:3 and C20:1 with indication of some CLA isomers obtained on a 25 m - CP-Wax 58 column; oven: 45°C (1 min isothermal) – 5°C/min – 225°C (15 min isothermal) – 5°C/min – 260°C

However, an overlap of c9t11 with a negligible proportion of c8t10 or t8c10, that possibly occurs only in cheese as a product of microbial isomerization during ripening (21, 26), can not be excluded.

As mentioned above, analyses of the possibly antiatherogenic CLA should always be regarded in connection with the probably atherogenic *trans*-C18:1 fatty acids and with *trans*-C18:2 fatty acids. Details on the gas chromatographic conditions, Ag-TLC separations and identifications of *trans*-C18:1 and *trans*-C18:2 positional isomers in bovine milk fat have already been published by us earlier (19, 29-31) and will not be repeated here. Particular emphasis was laid on the fact that literature data on *trans*-C18:1 contents frequently are too low, mainly due to a partial overlap of *trans* isomers with *cis* isomers (30, 32).

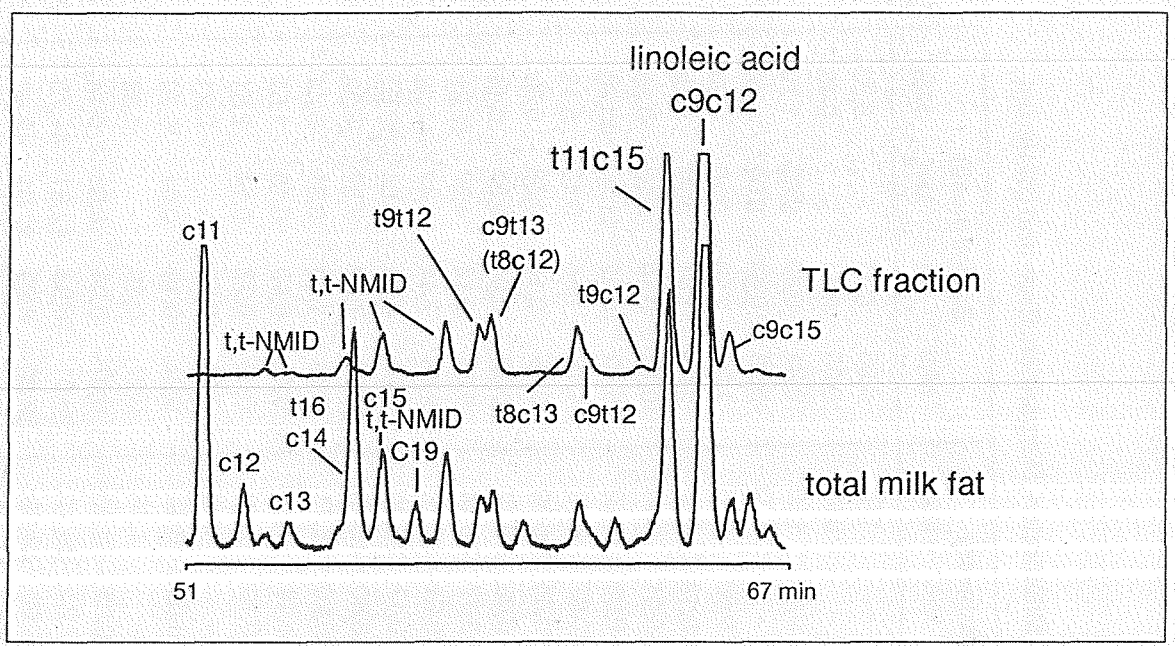

Fig. 7: Isomers of linoleic acid (FAME) of bovine milk fat in a TLC-fraction (dienoic fatty acids) and in total fat obtained on a CP-Sil 88 column (100 m x 0.25 mm); t8c13: tentative identification; t,t-NMID: unknown non methylene interrupted diene; c11 to c15 : *cis*C18:1 isomers

Fig. 7 shows the dienoic acid Ag-TLC fraction as well as the total fatty acids from a bovine milk fat. These partial chromatograms comprise the isomers of linoleic acid (without CLAs) and in case of the total fat some *cis*-/*trans*-C18:1 isomers (c11, c12, c13, c14, c15, t16) and C19 in addition. Identification of the dienoic peaks was achieved by several standards as well as on the basis of identifications established in particular by Ratnayake and Pelletier (33) or Ulberth and Henninger (34) as described earlier (20).

