

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

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

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

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26 **Summary**

27 The study reported in this Research Communication compared retail milks' FA profiles from
28 two neighbouring countries, estimated the potential contributions of these milks and a designer
29 milk (achieved by changing the diet of the dairy cow) to the recommended human dietary intake
30 of FA, and predicted (based on the milk FA profile) methane emission from dairy cows. Retail
31 milk in Estonia and Latvia was purchased from supermarkets monthly for one year. To compare
32 the FA composition of retail milk with designer milk with an increased PUFA content, the bulk
33 milk FA profile from a separate field trial was used. Milk FA concentrations of two
34 neighbouring countries were affected by state, season, and their interaction, while the main
35 influence on all these factors were different feeding practices (grazing availability, forage to
36 concentrate ratio and legume-rich silages vs. maize silages). Three cups (600 ml; fat content
37 2.5 g/100g) of Estonian, or Latvian retail milk or designer milk per day contributed more to the
38 recommended intakes of saturated FA (SFA) (42.5%, 42.7%, 38.7%, respectively) than other
39 FA. Compared to the retail milks, α -linolenic acid estimated intake was almost doubled by
40 designer milk consumption (19.7% of adequate intake) without influencing summed intakes of
41 SFA and *trans* FA. There were state and seasonal differences in the predicted methane outputs
42 of dairy cattle based on retail milk FA. Although the FA profiles of retail milks in the two
43 neighbouring countries were affected by state and season, an appreciable increase in human
44 dietary intakes of beneficial fatty acids from milk, and concomitant reduction in methane
45 emissions from dairy cows, can be achieved only by targeted feeding.

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

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49 Milk and dairy products are important sources of fat and fatty acids (FA) in the human diet.

50 When choosing healthier eating patterns, it is recommended to reduce the intake of SFA and

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

57 Milk production is often considered to have a substantial environmental impact due to methane
58 (CH₄) emissions by cattle as a result of rumen fermentation. The moderate relationship between
59 milk FA profile and CH₄ emissions (van Lingen *et al.*, 2014), both influenced by feeding of
60 dairy cows, indicates that the FA profile of retail milk could be used as a non-invasive method
61 to estimate CH₄ production in dairy cows.

62 The objectives of this study were, (i) to compare the detailed FA profiles of retail milks of two
63 neighbouring EU countries (Estonia and Latvia) with similar climate conditions but with some
64 differences in dairy cows' management and feeding practice, (ii) to compare how Estonian and
65 Latvian milks, and milk with a modified FA profile ('designer' milk) achieved by targeted
66 feeding of the dairy cow, are related to the recommended human dietary intakes of different FA
67 and FA groups, and (iii) to assess CH₄ emissions via prediction equations based on milk FA
68 composition.

69

70

71 **Material and methods**

72

73 *Retail milk collection and milk fatty acid analysis*

74 Homogenised and pasteurised retail milk (2.5 g/100 g fat, 1 l carton or bag) was purchased
75 from supermarkets in Estonia (Tartu) and Latvia (Riga) once a month for one year (March 2011

76 – February 2012). All seven brands from three processors (including supermarket own labels)
77 on sale in Estonia (total sample n=84) and six processors' brands in different regions in Latvia
78 (70% of the market; total n=72) were included in the study. Samples were kept frozen (–20 °C)
79 and analysed at the milk quality laboratory of the Estonian University of Life Sciences, Tartu,
80 Estonia.

81 The milk samples were prepared and analysed for FA profiles as described by Meremäe *et al.*
82 (2012). Results for all FA were expressed as g/100 g of total FA. Estimated contributions (%)
83 of recommended daily milk consumption to the recommended or adequate intakes of FA were
84 calculated.

85

86 *Designer milk production and sampling*

87 In a trial (April – May, 2007) on a dairy farm in Estonia (300 Estonian Holstein dairy cows,
88 loose housed, milked twice per day, average milk yield 26.8 kg per cow per day) cows were
89 fed a total mixed ration (TMR) *ad libitum* in three feeding groups based on days in milk (DIM):
90 1–100, 101–250 and 251 up to end of the lactation. The TMR for all feeding groups contained
91 grass-clover (50:50) silage, barley and maize meal, heat treated rapeseed cake (crude fat 100
92 g/kg DM), cold-pressed linseed cake (crude fat 200 g/kg DM) and a mineral-vitamin mixture.
93 Concentrate to forage ratio, metabolisable energy, crude protein and crude fat contents in DM
94 in the three diets were respectively 59:41, 50:50, 34:66; 11.8 MJ/kg, 11.2 MJ/kg, 10.5MJ/kg;
95 169 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
96 bulk milk samples collected was used for the comparison.

