

Essential and toxic minerals content and fatty acid profile of colostrum in dairy sheep

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Supplementary Material

Table S1. Microwave-assisted procedure of mineralization of sheep's colostrum and milk samples.

Content of each vessel	Sample weight: ca. 1 g (exactly weighted, uncertainty: 0.0001 g); HNO ₃ 67%: 2 cm ³ ; H ₂ O ₂ 30%: 2 cm ³ ; ultrapure water: 7 cm ³
Heating step	From room temperature to 200°C in 15 minutes, power: 1800W
Standing step	200°C for 15 minutes, power: 1800W
Cooling step	From 200°C to room temperature in 15 minutes.

Table S2. ICP-MS instrumental settings for the determination of oligoelements and toxic element in sheep's colostrum and milk.

RF power generator (W)	1300	KED gas	Helium, 99.999%
Ar plasma flow (dm³ min⁻¹)	17.995	Masses of optimization	⁷ Li, ⁸⁹ Y and ²⁰⁵ Tl
Ar auxiliary flow (dm³ min⁻¹)	1.203	Dwell time (ms)	50
Ar nebulizer flow (dm³ min⁻¹)	0.991	Number of points per peak	3
Nebulizer	Meinhard®, glass	Acquisition time (s)	3
Spray chamber	Cyclonic, glass	Quantification	External calibration
Skimmer and sampling cones	Nickel	KED gas flow (cm³ min⁻¹)	3.5
Sampling depth (mm)	0	Masses of optimization	⁷ Li, ⁸⁹ Y and ²⁰⁵ Tl

Element	Quantification isotopic ion (% elemental abundance)	Interferents	Analysing mode	Correction equation
Cd	¹¹¹ Cd ⁺ (12.80)	⁹⁵ Mo ¹⁶ O ⁺	Normal	
Cu	⁶³ Cu ⁺ (69.17)	³¹ P ¹⁶ O ₂ ⁺ ; ⁴⁷ Ti ¹⁶ O ⁺ ; ¹²⁶ Te ²⁺	Normal	
Mn	⁵⁵ Mn ⁺ (100)	⁴⁰ Ar ¹⁴ N ¹ H ⁺ ; ¹ H ³⁷ Cl ¹⁷ O ⁺ ; ³⁷ Cl ¹⁸ O ⁺	Normal	
Ni	⁶⁰ Ni ⁺ (26.22)	⁴⁴ Ca ¹⁶ O ⁺	Normal	
Pb	²⁰⁸ Pb ⁺ (52.40)		Normal	

Se	$^{82}\text{Se}^+$ (8.73)	$^{82}\text{Kr}^+$; $^{81}\text{Br}^1\text{H}^+$; $^{40}\text{Ar}_2\text{H}^+$; Ho^{2+} ; Gd^{2+} ; Er^{2+}	KED	$-0.00783x^{83}\text{Kr}$
Zn	$^{66}\text{Zn}^+$ (27.90)	$^{50}\text{Tl}^{16}\text{O}^+$; $^{50}\text{V}^{16}\text{O}^+$; $^{34}\text{S}^{16}\text{O}_2^+$; $^{132}\text{Ba}^{2+}$	Normal	

Table S3. Validation parameters for the determination of oligoelements and toxic element in sheep's colostrum and milk.