It follows from Fig. 7 that in bovine milk fat the main *trans*-C18:2 isomeric peaks are t9t12, c9t13+t8c12, t8c13+c9t12 and t11c15, with the identification of t8c12 and t8c13 being tentative. In comparison margarine exhibits the main peaks t9t12+c9t13+t8c12, c9t12 and t9c12 (20). Thus, there is only little agreement between milk fat and margarine. In particular t9c12, one of the main isomers in margarine, occurs only in traces in milk fat, whereas the isomer t11c15 occurring in strongly varying amounts in milk fat is not found in margarine (20).

Fig. 8 demonstrates that *trans*-C18:1 contents obtained under conditons usually applied in the literature, i.e. without Ag-TLC and using 50 or even 30 m - CP-Sil 88 columns, only comprise the isomers t6 to t11. In the right part of Fig. 8 all *trans*-C18:1

isomers from t4 to t16 are shown that can be baseline-resolved under optimal conditions on a 100 m - column after Ag-TLC fractionation of the total bovine milk fat. In the following, the *trans*-C18:1 contents given always relate to the sum of all isomers (t4 to t16) shown in Fig. 8. The registration of only t6 to t11 may lead to an under-estimation of the total *trans*-C18:1 content of up to 62 %. Specified information on such *trans*-C18:1 contents being too low have recently been published by us (30, 35). The mean correction factor for German bovine milk fats was calculated to be 1.55. Multiplication of the combined contents of t6 to t11 by this factor results in a good estimation of the total *trans*-C18:1 content.

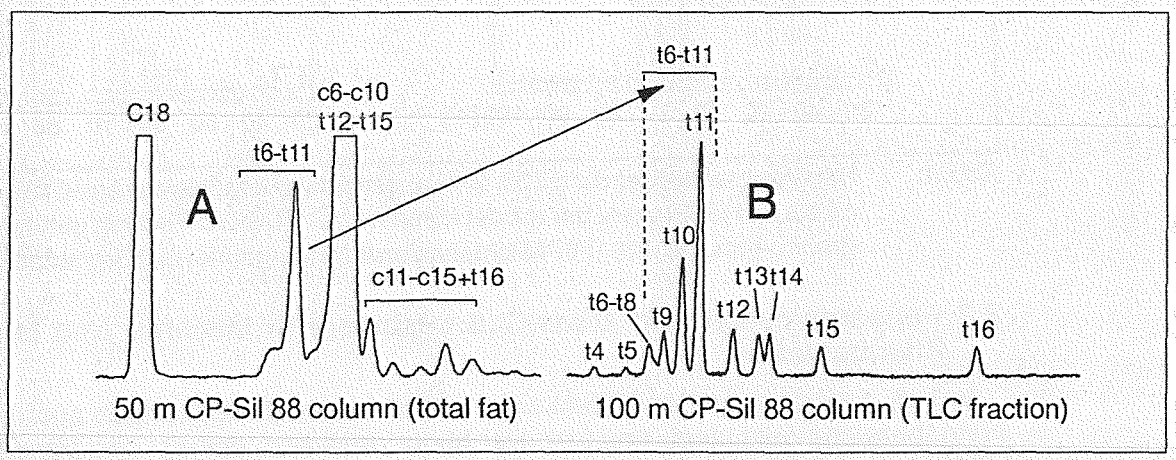

Fig. 8: Comparison of *trans*-C18:1 isomers from bovine milk fat being accessible to quantitation on a 50 m - Sil 88 column (A, without Ag-TLC) with the corresponding *trans*-monoenoic acid TLC fraction recorded on a 100 m - Sil 88 column (B)

3.2 CLA content of milk fats in relation to *trans*-C18:1 and C18:2 contents

Our analyses of 1756 milk fat resulted in an average c9t11 content of 0.75 % (range: 0.10-1.89 %). The mean content increased from typical barn feeding (winter: n = 927) to the transition period in spring and late autumn (n = 236) and further to pasture feeding (summer: n = 593) from 0.45 % to 0.76 % up to 1.20 % (Fig. 9).

