Effect of feeding on *trans* positional isomers of octadecenoic acid in milk fats

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1. Introduction

Since the beginning of the 1990's it has been increasingly reported that *trans* fatty acids (TFA), similarly as the long chain saturated fatty acids lauric, myristic and palmitic acid, shall cause a rise of the LDL cholesterol level in human serum. In addition, further negative effects, such as a decrease in the HDL cholesterol level as well as an increase in lipoprotein (a) and serum triglyceride levels resulting from the intake of TFA have been described. The multitude of controversial discussions have prompted a great number of studies to measure the contents of TFA in animal fats and partially hydrogenated vegetable fats in many countries. In this connection, more recent publications have reported on a marked decrease in the TFA contents of margarines in 1995/96 in France (1), Germany (2, 3) and Austria (4) compared with previous years.

In individual cases the literature provides indications of a physiological influence of special C18:1-*trans* positional isomers (TPI). So, it is reported that biosynthesis of long chain polyenoic fatty acids is restricted by an inhibition of the Δ6-desaturase by *trans, trans*-C18:2 or by elaidic acid (*trans* Δ9-C18:1) and vaccenic acid (*trans* Δ11-C18:1) (5, 6) and, thus, also synthesis of prostaglandins (7). According to WILLETT *et al.* (8) increased inhibition of the Δ6-desaturase by elaidic acid, compared with vaccenic acid (7), is also likely to be due to different influences of TFA from partially hydrogenated vegetable fats (higher elaidic acid contents) compared with TFA from animal fats (higher vaccenic acid contents). The evaluations by ASCHERIO *et al.* (9) of the habits in consumption of 239 patients suffering from myocardial infarction – compared with 282 healthy probands – showed a significant relation between TFA intake from partially hydrogenated vegetable fats but no relation between the intake of animal fats and the risk of infarction. According to WILLETT *et al.* (8) and ASCHERIO *et al.* (9) an explanation for this may be the higher contents of elaidic acid in partially hydrogenated vegetable fats compared with milk fat. All these conclusions have, however, frequently been criticized. Further, from the nutritional point of view, WATTS *et al.* (10) do not exclude a negative influence of vaccenic acid which occurs in higher amounts in bovine milk fat. Nutritional studies on the varying influences of defined TPI, such as elaidic or vaccenic acid are, however, not reported in the literature, although here different impacts are quite possible, in particular, in view of the few indications provided in the literature.

The aim of this work is to examine the seasonal effects of feeding in winter and summer or the transition period in spring and late autumn, and of energetic underfeeding of the cows on the contents of TPI *trans* Δ4, Δ5 to Δ16 in bovine milk fat. In this way, an influence on defined TPI contents might be possible. First indications of seasonal influences on the total *trans*-C18:1-content of German milk fats (11) and the levels of TPI *trans* Δ6-Δ9, *trans* Δ10-Δ11, *trans* Δ12, *trans* Δ13-Δ14, *trans* Δ15 and *trans* Δ16 in French milk fats have been provided recently (12, 13).

2. Materials and methods

2.1 Samples

Trial a) First, a typical barn feeding trial was performed using 5 cows (lactation stage approx. 4th month). Then, these cows were fed grass from the 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 under energy deficit conditions: 4 kg straw and 2 kg concentrate (20 MJ net energy/lactation). To exclude any influence caused by the weather, feeding was always done in the barn.

Trial b) 927 bulk milks originating from rather varying West German areas were obtained under typical winter feeding conditions (barn feeding) with much concentrate allocations, 593 milks under typical summer feeding conditions – mainly pasture feeding – and 236 milks were obtained during the transition period from winter to summer feeding and in late autumn from summer to winter feeding. Another 58 bulk milks were obtained from slightly underfed cows during the transition period in times of drought in summer or at the peak of lactation.

Trial c) Of 4 different West German milk collection areas the fat from bulk milks was extracted weekly over a period covering roughly 1 year; here, the bulk milk samples were respectively obtained from the same herds belonging to a large milk collection area.

Trial d) A herd of 10 cows (lactation stage approx. 4th month) was subdivided in 2 groups consisting of 5 animals, respectively. One group was held under conditions of sufficient energy supply, whilst the other one was subjected to energetic underfeeding. Sampling (bulk milks mixed from aliquots) was done 1, 4 and 7 days after the beginning of the special feeding trials.