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Element	Sensitivity		Linearity		Correlation coefficient R ²	Precision		Recovery (%)	CRM Certified concentration (mg kg ⁻¹)
	LoD (µg dm ⁻³)	LoQ (µg dm ⁻³)	Range (µg kg ⁻¹)	Regression line		Repeatability ^a (CV)	Intermediate precision ^b (CV)		
Cd	0.0002	0.0007	0.04-40	$y = (4920 \pm 10)x + (491 \pm 20)$	1	4.1 ^c 4.2 ^d	18 ^c 8.3 ^d	110 ^c 98 ^d	<i>0.002</i> ^c 0.106 ^d
Cu	0.014	0.048	0.2-200	$y = (20800 \pm 100)x + (8190 \pm 40)$	1	9.7 ^c 5.0 ^d	7.7 ^c 2.7 ^d	105 ^c 90 ^d	4.3 ^c 5.0 ^d
Mn	0.005	0.018	0.15-150	$y = (68570 \pm 200)x + (14270 \pm 150)$	1	3.0 ^c 8.0 ^d	3.6 ^c 6.9 ^d	107 ^c 102 ^d	<i>1.04</i> ^c 0.29 ^d
Ni	0.0008	0.0027	0.25-250	$y = (10490 \pm 300)x - (4610 \pm 100)$	1	7.1 ^c 8.0 ^d	8.4 ^c 9.0 ^d	10 ^c 107 ^d	<i>0.011</i> ^c 0.28 ^d
Pb	0.001	0.003	0.09-90	$y = (27720 \pm 250)x - (5510 \pm 200)$	0.9999	3.9 ^c 2.7 ^d	14 ^c 3.6 ^d	113 ^c 91 ^d	<i>0.12</i> ^c 0.207 ^d
Se	0.0008	0.0026	0.1-100	$y = (25 \pm 5)x + (20 \pm 1)$	0.9999	3.7 ^c 4.6 ^d	4.2 ^c 11 ^d	100 ^c 95 ^d	0.24 ^c 0.19 ^d
Zn	0.006	0.021	0.35-350	$y = (5310 \pm 50)x + (19260 \pm 400)$	1	4.4 ^c 2.3 ^d	2.5 ^c 5.6 ^d	97 ^c 95 ^d	13 ^c 44.9 ^d

LoD, limit of detection; LoQ, limit of quantification; CV, coefficient of variation; ^a parameter evaluated repeating the analysis of a CRM for five times within the same analytical session; ^b parameter evaluated repeating the analysis of a CRM on five different analytical sessions performed within one month; ^c measured on IAEA-A-13 certified reference sample, ^d measured on ERM-BD151 certified reference sample; CRM concentration in *italics* is a not certified data; n = 5.

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Table S4. Average concentration (% on total amount of FAs) of all FAs measured in colostrum of each of the eight farms

