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The fatty acid composition of Estonian and Latvian retail milk; implications for human nutrition compared with a designer milk

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Short title: **The fatty acid composition of retail milk**

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Summary

The study reported in this Research Communication compared retail milks' FA profiles from two neighbouring countries, estimated the potential contributions of these milks and a designer milk (achieved by changing the diet of the dairy cow) to the recommended human dietary intake of FA, and predicted (based on the milk FA profile) methane emission from dairy cows. Retail milk in Estonia and Latvia was purchased from supermarkets monthly for one year. To compare the FA composition of retail milk with designer milk with an increased PUFA content, the bulk milk FA profile from a separate field trial was used. Milk FA concentrations of two neighbouring countries were affected by state, season, and their interaction, while the main influence on all these factors were different feeding practices (grazing availability, forage to concentrate ratio and legume-rich silages *vs.* maize silages). Three cups (600 ml; fat content 2·5 g/100g) of Estonian, or Latvian retail milk or designer milk per day contributed more to the recommended intakes of saturated FA (SFA) (42·5%, 42·7%, 38·7%, respectively) than other FA. Compared to the retail milks, α-linolenic acid estimated intake was almost doubled by designer milk consumption (19·7% of adequate intake) without influencing summed intakes of SFA and *trans* FA. There were state and seasonal differences in the predicted methane outputs of dairy cattle based on retail milk FA. Although the FA profiles of retail milks in the two neighbouring countries were affected by state and season, an appreciable increase in human dietary intakes of beneficial fatty acids from milk, and concomitant reduction in methane emissions from dairy cows, can be achieved only by targeted feeding.

Keywords: Milk, retail milk, milk fatty acids, dietary intake.

Milk and dairy products are important sources of fat and fatty acids (FA) in the human diet. When choosing healthier eating patterns, it is recommended to reduce the intake of SFA and

TFA, and to increase the consumption of polyunsaturated fatty acids (PUFA) (EFSA, 2010; USHHS and USDA, 2010). It is well established that the diet of dairy cows, and their genetic variation, are the main factors influencing milk FA composition (Shingfield *et al*., 2013). Altering the diet of dairy cows offers the opportunity to reduce milk medium-chain and total SFA content, and increase C18:1 *cis*-9, total PUFA and conjugated linoleic acid (CLA) contents in milk, although to a variable extent.

Milk production is often considered to have a substantial environmental impact due to methane (CH_4) emissions by cattle as a result of rumen fermentation. The moderate relationship between milk FA profile and CH_4 emissions (van Lingen *et al.*, 2014), both influenced by feeding of dairy cows, indicates that the FA profile of retail milk could be used as a non-invasive method to estimate CH_4 production in dairy cows.

The objectives of this study were, (i) to compare the detailed FA profiles of retail milks of two neighbouring EU countries (Estonia and Latvia) with similar climate conditions but with some differences in dairy cows' management and feeding practice, (ii) to compare how Estonian and Latvian milks, and milk with a modified FA profile ('designer' milk) achieved by targeted feeding of the dairy cow, are related to the recommended human dietary intakes of different FA and FA groups, and (iii) to assess CH_4 emissions via prediction equations based on milk FA composition.

Material and methods

Retail milk collection and milk fatty acid analysis

Homogenised and pasteurised retail milk (2·5 g/100 g fat, 1 l carton or bag) was purchased from supermarkets in Estonia (Tartu) and Latvia (Riga) once a month for one year (March 2011

– February 2012). All seven brands from three processors (including supermarket own labels) on sale in Estonia (total sample n=84) and six processors' brands in different regions in Latvia (70% of the market; total n=72) were included in the study. Samples were kept frozen (–20 °C) and analysed at the milk quality laboratory of the Estonian University of Life Sciences, Tartu, Estonia.

The milk samples were prepared and analysed for FA profiles as described by Meremäe *et al.* (2012). Results for all FA were expressed as g/100 g of total FA. Estimated contributions (%) of recommended daily milk consumption to the recommended or adequate intakes of FA were calculated.