Milk fat was always obtained from butter by melting and filtering of the fat layer at 50 °C in an oven.

2.2 Analysis of trans fatty acids and triglycerides

Following thin layer chromatographic separation using silica-gel plates impregnated with $AgNO_3$ (TLC) the TFA were, on the one hand, analyzed gas chromatographically on a CP-Sil 88-column (100 m in length, 100% cyanopropyl polysiloxane). The procedure of separation and the conditions of FAME (fatty acid methyl esters) analysis have already been described elsewhere (3, 11, 14). On the other hand, a method based on the evaluation of triglyceride combinations was used for determining the TPI which as well allows all *trans* isomers in milk fat to be determined with high precision. The method is based on triglyceride formulae statistically derived from data obtained by TLC/GC of FAME and GC of triglycerides and has already been described elsewhere (15). The same publication provides information on gas chromatographic triglyceride analysis (separation by carbon number) using a short packed 50 cm column (3% OV-1).

The integration of chromatograms was performed using a Hewlett Packard 3365 II Chemstation. All results are given in g per 100 g total fatty acids.

3. Results and discussion

Fig. 1 shows gas chromatograms of the TPI of octadecenoic acid (FAME) after isolation of the *trans*-monoene fraction by Ag-TLC from milk fat resulting from typical barn feeding, pasture feeding and energetic underfeeding (trial a). The first 2 feeding conditions are typical of the winter and the summer period, respectively. An energy deficit is found especially at the peak of lactation in bulk milk, mainly if there are preferential times of calving. On the other hand, an energy deficit is often related to the change-over from barn to pasture and the low content of raw fibre in young grass. According to previous studies (16) an energy deficit can be recognized by a high triglyceride C52 content associated with a difference of >6% between C52 and C54.

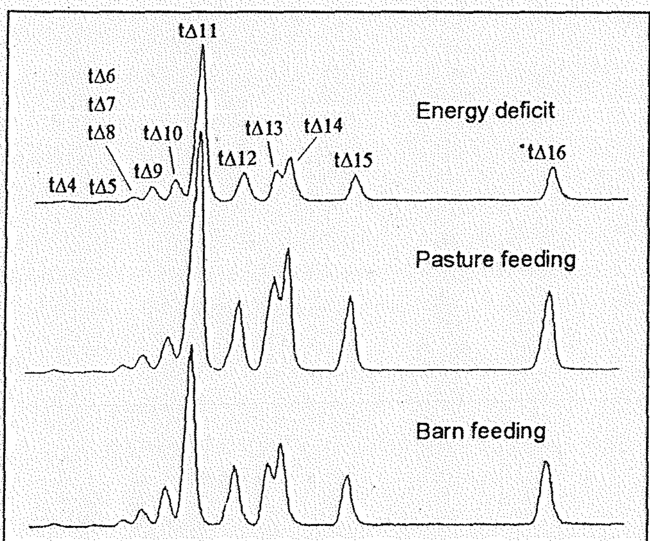

Fig. 1: Partial gas chromatograms of Ag-TLC fractions containing *trans* fatty acid methyl esters (FAME) derived from milk fat of the pasture and barn feeding period as well as after an energy deficit, respectively. Analysis on a CP Sil 88 100-m capillary column (125°C, H_2 pressure 220 kPa)

Well recognizable are the distinctly higher TFA contents during pasture feeding compared with barn feeding or the period of severe energetic underfeeding.

In a further trial using 927 milk fats from the winter feeding period (barn feeding), 236 milk fats from the transition period (barn to summer feeding in spring and reverse conditions in late autumn), 593 milk fats from feeding in summer (pasture feeding) and 58 milk fats obtained from cows suffering from a slight energy deficit all TPI *trans* Δ4 to Δ16 were determined (trial b). In Fig. 2 the results are summarized relative to total *trans*-C18:1 isomers. Above all, it is clearly shown that here the proportions of vaccenic acid (*trans* Δ11), being 35.09%, 46.38%, 55.30% and 51.45% for the different feedings, show the decisive differences and that the changes of other TPI, apart from slightly higher differences for *trans* Δ13/14, are almost negligible. Fig. 2B also shows the isomeric distribution of a herd suffering from severe energetic underfeeding (Fig. 1, trial a), the *trans* Δ11-content being 41.94%. Here, the distribution is similar to that established for feeding in winter.