The mean contents of *trans*-C18:2 (without CLA; sum of t9t12, c9t13, t8c12, t8c13, c9t12, t9c12, t11c15) and *trans*-C18:1 (sum of t4, t5 to t16) amounted to 0.63 and 3.62 %. Further, the 3 feeding periods barn, transition and pasture feeding exhibited *trans*-C18:2 and *trans*-C18:1 contents of 0.46, 0.66, 0.87 % and 2.65, 3.80, 5.08 %, respectively. Thus, comprising a representative number of German milk fats, Fig. 9 demonstrates that a fodder-related increase of CLA, probably being valuable with regard to nutritional physiology, is associated with a rather high increase of undesirable *trans*-C18:1 fatty acids. The relative increase of CLA by 167 % from winter to summer feeding further is accompanied by an increase of the *trans*-C18:2 isomers by 89 %, being physiologically undesirable as well.

Defined feeding conditions applied to a small herd of cows (n = 5) demonstrated a doubling of the c9t11 content during a slight energy deficit (young pasture) compared to barn feeding (Fig. 10). Feeding of older pasture even led to a CLA content being three times the basic content (barn). However, at a strong energy deficit (peak of lactation) the content of c9t11 decreased to the basic level again (additional experiment). For the three feeding variants (barn, young pasture, older pasture) *trans*-C18:1 contents of 2.83, 3.71 and 4.19 % were found. Fig. 10 illustrates the changes in fatty acid contents between the

basic diet and the two pasture variants. As already from Fig. 9 it follows from Fig. 10 that there is a high positive correlation between CLA and *trans*-C18:1 contents. Moreover, it becomes obvious that fodder-related changes in CLA contents also lead to very great changes for other fatty acids, particularly concerning the saturated medium-chain fatty acids C12-C16.

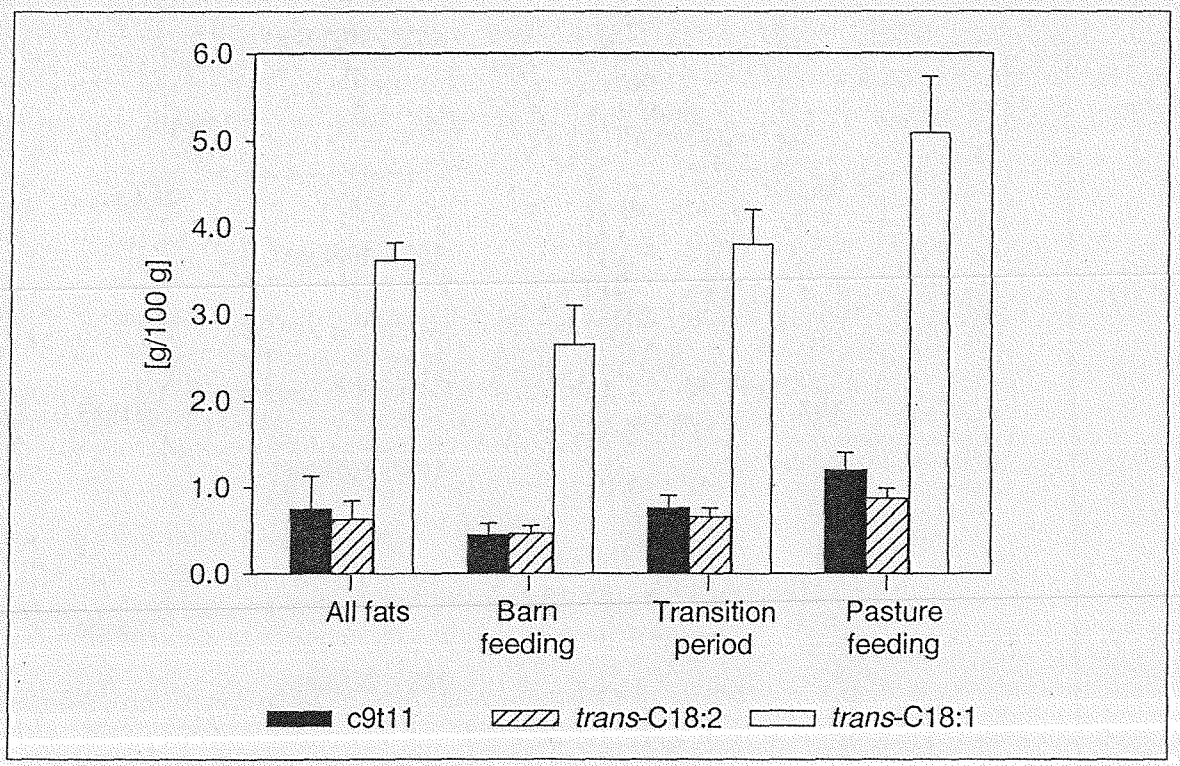

Fig. 9: Contents of CLA (c9t11), *trans*-C18:1 and *trans*-C18:2 (without CLA) in milk fat with indication of the standard deviations under typical barn feeding (n = 927), during the transition period (n = 236), at pasture feeding (n = 593) as well as in all samples combined (n = 1756)