Designer milk production and sampling

In a trial (April – May, 2007) on a dairy farm in Estonia (300 Estonian Holstein dairy cows, loose housed, milked twice per day, average milk yield 26·8 kg per cow per day) cows were fed a total mixed ration (TMR) *ad libitum* in three feeding groups based on days in milk (DIM): 1–100, 101–250 and 251 up to end of the lactation. The TMR for all feeding groups contained grass-clover (50:50) silage, barley and maize meal, heat treated rapeseed cake (crude fat 100 g/kg DM), cold-pressed linseed cake (crude fat 200 g/kg DM) and a mineral-vitamin mixture. Concentrate to forage ratio, metabolisable energy, crude protein and crude fat contents in DM in the three diets were respectively 59:41, 50:50, 34:66; 11·8 MJ/kg, 11·2 MJ/kg, 10·5MJ/kg; 169 g/kg, 156 g/kg, 147 g/kg and 57·7 g/kg, 45·4 g/kg, 41·6 g/kg. The mean FA profile of four bulk milk samples collected was used for the comparison.

Prediction of methane production

The equation of Van Lingen *et al.* (2014) was used for CH_4 prediction (CH_4 (g/kg of FPCM, fat-protein corrected milk) = 21·13 – 1·38 × C4:0 + 8·53 × C16:0-iso – 0·22 × cis-9 C18:1 – 0·59 × trans-10+11 C18:1; R^2 = 0·47). All FA in the equation were as g/100 g FA.

Statistical analysis

The effects of country, season (summer = May – Oct. *vs* winter = Nov. – April) and country by season interaction on the FA composition of retail milks and predicted CH_4 emissions were tested using fixed effect analysis of variance (ANOVA) including also the random effect of milk product brand (to consider any potential correlation between milk samples of the same brand) and two replicate measures of the same sample as repeated measures. The denominator degrees of freedom in ANOVA were calculated according to the Kenward-Roger method. To identify common patterns in milk FA profiles, and analyse their differences, principal component analysis (PCA) was performed. Additionally the FA compositions, predicted CH_4 emissions and estimated contributions (%) of recommended daily milk consumption to the recommended or adequate intakes of FA were compared between Estonian and Latvian retail milks and designer milk using the t-tests with degrees of freedom equal to number of samples and followed by Bonferroni correction for multiple testing. The modelling was carried out using SAS 9.4 procedure MIXED and PCA with R 3.2.3 package ade4. The results were considered statistically significant at $P \leq 0.05$.

Results and discussion

The fatty acid composition of retail milks

Even though Estonia and Latvia are neighbouring countries with similar landscape and climate, farming systems and feeding practices are different with grazing availability (16% and 60% of herds, respectively), forage to concentrate ratio (F:C) (55:45 and 70:30, respectively), use of legume-rich forage (40% of silages) and a lower proportion of maize silages in Estonia (5% vs 19% in Latvia). These differences were reflected in the FA composition of retail milks. The FA concentrations of most milk FA, as well as FA groups, were affected by state and season, and many by the interaction of state and season (online Supplementary Table S1). The effect of season on the FA composition of retail milk was more pronounced than the effect of state.

Results of PCA of the whole dataset are presented in online Supplementary Fig. S1, and the patterns of FA concentrations described by the first and the second PC according to state, season and state by season in Fig. 1.

(Figures 1 near here)

Relative to the overall FA pattern of Estonian retail milk, the FA pattern of Latvian retail milk was shifted towards a positive correlation with PC1 (Fig. 1A), which was related to the higher proportions of ruminal biohydrogenation intermediates including C18:1 *trans*-11, CLA and branched-chain FA (BCFA) originating from rumen microbes. The overall summer milk FA pattern was shifted towards the first and the second (C18:0, most of ruminal biohydrogenation intermediates, CLA and majority of the n-3 FA, n-6 FA) quarters (Q) of the plot (Fig. 1B). The same distinctive feature was present in both Estonian and Latvian summer milk reflecting the higher dietary supply of PUFA, especially that of C18:3 n-3, from fresh grass compared to winter diets (Dewhurst *et al*., 2006). Compared to Estonian summer milk, the FA pattern of Latvian summer milk was shifted towards the first and fourth (BCFA, C15:0 and C17:0) quarters of the plot (Fig. 1C). Regarding winter milk, the FA pattern of Latvian winter milk was shifted towards the higher proportions of BCFA, C15:0 and C17:0 (IVQ) compared to Estonian winter milk and lower proportions of *de novo* synthesised FA (IIIQ) also FA clustered in the second quarter (Fig. 1C). The higher concentrations of BCFA and linear odd-chain FA (C15:0, C17:0) in Latvian winter milk are in line with a previously reported (Vlaeminck *et al*., 2006) effect of higher F:C ratio increasing the proportions of bacteria-derived FA leaving the rumen.