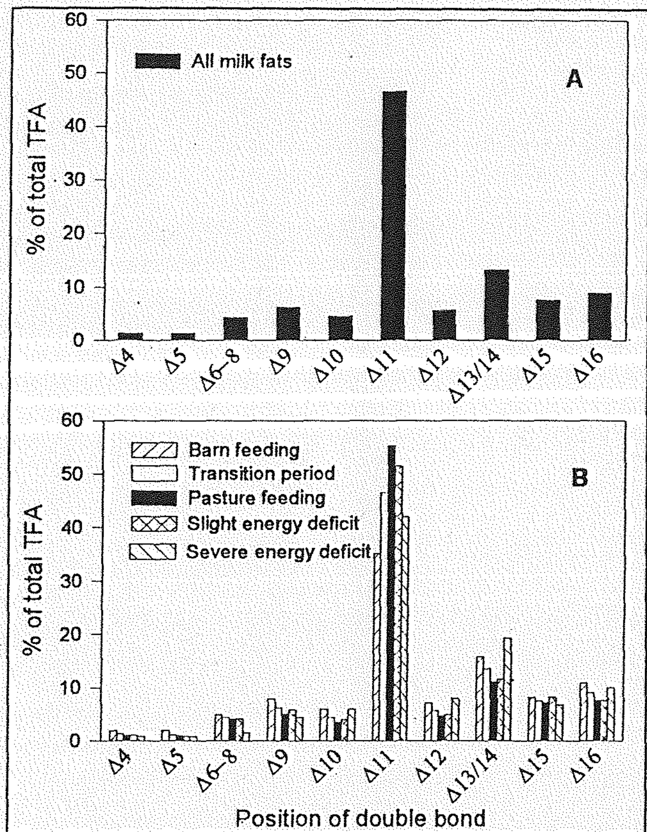

Fig. 2: (A) mean isomeric distribution of *trans*-C18:1 positional isomers in German milk fats (n=1756) relative to the mean total *trans*-C18:1 content (g/100 g of total *trans*-C18:1 fatty acids); (B) mean isomeric distribution in German milk fats subdivided in samples from the barn feeding period in winter (n=927), the transition period (n=236), the pasture feeding period in summer (n=593), a period of slight energy deficit (n=58) and of severe energy deficit of a herd of 5 cows

As regards French milk fats WOLFF *et al.* (13) have found a marked difference in the sum of *trans* Δ10+ *trans* Δ11 as well, being 48.4% in winter compared with 58.2% in May/June and 56.5% in July/August. The corresponding proportions established by us were 41.1% and 58.8% for winter and summer feeding. However, the corresponding isomeric proportion found for the transition period from barn to pasture feeding shown in Fig. 2 is 50.9%. Thus, the feeding period with high concentrate allocations corresponding to German winter milk fats is apparently lacking with the French milk fats. Moreover, in Germany starch-rich fodder, such as maize silage or whole plant silage from wheat, is increasingly fed during this period which results in a "hard" milk fat with low absolute TFA contents. The high content of TFA in German summer milk fats is associated with the high amount of polyunsaturated C18 fatty acids in the fodder occuring during pasture feeding, namely linoleic and linolenic acid, which contribute up to 75% to this fodder. Through biohydrogenation in the rumen these fatty acids are transferred into stearic and oleic acid but also into TFA proportions.

Table 1 shows the absolute TFA contents, the standard deviations and the TFA ranges during the different feeding conditions. Whilst from barn to pasture feeding roughly a doubling of the total *trans*-C18:1 contents has occurred, an almost threefold increase was observed for the vaccenic acid content, whereas the elaidic acid content was only minimally increased. Under conditions of a slight energy deficit the mean TFA content was comparable to that of the transition period. Only just a severe energy deficit (trial a) leads to TFA contents, corresponding roughly to barn feeding. However, the C54-triglyceride content of 14.36% observed here usually has to be assigned to an extremely soft summer fat. This finding can be explained by the fact that in the case of energetic underfeeding higher amounts of triglycerides with high C18 fatty acid contents are transferred from depot fat to the mammary gland without being hydrogenated by rumen bacteria.