A further feeding trial (17) with defined conditions (herd of 5 cows) also exhibited a clear increase of c9t11 as well as of *trans*-C18:1 at sufficient pasture feeding (Fig. 11). Whereas the difference between the triglyceride contents C52 and C54 did not indicate an energy deficit (36) at pasture feeding, such a deficit was found in the third feeding period (C52-C54 = 11.2 %). At this energetic under-supply the content of c9t11 and *trans*-C18:1 reached the basic level of barn feeding again, though an extremely high C54 content of 14.7 % was analysed that usually would indicate an extreme pasture feeding and an increase of *trans* fatty acids. During an energy deficit the mammary gland produces much C52 from fatty acids originating from the adipose tissue (C16,C18/C18:1, C18/C18:1). The stearic acid mobilized from the adipose tissue can only be converted into oleic acid by desaturase but not into *trans*-C18:1 leading to relatively higher oleic acid but lower *trans*-C18:1 contents. Thus, a simultaneous increase of c9t11 and *trans*-C18:1 with rising C54 content is only found at an energetically sufficient feeding or at most a slight deficit.

Fig. 11 exhibits the changes in contents of CLA and *trans*-C18:1 (increase) and additionally of palimitic acid (decrease). These trials as well as those presented in Fig. 10 demonstrate that it is very difficult to record changes in physical or technological properties of milk fat that are directly attributable to an fodder-related increase of the CLA

content. All the feeding variants caused rather small absolute changes of the CLA content but extreme decreases in the contents of C4 to C16 (sum) or even C16 alone as well as corresponding high increases of C16:1 to C24 (Fig. 10). Thus, differences in techological properties of spreadable fats resulting from such varying feeding conditions least of all characterize changes due to an increase of CLA.