Human consumption of health-related fatty acids

The estimated contribution of Estonian and Latvian retail milk to the recommended or adequate intakes of FA for adults (Table 1) confirmed previous suggestions (Shingfield *et al*., 2013) that milk fat is an important source for SFA (~43% of the recommended upper limit) if the recommended amount (600 mL, fat content 2·5 g/100g; Tervise Arengu Instituut, 2017) is consumed. Estimated contributions for desirable α-linolenic acid (ALA; C18:3 n-3) were relatively low but still provided above 8% of adequate intake (1·1 g or 0·5% of energy intake). Regarding the sum of long-chain n-3 PUFA (eicosapentaenoic acid (EPA; C20:5 n-3) and, docosahexaenoic acid (DHA; C22:6 n-3), the latter was not detected in this study, but estimated intake of long-chain n-3 PUFA was enhanced (~3·5% vs ~9·0% of 250 mg, Table 1) by docosapentaenoic acid (DPA; C22:5 n-3), the concentration of which was greater than EPA (online Supplementary Table S1). The function of dietary DPA remains uncertain, although some reports indicate it may be beneficial to health (Howe *et al*., 2007)

In line with the results of feeding trial with dairy cow diet supplemented with linseed (Stergiadis *et al*., 2014) the FA profile of designer milk differed substantially from retail milk profiles (Fig. 1). Our designer milk contained more ALA, long-chain n-3 FA, *trans* FA and less SFA compared with retail milks (online Supplementary Table S1). Designer milk consumption would increase the estimated intake of C18:3 n-3, Σ n-3 and sum of essential FA [ALA+linoleic acid (LA; C18:2 n-6)] but also EPA and DPA (Table 1). The estimated contribution of milk fat to the recommended upper limit for dietary intake of SFA was lower for designer milk compared with retail milk, and it was also lower for designer milk for SFA+TFA.

Even though the effect of the state on the FA composition of retail milk was observed for most FA, only small differences were observed in estimated consumption of discussed FA and FA groups at the recommended milk intake level of 600 mL/d. However, while consuming designer milk ALA intake would be almost doubled to 19.7% of AI.

Predicted CH_4 emissions in dairy cows

Although van Lingen et al. (2014) indicated that milk FA composition has only a moderate potential for CH_4 prediction per unit of milk, the method still enables to roughly assess regional differences, modify feeding strategies and mitigate CH_4 emissions. Despite the dissimilarities in feeding strategies, there were no differences in yearly mean CH_4 output values from dairy cows (g/kg FPCM; $P = 0{\cdot}51$) between the two states. The predicted CH_4 emissions were higher ($P < 0{\cdot}001$) during the winter period compared to the summer 12·19 vs 11·92 and 12·62 vs 11.65 in Estonia and Latvia, respectively. Our simulation showed notably lower enteric CH_4 emissions when producing designer milk (11.06; $P < 0.001$) compared to conventional production (12.06 for Estonia and 12.11 for Latvia). Lower predicted CH_4 emission while producing designer milk, caused by feeding oilseed (Meale *et al*., 2013), shows that production of favourable for human health designer milk with higher PUFA content has also environmental advantages.

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Table 1. Estimated contribution (%) of recommended daily consumption (600 mL, fat content 2.5 g/100g) of Estonian and Latvian retail milk and designer milk, to the recommended or adequate intakes of FA for females with energy intake of ~8.37 MJ/d