In a further trial butter samples from bulk milks, based on always the same herds, were obtained from 4 large milk collection areas (region 1 to 4) and analyzed weekly (trial c). Fig. 3 provides an example of the results of the weekly measurements of region 1 as regards pure oleic acid as well as the TPI and the total *trans*-C18:1 contents. It can be seen that, apart from *trans* Δ10, all *trans*-C18:1 fatty acids as well as oleic acid take a similar course with, compared with summer, considerably lower

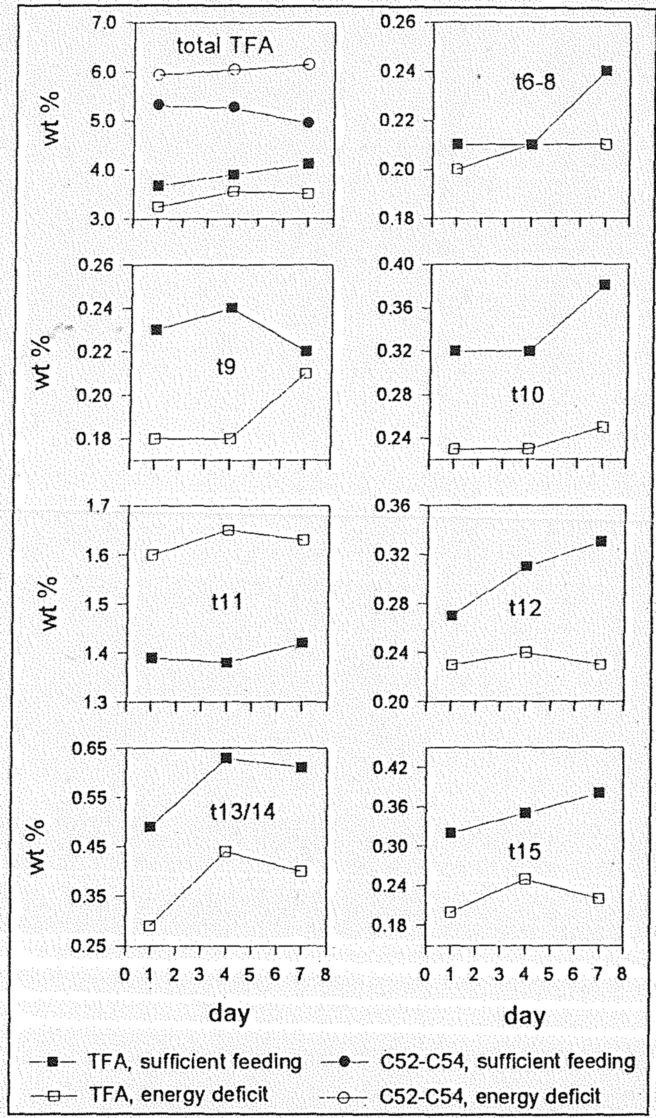

Fig. 3: Weekly variation in the contents of oleic acid, of total *trans*-C18:1 and of the different positional isomers of *trans*-C18:1 in milk fats from a large milk collection area (region 1) (g/100 g of total fatty acids)

Table 1: Contents of the positional isomers *trans* Δ4 to Δ16-C18:1 and of C54-triglycerides in milk fats from different feeding periods from bulk milk of different herds of cows (g/100 g total fatty acids)