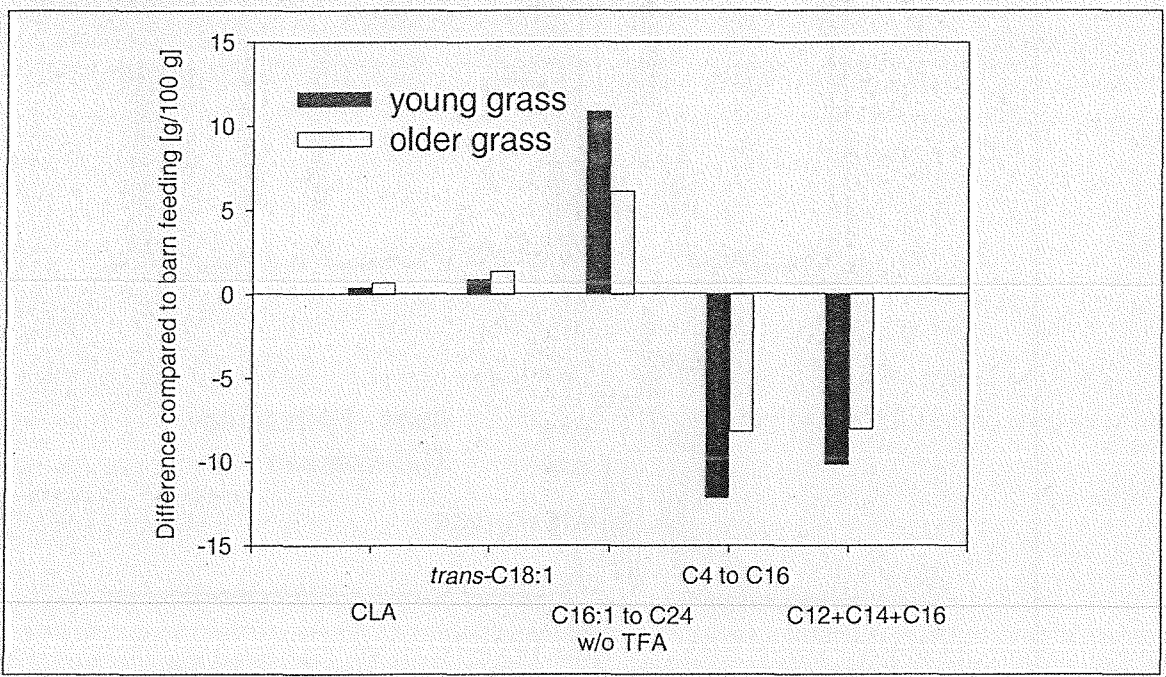

Fig. 10: Changes in the contents of CLA (c9t11), *trans*-C18:1, C16:1 to C24 w/o TFA (TFA = *trans* fatty acids), C4 to C16 and C12+C14+C16 in milk fat at feeding with young and older grass compared to a basic diet (barn feeding)

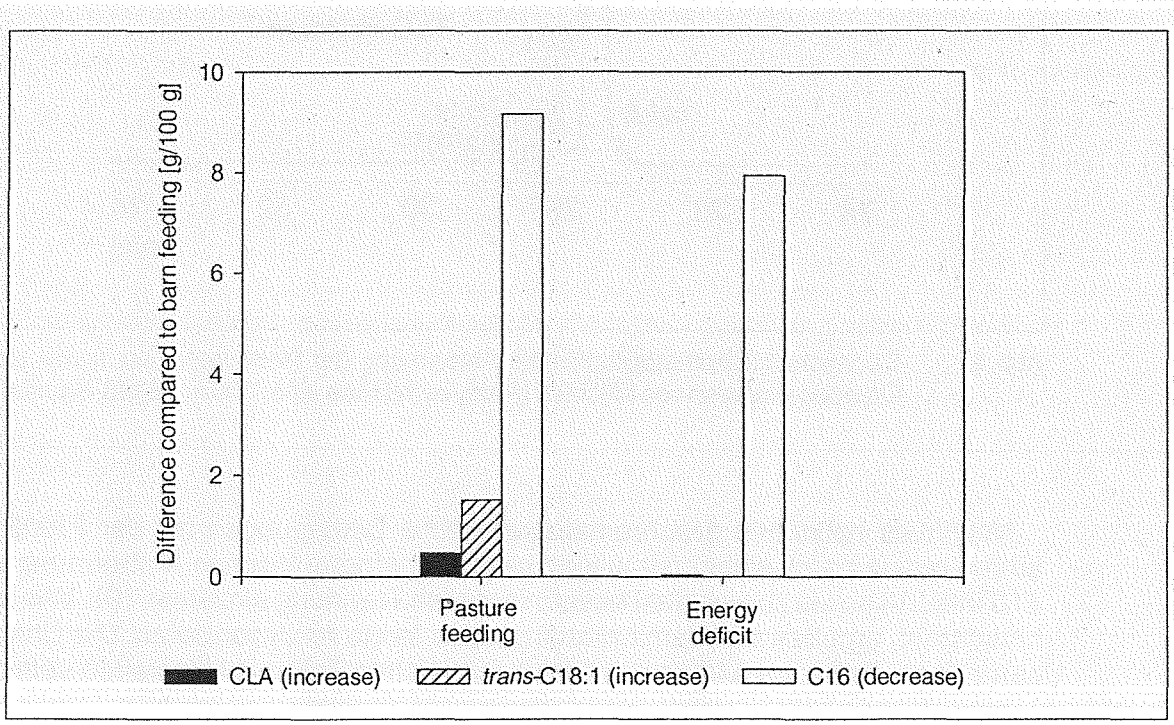

Fig. 11: Changes in the contents of CLA (c9t11), *trans*-C18:1 and C16 in milk fat at pasture feeding and energetic deficit in comparison to barn feeding; means of experiments with the same 5 cows, respectively.