	Farms								P value	SEM
	A	B	C	D	E	F	G	H		
Fat, %	5.3 c	7.6 abc	7.7 abc	9.6 ab	6.9 abc	5.5 bc	9.1 abc	10.2 a	0.0013	0.3575
FA (% on Total FAs)										
<i>Short chain FA</i>										
C4:0	1.93 ab	1.96 ab	1.70 b	1.83 bc	2.22 a	1.99 ab	1.66 b	1.60 b	0.0041	0.0427
C6:0	0.69 b	0.84 b	0.73 b	0.67 b	1.13 a	0.60 b	0.71 b	0.58 b	<0.0001	0.0270
C7:0	0.04 bc	0.04 bc	0.03 c	0.05 bc	0.05 bc	0.12 a	0.05 b	0.04 c	<0.0001	0.0027
C8:0	0.49 b	0.66 b	0.57 b	0.50 b	0.92 a	0.39 b	0.53 b	0.43 b	<0.0001	0.0259
C9:0	0.08 b	0.10 b	0.08 b	0.10 b	0.11 b	0.22 a	0.10 b	0.08 b	<0.0001	0.0053
C10:0	1.54 bc	2.14 b	1.78 bc	1.55 bc	3.04 a	1.23 bc	1.68 bc	1.28 c	<0.0001	0.0885
C10:1	0.07 ab	0.06 abc	0.06 abc	0.07 ab	0.08 ab	0.03 c	0.05 bc	0.08 a	<0.0001	0.0028
<i>Medium chain FA</i>										
C11:0	0.11 bc	0.14 b	0.11 bc	0.12 bc	0.22 a	0.09 bc	0.10 bc	0.09 c	<0.0001	0.0063
C12:0	1.98 b	2.12 b	2.01 b	2.13 b	2.74 a	1.72 b	1.93 b	1.77 b	<0.0001	0.0509
isoC13:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.4431	0.0004
anteisoC13:0	0.03 b	0.03 ab	0.03 b	0.04 ab	0.05 a	0.03 b	0.03 b	0.03 b	0.0003	0.0013
isoC14:0	0.06 ab	0.06 ab	0.06 ab	0.05 ab	0.05 ab	0.05 ab	0.07 a	0.04 b	0.0352	0.0018
C14:0	11.01 bc	11.75 ab	9.96 bc	11.78 ab	14.35 a	10.62 bc	8.86 c	9.19 bc	<0.0001	0.2857
isoC15:0	0.19	0.19	0.16	0.17	0.16	0.20	0.19	0.17	0.0188	0.0038
anteisoC15:0	0.20	0.23	0.17	0.20	0.22	0.21	0.22	0.17	0.0561	0.0055
C14:1c9	0.39 b	0.47 ab	0.26 b	0.48 ab	0.73 a	0.42 b	0.23 b	0.31 b	<0.0001	0.0258
C15:0	0.55 bc	0.64 ab	0.52 bc	0.52 bc	0.72 a	0.49 bc	0.61 abc	0.47 c	<0.0001	0.0150
C15:1	0.03 b	0.03 b	0.03 b	0.02 b	0.05 a	0.03 b	0.03 b	0.03 b	<0.0001	0.0013
isoC16:0	0.20 ab	0.21 ab	0.19 ab	0.19 ab	0.19 ab	0.21 ab	0.22 a	0.17 b	0.0473	0.0040
C16:0	27.19 bc	31.47 b	25.67 c	29.11 bc	37.12 a	27.92 bc	25.56 c	25.84 c	<0.0001	0.5818
isoC17:0	0.42 bc	0.40 cd	0.41 bcd	0.39 cd	0.34 d	0.49 ab	0.50 a	0.43 abc	<0.0001	0.0078