	Barn feeding (n=927)			Transition period (n=236)			Pasture feeding (n=593)			Slight energy deficit (n=58)		
	Mean	SD[1]	Range	Mean	SD[1]	Range	Mean	SD[1]	Range	Mean	SD[1]	Range
Total	2.65	0.45	1.29–4.21	3.80	0.40	2.71–4.94	5.08	0.65	3.28–6.75	4.35	0.53	2.18–5.18
Δ4	0.05	0.01	0.02–0.07	0.05	0.01	0.04–0.07	0.05	0.01	0.03–0.08	0.05	0.00	0.04–0.06
Δ5	0.05	0.01	0.02–0.11	0.04	0.01	0.01–0.06	0.05	0.01	0.00–0.07	0.04	0.00	0.04–0.05
Δ6-8	0.13	0.02	0.07–0.20	0.17	0.01	0.12–0.22	0.21	0.02	0.16–0.27	0.19	0.02	0.11–0.22
Δ9	0.21	0.01	0.16–0.29	0.24	0.01	0.21–0.27	0.26	0.01	0.20–0.30	0.26	0.01	0.22–0.27
Δ10	0.16	0.03	0.04–0.33	0.17	0.03	0.04–0.26	0.18	0.03	0.03–0.29	0.18	0.01	0.14–0.21
Δ11	0.93	0.30	0.35–1.96	1.78	0.35	0.85–2.73	2.87	0.55	1.45–4.43	2.23	0.43	0.44–2.88
Δ12	0.19	0.02	0.10–0.26	0.22	0.02	0.18–0.29	0.24	0.02	0.19–0.31	0.22	0.01	0.19–0.25
Δ13/14	0.42	0.06	0.00–0.63	0.52	0.05	0.38–0.81	0.57	0.06	0.41–0.85	0.53	0.04	0.44–0.62
Δ15	0.22	0.04	0.04–0.33	0.29	0.03	0.18–0.46	0.37	0.04	0.19–0.48	0.36	0.04	0.23–0.43
Δ16	0.29	0.04	0.11–0.44	0.35	0.03	0.21–0.46	0.39	0.04	0.24–0.52	0.34	0.02	0.29–0.41
C54	3.45	0.64	1.42–4.50	4.96	0.29	4.51–5.50	6.66	0.67	5.51–9.02	6.67	0.70	3.91–7.80

[1]SD: standard deviation

Table 2: Contents of the positional isomers *trans* Δ4 to Δ16-C18:1 in milk fats from different regions (g/100 g total fatty acids) subdevided in different feeding conditions for each region with the same herd of cows

	Region 1			Region 2			Region 3			Region 4		
	Barn[1] n[4]=23	TP[2] n=19	Past[3] n=22	Barn[1] n=22	TP[2] n=17	Past[3] n=15	Barn[1] n=21	TP[2] n=13	Past[3] n=18	Barn[1] n=24	TP[2] n=12	Past[3] n=9
Total	3.33	4.67	5.94	2.73	3.60	4.16	3.09	4.30	5.92	2.55	3.60	4.55
Δ4	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.04
Δ5	0.04	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.05	0.04	0.04	0.04
Δ6-8	0.15	0.19	0.24	0.14	0.17	0.19	0.14	0.18	0.24	0.13	0.17	0.19
Δ9	0.22	0.25	0.27	0.21	0.23	0.25	0.22	0.25	0.28	0.22	0.24	0.26
Δ10	0.17	0.17	0.17	0.19	0.20	0.20	0.17	0.17	0.18	0.19	0.20	0.18
Δ11	1.40	2.47	3.60	0.90	1.57	2.06	1.12	2.09	3.41	0.67	1.53	2.45
Δ12	0.21	0.25	0.27	0.21	0.24	0.25	0.22	0.25	0.26	0.21	0.24	0.24
Δ13/14	0.46	0.57	0.63	0.46	0.55	0.58	0.52	0.58	0.63	0.48	0.57	0.57
Δ15	0.26	0.35	0.39	0.22	0.27	0.29	0.24	0.32	0.43	0.21	0.28	0.27
Δ16	0.33	0.40	0.42	0.31	0.36	0.37	0.35	0.39	0.46	0.32	0.36	0.36
C54	4.24	5.94	7.52	3.57	4.82	5.66	3.91	5.37	7.29	3.54	4.77	5.71

[1]Barn: barn feeding period; [2]TP: transition period; [3]Past: pasture feeding period; [4]number of samples

TFA and oleic acid contents during the winter weeks. In Table 2 the means of all TPI for the feeding periods barn feeding, transition period and summer feeding are summarized for all 4 milk collection areas studied (e.g., region 1: weeks 1-16 and 47-52, 17-25 and 41-46 as well as 26-40 of the year). Between the different regions pronounced differences have been established, in particular for the vaccenic acid contents under barn feeding conditions. Comparable to the studies performed using bulk milks from a great number of different herds (Table 1), Table 2 illustrates only slight absolute deviations of the low *trans* Δ6-8, *trans* Δ9 and *trans* Δ10 contents between the 4 regions. Contrary to this, these TPI occur in higher absolute amounts and are greatly varying in margarines and shortenings/cooking fats (17).