Other investigations with 6 defined variants of rape feeding already described earlier (17, 18) showed particularly high CLA contents during feeding of 275 g and especially of 550 g rape oil or rape-seed pellets (up to three times the content resulting from the basic diet without any rape), whereas smaller increases were found with wholemeal from rape-seed. These 6 feeding variants exhibited *trans*-C18:1 contents of 2.87 % (basic diet), 3.75 % (275 g rape oil), 5.65 % (550 g rape oil), 3.36 % (275 g oil in 650 g wholemeal from rape-seed), 4.10 % (550 g oil in 1300 g wholemeal from rape-seed) and 6.51 % (550 g oil in pelleted rape-seed) in the milk fat. Fig.12 again demonstrates the absolute changes in fatty acid contents for CLA, *trans*-C18:1, C4 to C16 (decrease) as well as for the medium-chain saturated fatty acids C12+C14+C16 in comparison to the basic diet. Although in these trials considerable increases of CLA by relatively up to 234 % were found, the greatest changes by far are to be found for *trans*-C18:1 of absolutely up to 3.6 % (feeding of rape-seed pellets: *trans*-C18:1 = 6.5 %) as well as particularly for the other fatty acids (decrease of C4-C16, increase of C16:1-C24).

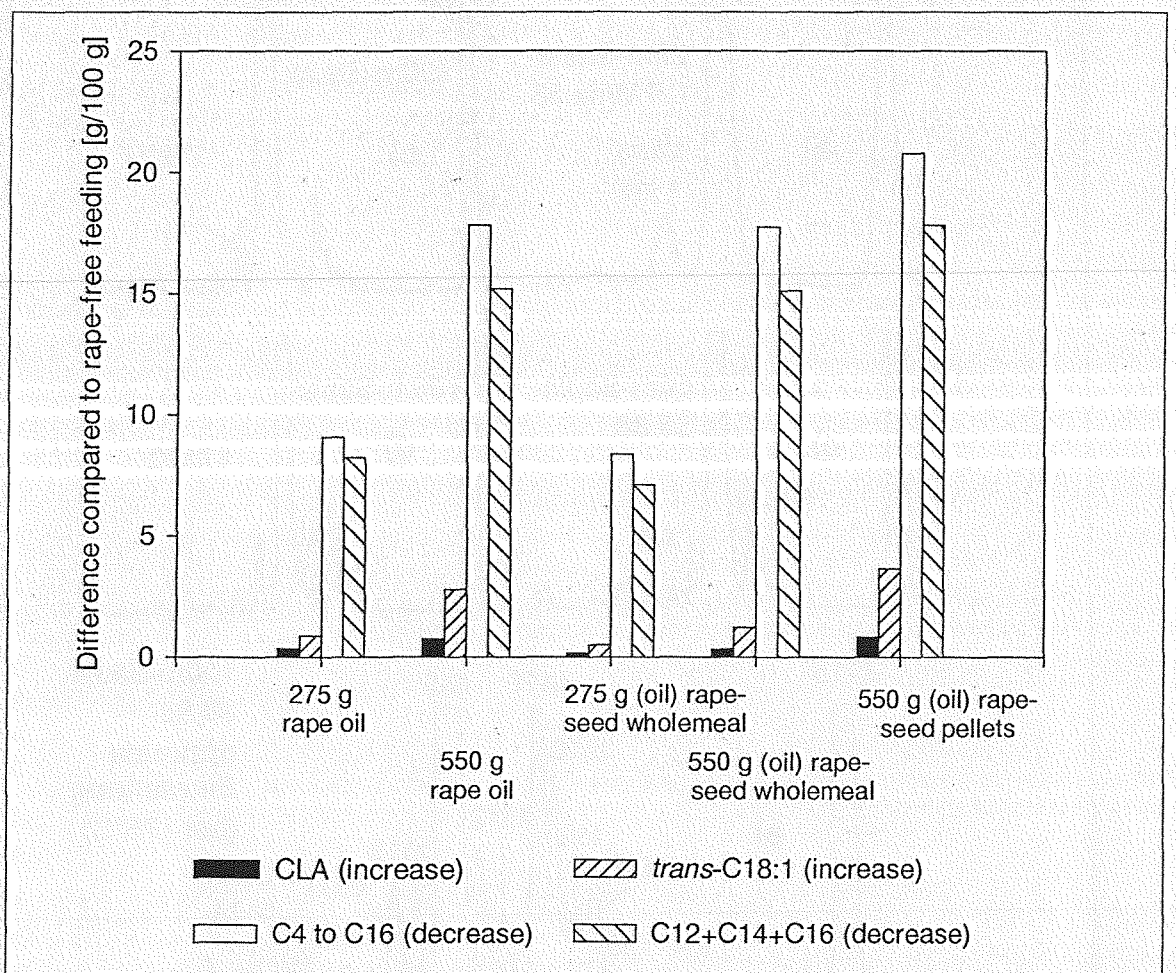

Fig. 12: Changes in the contents of CLA (c9t11), *trans*-C18:1, C4 to C16 and C12+C14+C16 in milk fat at different feeding of rape (rape oil, wholemeal from rape-seed, rape-seed pellets) in comparison to a basic diet without rape