anteisoC17:0	0.46 b	0.45 bc	0.44 bc	0.45 bc	0.36 c	0.51 ab	0.57 a	0.51 ab	<0.0001	0.0097
C16:1c9	1.38 b	1.85 ab	1.08 b	1.78 ab	2.63 a	1.72 ab	1.08 b	1.24 b	<0.0001	0.0872
C17:0	0.81 b	0.86 ab	0.83 b	0.78 b	0.68 b	0.84 ab	1.03 a	0.87 ab	0.0003	0.0185
isoC18:0	0.11 bc	0.12 ab	0.12 bc	0.12 bc	0.08 c	0.12 abc	0.15 a	0.13 ab	<0.0001	0.0035
C17:1c9	0.43 bc	0.43 bc	0.46 ab	0.45 abc	0.33 c	0.48 ab	0.54 a	0.48 ab	<0.0001	0.0107
<i>Long chain FA</i>										
C18:0	7.51 ab	7.29 ab	8.06 a	7.19 ab	5.02 b	6.64 ab	7.78 a	8.18 a	0.0130	0.2206
C18:1t9	0.22 ab	0.17 c	0.26 a	0.20 bc	0.15 c	0.21 abc	0.23 ab	0.24 ab	<0.0001	0.0057
C18:1t10	0.24	0.23	0.34	0.20	0.30	0.20	0.29	0.25	0.4630	0.0183
C18:1t11	0.81 a	0.59 ab	0.78 ab	0.56 ab	0.49 b	0.82 ab	0.71 ab	0.57 ab	0.0068	0.0276
C18:1t12	0.27 ab	0.16 c	0.31 a	0.20 bc	0.18 c	0.24 abc	0.27 ab	0.23 abc	<0.0001	0.0083
C18:1t13:t14	0.36 a	0.25 ab	0.30 ab	0.29 ab	0.34 ab	0.33 ab	0.28 ab	0.22 b	0.0350	0.0118
C18:1c9	30.52 ab	25.99 b	32.68 a	29.40 ab	17.18 c	31.26 ab	33.35 a	35.76 a	<0.0001	0.7795
C18:2n6 (LA)	2.24 b	2.21 b	2.92 ab	2.43 b	2.34 b	2.08 b	3.24 b	2.70 ab	0.0002	0.0744
C20:0	0.23 ab	0.22 ab	0.22 ab	0.19 bc	0.17 c	0.26 a	0.26 a	0.19 bc	<0.0001	0.0051
C18:3n6	0.04 b	0.05 a	0.05 ab	0.04 ab	0.05 ab	0.04 b	0.05 ab	0.05 ab	0.0107	0.0014
C20:1c9	0.04 a	0.03 abc	0.03 abc	0.03 bc	0.02 c	0.03 abc	0.03 ab	0.02 c	0.0001	0.0009
C18:3n3 (ALA)	0.61 a	0.46 bcd	0.33 de	0.37 cd	0.41 bcd	0.56 ab	0.49 abc	0.21 e	<0.0001	0.0170
CLAc9t11	0.89 a	0.56 cd	0.81 ab	0.61 bcd	0.47 d	0.92 a	0.71 abc	0.58 cd	<0.0001	0.0239
CLAt10c12	0.03 ab	0.03 bc	0.03 bc	0.02 cd	0.02 cd	0.04 a	0.03 cd	0.02 d	<0.0001	0.0009
CLAt12t14	0.02 a	0.01 c	0.01 abc	0.01 bc	0.01 bc	0.02 a	0.01 ab	0.01 c	<0.0001	0.0005
CLAt11t13	0.03 ab	0.04 a	0.03 ab	0.03 ab	0.03 ab	0.04 ab	0.03 b	0.03 b	0.011	0.0009
CLAt9t11	0.02 a	0.02 ab	0.02 b	0.02 b	0.02 ab	0.02 ab	0.02 b	0.01 b	<0.0001	0.0007
C18:4n3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.3227	0.0003