Although under conditions of severe energetic underfeeding (Fig. 2) the total *trans*-C18:1 proportion approximates the content resulting from barn feeding, the vaccenic acid content is slightly higher compared with barn feeding. To obtain more detailed information on this aspect, a herd comprising 5 cows received their normal feed, whilst further 5 cows were slightly underfed. Before, both groups had received the same basal ration (trial d). Whilst for the herd suffering from severe energetic underfeeding in Fig. 1 C54 contents of 10.03% and 14.36% were found after feeding the basal ration resp. after energetic underfeeding, in trial d C54 contents of 5.32% and 6.51% were established for normal feeding and energetic underfeeding, respectively. According to Table 1 these contents correspond by far more to normal feeding conditions. It can be seen from Fig. 4 that the total *trans*-C18:1 content is – under conditions of a slight energy deficit – again lower compared with sufficient energy supply. Also for the TPI altogether lower contents – apart from vaccenic acid – were established in the case of energetic underfeeding. This latter finding may indicate that vaccenic acid is likely to be accumulated to a greater extent in depot fat than, e.g., elaidic acid. Here the *trans* Δ11/ *trans* Δ9-ratios were 6.1 and 8.6 for sufficient and insufficient energetic feeding, respectively. The results given in Table 1 cannot be entirely compared with the above finding because here the basal rations were not the same for all herds. Further, the bulk milks were obtained from a great number of different herds. A high value of 8.8 for the *trans* Δ11/ *trans* Δ9-ratio established by us as a mean for beef tallow (mean of 5 different fats) underlines the above finding. However, this conclusion con-

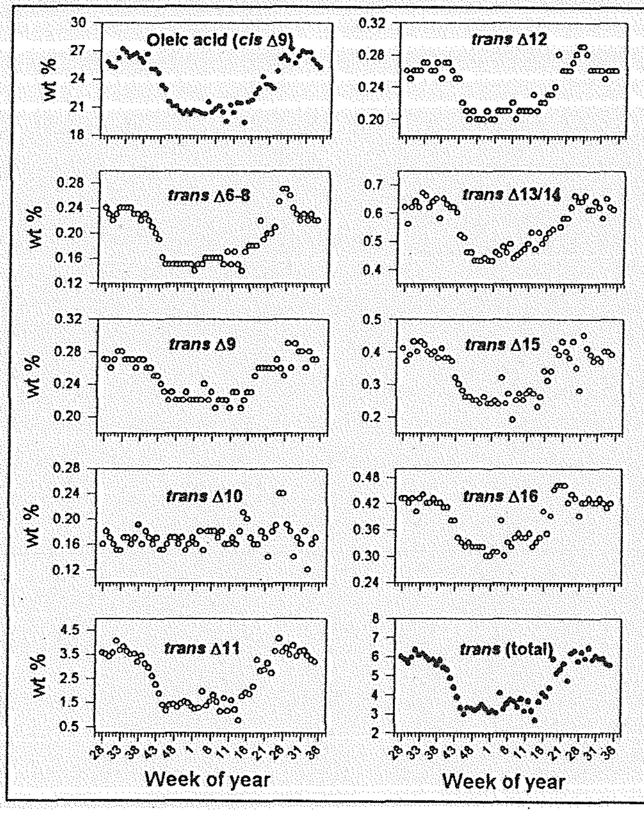

Fig. 4: Triglyceride content differences C52-C54, contents of total *trans*-C18:1 (TFA) as well as of all *trans* positional isomers (*trans* Δ6 to Δ15: t6 to t15) under conditions of sufficient energy supply and a slight energy deficit after 1, 3 and 7 days in milk fats from bulk milks of 2 herds comprising 5 cows, respectively (g/100 g of total fatty acids)

cerning *trans* Δ11 resulting from Fig. 4 should only be regarded as a first indication of the different ways of transition of TPI into depot fat.