All feeding trials show a close correlation between the increase of conjugated linoleic acid c9t11 and the undesirable *trans*-C18:1 or C18:2 isomers, except for periods with a strong energy deficit usually occurring with high-performing cows in the peak of lactation. Our calculations (37) resulted in correlation coefficients of r = 0.95 or r = 0.98 between

c9t11 and total *trans*-C18:1 or *trans* Δ11-C18:1 (t11). In feeding experiments comparing barn feeding, pasture feeding and ecological feeding also Jahreis et al. (38) found a close positive correlation between CLA and t11. The same conclusion was drawn by Jiang et al. (39) from feeding variants with different forage to concentrate ratios as well. The content of c9t11 could almost be doubled in this study by feeding restricted amounts of the trial diet instead of admitting for ad libitum consumption, with a correlation of r = 0.78 between c9t11 and t11. Stanton et al. (40) demonstrated that the c9t11 CLA level increased in milk fat from cows on a diet highly supplemented with rape-seed (1650 g/d full fat rape-seed) compared to a pasture or a low rape-seed diet (825 g/d full fat rape-seed) from 4.78 or 5.23 to 7.89 mg c9t11/g fat. Further, Kelly et al. (41) showed that a herd of cows that consumed only pasture doubled the CLA concentration in their milk fat compared to a control group of cows that were fed a mixed diet. Wolff et al. (42) had followed the seasonal variation of CLA in milk fat by analysing sixty samples of French butter collected at five different periods of the year. When changing from forage and concentrates during barn feeding to pasture feeding, the CLA content rose from 0.38 % (January) to 0.74 % (May-June).

In contrast to that findings Banni et al. (43) detected highest CLA contents in Italian milk fat in the winter season. This is due to the fact that in the south of Italy the cows are at pasture during the mild winter rather than during the dry summer.

Our results and in particular Figs.10-12 further demonstrate that a CLA increase is associated with a high decrease of the medium-chain saturated fatty acid C12, C14 and C16 of absolutely up to 18 %. According to a large number of publications these fatty acids were found to have an atherogenic effect by increasing serum and LDL cholesterol levels (e.g. 44, 45). Thus, all in all milk fats enriched with CLA are to be considered positively with regard to nutritional physiology. However, efforts should be made to restrict the undesirable *trans* content e.g. to 6 % *trans*-C18:1 (t4 to t16) by suitable unconventional means.

4. Conclusions

The content of the conjugated linoleic acid c9t11-C18:2 can considerably be increased by feeding management. As was shown by all experiments, the contents of undesirable *trans*-C18:1 acids simultaneously increased, while saturated medium-chain fatty acids were reduced. Future feeding experiments aiming at an increase of CLA in milk fat should not be performed without taking into account the changes in *trans*-C18:1/C18:2 contents.

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6. Summary

Precht, D., Molkentin, J: **Analysis and seasonal variation of conjugated linoleic acid and further *cis-/trans*-isomers of C18:1 and C18:2 in bovine milk fat.** Kieler Milchwirtschaftliche Forschungsberichte **51**(1) 63-78 (1999)

24 Conjugated linoleic acid (milk fat, *trans* fatty acids, saturated fatty acids, gas chromatography)

Conjugated linoleic acids (CLA) are reported to have anticarcinogenic and antiatherogenic properties. Different gas chromatographic conditions enabling extensive separation of the numerous CLA isomers were checked by using a high polar 100 m-capillary column (Sil 88). Errors in CLA quantitation, particularly caused by overlaps with *cis*-C20:1 isomers or C21, were discussed. Under optimal conditions, it has already been possible to separate the closely eluting isomers *cis* Δ9,*trans* Δ11 (c9t11) and *trans* Δ9,*cis* Δ11 (t9c11) in bovine and human milk fat by using a shorter medium-polar 25 m-capillary column (Wax 58) in routine analyses. Additionally, it could be demonstrated in several feeding trials with cows that an increase in the content of CLA c9t11 in milk fat is usually associated with an increase of the possibly atherogenic fatty acids *trans*-C18:1 and *trans*-C18:2. Simultaneously, a strong decrease of the medium-chain fatty acids, e.g. lauric, myristic and palmitic acid, causing an increase of the cholesterol level, was observed.

