

**Focusing on fatty acids profile in milk from different species after *in vitro* digestion**

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**SUPPLEMENTARY FILE**

**santillo4972**

## Supplementary Materials & Methods

A calibration curve with a standard mixture of 50 fatty acids (GLC Reference standard 674, Nu-Check Prep, Inc. Elysian MN 56028, USA GLC standard Reference 674, Nu-Check Prep, Inc., Elysian MN 560, USA) and a standard of 12 conjugated linoleic acids (CLA) (C18:2 trans-8, cis-10; C18:2 cis-9, trans-11; C18:2 cis-11, trans-13; C18:2 trans-9, cis-11; C18:2 cis-8, cis-10; C18:2 cis-10, cis-12; C18:2 cis-9, cis-11; C18:2 trans-10, cis-12; C18:2 trans-8, trans-10; C18:2 trans-9, trans-11; C18:2 trans-10, trans-12; C18:2 trans-11, trans-13; GLC Reference standard UC-59M, Nu-Check Prep, Inc. Elysian MN 56028, USA) was used for quantitative determination of FAME. The fatty acids were separated using a capillary column (HP88; 100 m x 0.25mm i.d., 0.20  $\mu$  m film thickness, Agilent Technologies Inc., Santa Clara, USA) installed on an Agilent Technologies 6890N GC equipped with a flame ionization detector (FID) and a split injector. The temperature of the injector and FID detector was 250°C, while the temperature ramp of the column was the following (Eulitz et al., 1999): 70° C for 4 min, from 70° C to 175° C (13° C/min), maintained at 175° C for 27 min, from 175° C to 215° C (4° C/min), maintained at 215° C for 45 min. The injection was performed in split mode 1:20 injecting 1  $\mu$  L. The pressure of the carrier gas (helium) was maintained constant at 175 kPa.

Milk source<sup>1</sup>

Fatty Acids <sup>2</sup>	FM	HM	DM	BM	OM	CM	SEM	Effect, P <sup>3</sup>
C4:0	1.06 bcd	0.76 cd	n.d.	4.25 a	1.37 bc	2.09 b	0.17	***
C6:0	0.15 d	0.34 cd	0.56 c	4.15 a	2.81 b	3.06 b	0.05	***
C8:0	0.79 d	0.33 d	4.64 a	2.36 c	2.46 c	3.30 b	0.05	***
C10:0	0.82 f	2.22 e	7.91 b	5.11 d	6.73 c	11.40 a	0.07	***
C11:0	0.02 b	0.03 b	n.d.	0.11 a	n.d.	n.d.	0.002	***
C12:0	9.34 a	7.84 b	5.62 cd	5.21 d	4.17 e	6.34 c	0.07	***
C14:0	3.61 d	7.24 c	3.68 d	14.02 a	12.29 b	11.93 b	0.14	***
C14:1	n.d.	n.d.	0.01 b	0.71 a	0.64 a	0.01 b	0.01	***
C15:0	n.d.	0.10 b	n.d.	n.d.	n.d.	0.56 a	0.01	***
C15:1	0.01 b	0.02 a	n.d.	n.d.	n.d.	n.d.	0.001	***
C16:0	16.40 cd	21.82 bc	13.35 d	31.60 a	26.00 b	32.07 a	0.55	***
C16:1	0.16 e	3.08 a	1.54 c	1.82 b	0.55 d	0.38 de	0.03	***
C17:0	0.01 e	0.15 d	0.03 e	0.50 b	0.74 a	0.38 c	0.01	***
C17:1	0.01	n.d.	0.01	0.01	n.d.	n.d.	0.001	**
C18:0	1.95 d	2.05 d	0.17 e	6.41 b	8.29 a	5.98 c	0.02	***
C18:1trans-9	0.02 cd	0.05 cd	0.16 bc	0.28 ab	0.02 d	0.31 a	0.02	***
C18:1trans-11	0.18 c	0.17 c	n.d.	1.45 b	2.60 a	0.13 c	0.01	***
C18:1cis-9	40.77 a	33.63 b	15.68 e	17.74 d	23.28 c	17.93 d	0.20	***
C18:2trans-9,trans-12	n.d.	n.d.	0.14 b	0.02 d	0.60 a	0.06 c	0.003	***
C18:2cis-9,cis-12	20.26 a	14.84 b	9.73 c	1.34 d	4.16 d	2.22 d	0.43	***
C20:0	0.11 b	0.06 b	1.58 a	0.09 b	0.22 b	0.09 b	0.03	***
C18:3n-6	0.14 b	0.50 b	1.60 a	0.05 b	0.08 b	0.08 b	0.07	***
C18:3n-3	2.60 b	0.82 c	11.18 a	0.97 bc	1.06 bc	0.41 c	0.16	***
C20:1	0.13 ab	0.13 ab	0.29 a	0.06 b	0.05 b	0.05 b	0.02	**
CLA cis-9, trans-11	0.02 e	0.12 d	n.d.	0.95 b	1.28 a	0.45 c	0.01	***
CLA trans-10,cis-12	n.d.	0.01	n.d.	0.01	0.01	n.d.	0.002	NS
CLA cis-9,cis-11	0.04	0.04	n.d.	0.10	0.03	0.02	0.01	NS
C21:0	0.08 a	0.01 b	n.d.	0.01 b	n.d.	0.01 b	0.004	**
CLA trans-9, trans-11	0.03 b	0.10 b	1.53 a	0.05 b	0.01 b	0.08 b	0.04	***
C20:2n-6	0.25 b	0.14 b	0.85 a	0.04 b	0.05 b	0.05 b	0.04	***
C22:0	0.02 b	0.03 b	0.22 a	0.02 b	0.04 b	0.02 b	0.01	***
C20:3n-6	0.01 b	0.53 a	0.26 b	n.d.	n.d.	0.01 b	0.02	***
C22:1	n.d.	0.10 b	2.08 a	0.11 b	n.d.	0.17 b	0.04	***
C20:4n-6	0.10 b	1.06 a	0.08 b	0.02 b	n.d.	0.01 b	0.03	***
C20:3n-3	0.11 b	0.04 b	0.88 a	0.13 b	0.25 b	0.04 b	0.04	***
C23:0	0.06	0.01	0.11	0.04	n.d.	n.d.	0.02	NS
C22:2n-6	0.16 b	0.12 b	3.22 a	0.05 b	0.05 b	0.09 b	0.08	***
C20:5n-3	0.08 ab	0.22 a	n.d.	0.17 a	0.10 ab	0.11 ab	0.02	*
C24:0	0.06 b	0.18 b	0.79 a	n.d.	0.03 b	0.02 b	0.02	***
C24:1	0.10 b	0.04 b	12.12 a	0.01 b	0.01 b	0.12 b	0.72	*
C22:6n-3	0.34 b	1.08 a	n.d.	0.01 c	0.01 c	0.01 c	0.01	***
Ar.I.	0.61 e	1.03 d	0.55 e	3.55 b	2.27 c	3.78 a	0.02	***
T.I.	0.54 e	0.92 d	0.28 f	3.11 b	2.19 c	3.84 a	0.03	***