4. Conclusions

Compared to summer feeding the total *trans*-C18:1 content is half as high during typical barn feeding. As regards the TPI greater differences resulting from the different normal feeding conditions barn feeding, transition period, pasture feeding and energetic underfeeding are here established only for the vaccenic acid contents, whilst the other TPI contents showed only slight absolute variations. A comparison of physiological effects in humans resulting from the intake of bovine milk fats compared with partially hydrogenated vegetable fats is, thus, confined to a comparison of the influence of *trans* Δ11 and the TFA group *trans* Δ6 to Δ10 present in vegetable fats in higher amounts or elaidic acid alone (*trans* Δ9). The present analyses propose that vaccenic acid is preferentially transferred into body fat. This finding should be interpreted as an incitement to perform corresponding studies with human subjects.

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5. References

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6. Summary

PRECHT, D., MOLKENTIN, J.: **Effect of feeding on *trans* positional isomers of octadecenoic acid in milk fats.** Milchwissenschaft **52** (10) 564–568 (1997).

44 Milk fat (*trans* fatty acids)

The seasonal influence of feeding (barn feeding, pasture feeding, transition period and energy deficit) on the content of the C18:1 *trans* positional isomers (TPI) *trans* Δ4, Δ5, Δ6 to Δ16 in bovine milk fat was investigated. From almost 1800 analyzed German milk fats vaccenic acid (*trans* Δ11) with contents of 35.09%, 46.38%, 55.30% and 51.45% relative to total *trans*-C18:1 made the decisive difference, whilst the differences of all other TPI were markedly less pronounced. During transition from barn to pasture feeding the absolute content of *trans* Δ11 increased threefold from 0.93% to 2.87% (elaidic acid, *trans* Δ9: 0.21% to 0.26%), whilst the total TPI content increased from 2.65% to 5.08%. Further, measurements of the seasonal course of all TPI contents were made weekly over a whole year in 4 large milk collection areas using the same cows. Apart from the almost unchanged contents of *trans* Δ10 – a seasonal course with low TPI contents in winter and up to at least twice as high contents (*trans* Δ11) during summer feeding was established here. With cows suffering from severe underfeeding only low TPI contents comparable to those established during the barn feeding period were found despite extremely high oleic acid- and C54-triglyceride contents. Such an energy deficit occurs frequently at the peak of lactation.

PRECHT, D., MOLKENTIN, J.: **Fütterungsbedingte Variation der *trans*-Positionsisomere der Octadecensäure in Milchfetten.** Milchwissenschaft **52** (10) 564–568 (1997).

44 Milchfett (*trans*-Fettsäuren)

In bovinen Milchfetten wurden die saisonalen Fütterungseinflüsse (Stall-, Weide-, Übergangsfütterung und energetische Unterfütterung) auf den Gehalt der *trans*-C18:1-Positionsisomere (TPI) *trans* Δ4, Δ5, Δ6 bis hin zu Δ16 untersucht. Bei den etwa 1800 analysierten deutschen Milchfetten machte hierbei mit relativen Anteilen von 35,09%, 46,38 %, 55,30% und 51,45% aller TPI die Vaccensäure (*trans* Δ11) den entscheidenden Unterschied aus, während Unterschiede aller anderen TPI wesentlich geringer ausfielen. Beim Übergang von Stall- zur Weidefütterung verdreifachte sich der absolute Anteil von *trans* Δ11 von 0,93% auf 2,87% (Elaidinsäure, *trans* Δ9: 0,21% auf 0,26%), während der Gesamt-TPI-Anteil von 2,65% auf 5,08% anstieg. Bei Messungen des wöchentlich über ein Jahr untersuchten jahreszeitlichen Verlaufs aller TPI-Gehalte in 4 großen Milcheinzugsgebieten bei jeweils gleichbleibender Herdenzusammensetzung wurde hier bis auf die nahezu unveränderten Anteile von trans Δ10 jeweils ein Verlauf mit geringen TPI-Anteilen im Winter und mindestens doppelt so hohen Gehalten (*trans* Δ11) während der Sommerfütterung gefunden. Bei stark unterfütterten Kühen traten trotz extrem hoher Ölsäure- und C54-Triglyeridanteile nur geringe TPI-Gehalte in der Größenordung der Stallfütterungsperiode auf. Ein derartiges Energiedefizit liegt häufig in der Spitze der Laktation vor.