Fatty acid	Recommendations, adequate intake (AI[†])	Means of retail milk		Designer milk, %
		Estonia, %	Latvia, %	
SFA	< 10% of energy intake[‡, §, ¶]	42·5[a]	42·7[a]	38·7[b]
SFA+TFA	≤ 10% of energy intake[††]	45·2[a]	45·6[b]	43·4[c]
cis PUFA	5-10% of energy intake[§, ¶]	3·62-1·81	3·39-1·69	5·53-2·76
cis MUFA	10-20% of energy intake[§, ¶]	15·7-10·4	15·2-10·2	16·6-11·1
C18:3 n-3	AI: 1·1 g[‡]	8·57[a]	8·70[a]	19·7[b]
C18:3 n-3	AI: 0·5% of energy intake[§, ‡‡¶]	8·49[a]	8·62[a]	19·5[b]
C18:2 n-6	AI: 11 g[‡]	2·14[a]	1·93[b]	2·93[c]
C18:2 n-6	AI: 4% of energy intake[‡‡¶]	2·65[a]	2·39[b]	3·62[c]
EPA+DHA	AI: 250 mg[‡‡]	3·44[a]	3·59[b]	5·03[c]
EPA+DHA+DPA	AI: 250 mg[††]	8·81[a]	8·92[a]	12·2[b]
Σ n-3	≥ 1% of energy intake[§, ¶]	5·36[a]	5·44[a]	11·3[b]
n-3 + n-6 (essential)	3% of energy intake[§]	4·94[a]	4·62[b]	8·07[c]

[†]AI, adequate intake.

[‡]2015–2020 Dietary Guidelines for Americans (USHHS and USDA, 2015).

[§]Nordic Nutrition Recommendations 2012. Recommended intake of macronutrients (excluding energy from alcohol) (Nordic Council of Ministers, 2014).

[¶]Estonian nutrition and physical activity recommendations 2015 (Tervise Arengu Instituut, 2017).

[††]Not used in cited recommendations.

[‡‡]Scientific opinion on dietary values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol (EFSA, 2010).

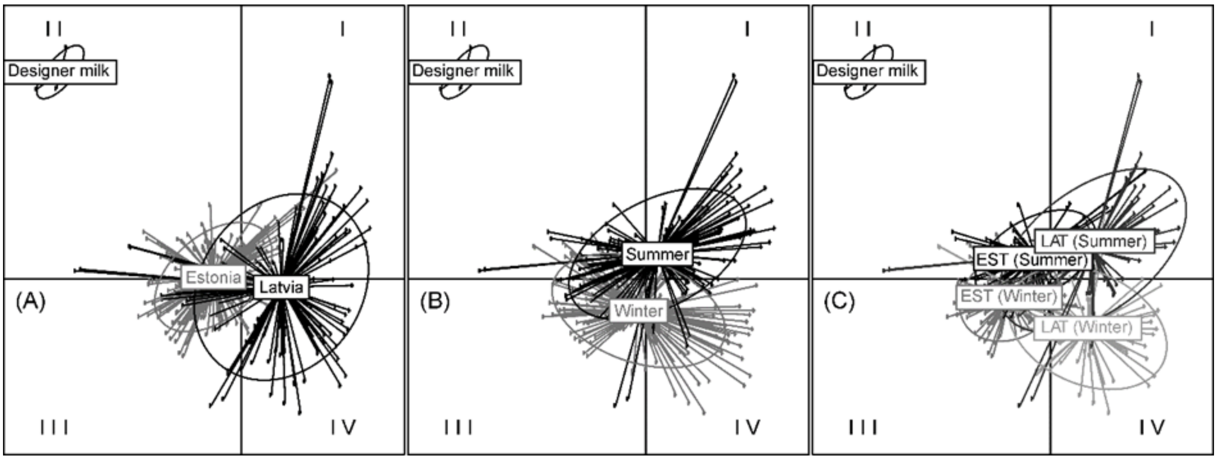
[a,b,c] Means with different superscript letters are statistically significantly different ($P < 0{\cdot}05$, t-tests with degrees of freedom equal to number of samples and followed by Bonferroni correction for multiple testing).

Figure legend:

Fig. 1:

The patterns of FA concentrations described by the first and the second principal component: (A) fatty acid patterns by national state, (B) fatty acid patterns by season, (C) fatty acid patterns by national state and season. In all figures, also the location of designer milk samples is presented. The factor loadings showing the relative importance of fatty acids in first two principal components are presented in Supplementary Figure S1.

Fig. 1


II
I
Designer milk
Estonia
Latvia
(A)
III
IV
II
I
Designer milk
Summer
Winter
(B)
III
IV
II
I
Designer milk
LAT (Summer)
EST (Summer)
EST (Winter)
LAT (Winter)
(C)
III
IV