Supplementary File Table S1 legend.

Table S1. Effect of milk source on fatty acid composition (% of FAME).

<sup>1</sup>FM = formula milk; HM = human milk; DM = donkey milk; BM = bovine milk; OM = ovine milk; CM = caprine milk.

<sup>2</sup> n.d. = not detectable for value < 0.01; Ar.I. = atherogenic index; T.I. = thrombogenic index.

<sup>3</sup> NS,  $P < 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

Free Fatty Acids <sup>2</sup>	Milk source <sup>1</sup>						SEM	Effect, P <sup>3</sup>
	FM	HM	DM	BM	OM	CM		
C6:0	4.91 ab	0.43 ab	n.d. b	6.99 ab	9.44 a	n.d. b	1.21	*
C8:0	4.85	3.50	4.93	6.69	12.22	5.17	0.91	NS
C10:0	7.11	10.72	5.99	12.03	15.61	11.47	1.79	NS
C12:0	15.02 b	21.68 a	3.36 c	7.03 c	6.15 c	7.82 c	1.40	**
C14:0	4.93 b	11.00 a	5.20 b	9.85 ab	8.88 ab	11.32 a	0.90	**
C16:0	73.47 c	111.74 b	131.46 a	117.14 b	99.93 b	108.96 b	3.07	***
C16:1	n.d.	3.14 a	n.d.	0.42 b	n.d.	0.48 b	0.15	***
C18:0	44.78 c	66.13 b	89.56 a	69.30 b	62.32 b	56.84 bc	2.87	***
C18:1trans-11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	
C18:1cis-9	6.90 b	20.97 a	2.60 b	3.94 b	3.74 b	3.18 b	1.40	***
C18:2cis-9,cis-12	0.43 b	1.04 a	0.16 b	0.14 b	0.29 b	0.14 b	0.08	***
C20:0	5.06	3.26	n.d.	n.d.	3.57	n.d.	0.76	NS
C18:3n-6	0.80	0.79	n.d.	n.d.	n.d.	n.d.	0.17	NS
C18:3n-3	n.d.	1.90	5.87	2.90	1.96	5.90	1.03	NS
C20:1	1.62	1.57	n.d.	n.d.	1.62	n.d.	0.80	NS
C20:2n-6	4.76 b	5.00 ab	n.d.	1.47 b	7.19 ab	9.17 a	1.21	*
C22:0	1.61 a	1.66 a	n.d.	0.86 ab	0.67 b	1.34 ab	0.17	**
C20:3n-6	1.05	1.26	n.d.	1.06	0.75	0.75	0.21	NS
C22:1	3.64	1.40	n.d.	0.29	0.68	1.21	0.94	NS
C20:4n-6	8.79	4.08	n.d.	4.42	4.78	1.95	2.64	NS
C20:3n-3	0.38	0.67	n.d.	n.d.	n.d.	1.91	1.17	NS
C22:2n-6	2.10	0.00	n.d.	n.d.	3.93	2.82	0.57	NS
C20:5n-3	0.00	0.89	n.d.	n.d.	0.55	n.d.	0.17	NS
C24:0	2.08	1.55	n.d.	n.d.	n.d.	n.d.	0.65	NS
C24:1	0.16 b	7.93 a	n.d.	n.d.	5.69 a	5.56 a	0.69	*
C22:6n-3	0.08	0.36	n.d.	n.d.	0.12	0.30	0.09	NS

Supplementary File Table S2 legend.

Table S2. Effect of milk source in free fatty acids of digested milk ( $\mu\text{g/mL}$  of extract).

<sup>1</sup>FM = formula milk; HM = human milk; DM = donkey milk; BM = bovine milk; OM = ovine milk; CM = caprine milk.

<sup>2</sup> n.d. = not detectable for value  $< 0.01$ .

<sup>3</sup> NS,  $P < 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$