97

98 *Prediction of methane production*

99 The equation of Van Lingen *et al.* (2014) was used for CH₄ prediction (CH₄ (g/kg of FPCM,
100 fat-protein corrected milk) = $21 \cdot 13 - 1 \cdot 38 \times C4:0 + 8 \cdot 53 \times C16:0\text{-iso} - 0 \cdot 22 \times \text{cis-9 C18:1} -$
101 $0 \cdot 59 \times \text{trans-10+11 C18:1}$; $R^2 = 0 \cdot 47$). All FA in the equation were as g/100 g FA.

102

103 *Statistical analysis*

104 The effects of country, season (summer = May – Oct. vs winter = Nov. – April) and country by
105 season interaction on the FA composition of retail milks and predicted CH₄ emissions were
106 tested using fixed effect analysis of variance (ANOVA) including also the random effect of
107 milk product brand (to consider any potential correlation between milk samples of the same
108 brand) and two replicate measures of the same sample as repeated measures. The denominator
109 degrees of freedom in ANOVA were calculated according to the Kenward-Roger method. To
110 identify common patterns in milk FA profiles, and analyse their differences, principal
111 component analysis (PCA) was performed. Additionally the FA compositions, predicted CH₄
112 emissions and estimated contributions (%) of recommended daily milk consumption to the
113 recommended or adequate intakes of FA were compared between Estonian and Latvian retail
114 milks and designer milk using the t-tests with degrees of freedom equal to number of samples
115 and followed by Bonferroni correction for multiple testing. The modelling was carried out using
116 SAS 9.4 procedure MIXED and PCA with R 3.2.3 package ade4. The results were considered
117 statistically significant at $P \leq 0.05$.

118

119

120 **Results and discussion**

121

122 *The fatty acid composition of retail milks*

123 Even though Estonia and Latvia are neighbouring countries with similar landscape and climate,
124 farming systems and feeding practices are different with grazing availability (16% and 60% of
125 herds, respectively), forage to concentrate ratio (F:C) (55:45 and 70:30, respectively), use of
126 legume-rich forage (40% of silages) and a lower proportion of maize silages in Estonia (5% vs
127 19% in Latvia). These differences were reflected in the FA composition of retail milks. The FA
128 concentrations of most milk FA, as well as FA groups, were affected by state and season, and
129 many by the interaction of state and season (online Supplementary Table S1). The effect of
130 season on the FA composition of retail milk was more pronounced than the effect of state.

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

134 (*Figures 1 near here*)

135 Relative to the overall FA pattern of Estonian retail milk, the FA pattern of Latvian retail milk
136 was shifted towards a positive correlation with PC1 (Fig. 1A), which was related to the higher
137 proportions of ruminal biohydrogenation intermediates including C18:1 *trans*-11, CLA and
138 branched-chain FA (BCFA) originating from rumen microbes. The overall summer milk FA
139 pattern was shifted towards the first and the second (C18:0, most of ruminal biohydrogenation
140 intermediates, CLA and majority of the n-3 FA, n-6 FA) quarters (Q) of the plot (Fig. 1B). The
141 same distinctive feature was present in both Estonian and Latvian summer milk reflecting the
142 higher dietary supply of PUFA, especially that of C18:3 n-3, from fresh grass compared to
143 winter diets (Dewhurst *et al.*, 2006). Compared to Estonian summer milk, the FA pattern of
144 Latvian summer milk was shifted towards the first and fourth (BCFA, C15:0 and C17:0)
145 quarters of the plot (Fig. 1C). Regarding winter milk, the FA pattern of Latvian winter milk was
146 shifted towards the higher proportions of BCFA, C15:0 and C17:0 (IVQ) compared to Estonian
147 winter milk and lower proportions of *de novo* synthesised FA (IIIQ) also FA clustered in the

148 second quarter (Fig. 1C). The higher concentrations of BCFA and linear odd-chain FA (C15:0,
149 C17:0) in Latvian winter milk are in line with a previously reported (Vlaeminck *et al.*, 2006)
150 effect of higher F:C ratio increasing the proportions of bacteria-derived FA leaving the rumen.

