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 (CH_4) emissions by cattle as a result of rumen fermentation. The moderate relationship between

59 milk FA profile and CH_4 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 CH_4 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 CH_4 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 CH_4 prediction (CH_4 (g/kg of FPCM,

100 fat-protein corrected milk) = $21 \cdot 13 - 1 \cdot 38 \times C4:0 + 8 \cdot 53 \times C16:0$-iso $- 0 \cdot 22 \times$ cis-9 C18:1 $-$

101 $0 \cdot 59 \times$ 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 CH_4 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 CH_4

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 CH_4 emissions in dairy cows*

179   Although van Lingen et al. (2014) indicated that milk FA composition has only a moderate

180   potential for CH_4 prediction per unit of milk, the method still enables to roughly assess regional

181   differences, modify feeding strategies and mitigate CH_4 emissions. Despite the dissimilarities

182   in feeding strategies, there were no differences in yearly mean CH_4 output values from dairy

183   cows (g/kg FPCM; $P = 0·51$) between the two states. The predicted CH_4 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 CH_4

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 CH_4 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

196   **References**

197

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231

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.

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.

255   **Fig. 1**

256