Zusammenfassung

Precht, D., Molkentin, J: **Analytik und jahreszeitliche Variation der konjugierten Linolsäure sowie weiterer *cis-/trans*-Isomere von C18:1 und C18:2 in bovinem Milchfett.** Kieler Milchwirtschaftliche Forschungsberichte **51** (1) 63-78 (1999)

24 Konjugierte Linolsäure (Milchfett, *trans*-Fettsäuren, gesättigte Fettsäuren, Gaschromatographie)

Konjugierten Linolsäuren (CLA) werden antikarzinogene und antiatherogene Eigenschaften zugesprochen. Unter Verwendung einer hochpolaren 100 m-Kapillarsäule (Sil 88) wurden unterschiedliche gaschromatographische Bedingungen geprüft, die eine weitgehende Trennung der zahlreichen CLA-Isomere ermöglichen. Insbesondere werden mögliche Fehler bei der Quantifizierung von CLA diskutiert, die vor allem durch eine Überlagerung mit *cis*-C20:1-Isomeren sowie C21 verursacht werden können. Allerdings konnte bei bovinem Milchfett sowie Humanmilchfett eine Trennung der dicht nebeneinanderliegenden Isomere *cis* Δ9,*trans* Δ11 (c9t11) und *trans* Δ9,*cis* Δ11 (t9c11) unter optimalen Bedingungen schon mit einer kürzeren mittelpolaren 25 m-Kapillarsäule (Wax 58) im Routinebetrieb erreicht werden. Darüber hinaus konnte in einer Reihe von Fütterungsversuchen an Kühen gezeigt werden, daß ein Anstieg des Gehaltes der CLA c9t11 im Milchfett in der Regel mit einer Zunahme der möglicherweise atherogenen *trans*-C18:1-Fettsäuren sowie von *trans*-C18:2 einhergeht, während gleichzeitig eine starke Abnahme der cholesterinspiegelsteigernden, mittelkettigen Fettsäuren Laurin-, Myristin und Palmitinsäure beobachtet wurde.

Résumé

Precht, D., Molkentin, J: **Analyse et variation saisonnière de l'acide linoléique conjugué et d'autres isomères *cis-/trans* de C18:1 et C18:2 dans la matière grasse bovine.** Kieler Milchwirtschaftliche Forschungsberichte **51** (1) 63-78 (1999)

24 Acides linoléiques conjugués (matière grasse laitière, acides gras *trans*-isomères, acides gras saturés, chromatographie gazeuse)

On dit des acides linoléiques conjugués d'avoir un effet anti-carcinogène et anti-athérogénique Différentes conditions de chromatographie gazeuse, permettant une séparation poussée des nombreux isomères d'acides linoléiques conjugués, ont été examinées en utilisant une colonne capillaire à haute polarité 100 m (Sil 88). Des erreurs de quantitation de l'acide linoléique conjugué, avant tout causées par des imbrications avec des isomères *cis*-C20:1 ou C21, ont été discutées. Sous des conditions optimales, il a déjà été possible de séparer des isomères *cis* Δ9,*trans* Δ11 (c9t11) et *trans* Δ9,*cis* Δ11 (t9c11),étroitement élués dans la graisse bovine et la matière grasse laitière, en utilisant une colonne capillaire plus courte à polarité moyenne 25 m (Wax 58) dans des analyses de routine. En plus, il a pu être démontré dans plusieurs essais d'alimentation de vaches, qu'une augmentation du contenu de l'acide linoléique conjugué dans la matière grasse laitière est normalement asscociée à une augmentation des acides gras *trans*-C18:1 et *trans*-C18:2 à effet probablement anti-athérogénique. En même temps, une forte baisse des acides gras de chaîne moyenne, comme l'acide laurique, myristique et palmitique, causant une hausse du niveau du cholestérol, a été observée.