151

152 *Human consumption of health-related fatty acids*

153 The estimated contribution of Estonian and Latvian retail milk to the recommended or adequate
154 intakes of FA for adults (Table 1) confirmed previous suggestions (Shingfield *et al.*, 2013) that
155 milk fat is an important source for SFA (~43% of the recommended upper limit) if the
156 recommended amount (600 mL, fat content 2.5 g/100g; Tervise Arengu Instituut, 2017) is
157 consumed. Estimated contributions for desirable α -linolenic acid (ALA; C18:3 n-3) were
158 relatively low but still provided above 8% of adequate intake (1.1 g or 0.5% of energy intake).
159 Regarding the sum of long-chain n-3 PUFA (eicosapentaenoic acid (EPA; C20:5 n-3) and,
160 docosahexaenoic acid (DHA; C22:6 n-3), the latter was not detected in this study, but estimated
161 intake of long-chain n-3 PUFA was enhanced (~3.5% vs ~9.0% of 250 mg, Table 1) by
162 docosapentaenoic acid (DPA; C22:5 n-3), the concentration of which was greater than EPA
163 (online Supplementary Table S1). The function of dietary DPA remains uncertain, although
164 some reports indicate it may be beneficial to health (Howe *et al.*, 2007)

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

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

177

178 *Predicted CH₄ emissions in dairy cows*

179 Although van Lingen et al. (2014) indicated that milk FA composition has only a moderate
180 potential for CH₄ prediction per unit of milk, the method still enables to roughly assess regional
181 differences, modify feeding strategies and mitigate CH₄ emissions. Despite the dissimilarities
182 in feeding strategies, there were no differences in yearly mean CH₄ output values from dairy
183 cows (g/kg FPCM; $P = 0.51$) between the two states. The predicted CH₄ emissions were higher
184 ($P < 0.001$) during the winter period compared to the summer 12.19 vs 11.92 and 12.62 vs
185 11.65 in Estonia and Latvia, respectively. Our simulation showed notably lower enteric CH₄
186 emissions when producing designer milk (11.06; $P < 0.001$) compared to conventional
187 production (12.06 for Estonia and 12.11 for Latvia). Lower predicted CH₄ emission while
188 producing designer milk, caused by feeding oilseed (Meale *et al.*, 2013), shows that production
189 of favourable for human health designer milk with higher PUFA content has also environmental
190 advantages.

191

192

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231

232 **Table 1.** Estimated contribution (%) of recommended daily consumption (600 mL, fat content
 233 2.5 g/100g) of Estonian and Latvian retail milk and designer milk, to the recommended or
 234 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
<i>cis</i> PUFA	5-10% of energy intake ^{§, ¶}	3.62-1.81	3.39-1.69	5.53-2.76
<i>cis</i> 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

235 [†]AI, adequate intake.

236 [‡]2015–2020 Dietary Guidelines for Americans (USHHS and USDA, 2015).

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

239 [¶]Estonian nutrition and physical activity recommendations 2015 (Tervise Arengu Instituut,
 240 2017).

241 ^{††}Not used in cited recommendations.

242 ^{‡‡}Scientific opinion on dietary values for fats, including saturated fatty acids, polyunsaturated
243 fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol (EFSA, 2010).

244 ^{a,b,c} Means with different superscript letters are statistically significantly different ($P < 0.05$, t-
245 tests with degrees of freedom equal to number of samples and followed by Bonferroni
246 correction for multiple testing).

247 **Figure legend:**

248

249 **Fig. 1:**

250 The patterns of FA concentrations described by the first and the second principal component:

251 (A) fatty acid patterns by national state, (B) fatty acid patterns by season, (C) fatty acid patterns

252 by national state and season. In all figures, also the location of designer milk samples is

253 presented. The factor loadings showing the relative importance of fatty acids in first two

254 principal components are presented in Supplementary Figure S1.