C20:2n6	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.2705	0.0007
C20:3n9	0.07	0.07	0.06	0.08	0.06	0.08	0.07	0.06	0.0284	0.0016
C22:0	0.07 ^{ab}	0.07 ^{ab}	0.06 ^{ab}	0.05 ^b	0.06 ^{ab}	0.07 ^{ab}	0.09 ^a	0.05 ^b	0.0013	0.0027
C20:3n6	0.03 ^b	0.03 ^{ab}	0.04 ^a	0.04 ^{ab}	0.04 ^{ab}	0.03 ^b	0.03 ^{ab}	0.04 ^{ab}	0.0009	0.0008
C20:4n6 (ARA)	0.26 ^c	0.27 ^{bc}	0.33 ^{ab}	0.32 ^{abc}	0.28 ^{bc}	0.24 ^c	0.39 ^a	0.37 ^a	<0.0001	0.0081
C23:0	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.01	0.0579	0.0013
EPA	0.10 ^a	0.08 ^b	0.07 ^b	0.07 ^{bc}	0.07 ^b	0.08 ^{ab}	0.07 ^b	0.05 ^c	<0.0001	0.0023
DPA	0.24 ^a	0.16 ^{bc}	0.18 ^{bc}	0.14 ^{cd}	0.16 ^{bcd}	0.20 ^{ab}	0.20 ^{ab}	0.11 ^d	<0.0001	0.0059
DHA	0.09 ^a	0.06 ^b	0.06 ^b	0.05 ^{bc}	0.06 ^b	0.06 ^{ab}	0.06 ^b	0.03 ^c	<0.0001	0.0024
Groups of FA										
SCFA	4.84 ^{bc}	5.80 ^b	4.96 ^{bc}	4.77 ^{bc}	7.55 ^a	4.59 ^{bc}	4.79 ^{bc}	4.09 ^c	<0.0001	0.1653
MCFA	46.47 ^{bc}	52.23 ^b	43.35 ^c	49.58 ^{bc}	61.79 ^a	47.02 ^{bc}	42.77 ^c	42.73 ^c	<0.0001	0.9431
LCFA	48.69 ^{ab}	41.97 ^b	51.68 ^a	45.65 ^{ab}	30.66 ^c	48.40 ^{ab}	52.44 ^a	53.18 ^a	<0.0001	1.0364
SFA	56.17 ^{bc}	62.25 ^b	54.15 ^c	58.41 ^{bc}	70.21 ^a	55.27 ^{bc}	53.16 ^c	52.50 ^c	<0.0001	0.8262
MUFA	37.62 ^{ab}	32.72 ^b	39.57 ^a	36.27 ^{ab}	24.82 ^c	38.73 ^{ab}	40.07 ^a	42.17 ^a	<0.0001	0.7501
PUFA	6.19 ^{ab}	5.01 ^b	6.27 ^{ab}	5.31 ^b	4.95 ^b	5.99 ^{ab}	6.75 ^a	5.32 ^b	<0.0001	0.1261
UFA	43.81 ^{ab}	37.74 ^b	45.83 ^a	41.58 ^{ab}	29.78 ^c	44.72 ^{ab}	46.82 ^a	47.49 ^a	<0.0001	0.8264
OCFA	1.62 ^{bc}	1.82 ^{ab}	1.60 ^{bc}	1.60 ^{bc}	1.82 ^{abc}	1.80 ^{abc}	1.94 ^a	1.56 ^c	<0.0001	0.0240
BCFA	1.69 ^{bc}	1.71 ^{bc}	1.59 ^{bc}	1.62 ^{bc}	1.47 ^c	1.83 ^{ab}	1.97 ^a	1.66 ^{bc}	<0.0001	0.0253
OBCFA	3.32 ^b	3.53 ^{ab}	3.19 ^b	3.22 ^b	3.28 ^b	3.63 ^{ab}	3.91 ^a	3.22 ^b	<0.0001	0.0434
PUFA6	2.67 ^{bc}	2.67 ^{bc}	3.45 ^{ab}	2.95 ^{bc}	2.81 ^{bc}	2.47 ^c	3.83 ^a	3.28 ^{abc}	<0.0001	0.0818
PUFA3	1.07 ^a	0.80 ^{bcd}	0.67 ^{cd}	0.64 ^d	0.74 ^{bcd}	0.94 ^{ab}	0.84 ^{bc}	0.42 ^e	<0.0001	0.0257
n6/n3	2.50 ^e	3.31 ^{cd}	5.20 ^b	4.61 ^b	3.77 ^c	2.63 ^{de}	4.68 ^b	7.76 ^a	<0.0001	0.1847
n3/n6	0.40 ^a	0.31 ^b	0.19 ^d	0.22 ^{cd}	0.27 ^{bc}	0.38 ^a	0.22 ^d	0.13 ^e	<0.0001	0.0104
CLA	1.05 ^a	0.70 ^{cd}	0.94 ^{ab}	0.73 ^{bcd}	0.60 ^d	1.09 ^a	0.84 ^{abc}	0.68 ^{cd}	<0.0001	0.0255
TFA	4.01 ^a	2.74 ^d	3.94 ^{ab}	2.97 ^{bcd}	2.77 ^{cd}	3.90 ^{abc}	3.63 ^{abcd}	3.07 ^{abcd}	<0.0001	0.0980
TFA (without VA)	3.20 ^a	2.15 ^c	3.16 ^a	2.41 ^{bc}	2.28 ^{bc}	3.08 ^{ab}	2.92 ^{ab}	2.50 ^{abc}	<0.0001	0.0724
AI	1.70 ^{bc}	2.22 ^b	1.52 ^{bc}	2.01 ^{bc}	3.45 ^a	1.69 ^{bc}	1.35 ^c	1.39 ^c	<0.0001	0.0945
TI	1.63 ^{bc}	2.20 ^b	1.55 ^{bc}	2.00 ^{bc}	3.33 ^a	1.68 ^{bc}	1.41 ^c	1.48 ^{bc}	<0.0001	0.0883
h/H	0.94 ^{ab}	0.72 ^{bc}	1.08 ^c	0.87 ^{ab}	0.44 ^c	0.97 ^{ab}	1.13 ^a	1.17 ^a	<0.0001	0.0357
DI C10:1	4.23 ^{ab}	3.12 ^b	3.67 ^b	4.26 ^{ab}	2.54 ^b	2.69 ^b	2.80 ^b	5.96 ^a	<0.0001	0.1812
DI C14:1	3.25 ^{ab}	3.75 ^{ab}	2.52 ^b	3.78 ^{ab}	4.62 ^a	3.48 ^{ab}	2.51 ^b	3.10 ^b	0.0001	0.1279
DI C16:1	4.79 ^{ab}	5.41 ^{ab}	4.01 ^b	5.46 ^{ab}	6.42 ^a	5.48 ^{ab}	4.03 ^b	4.54 ^b	0.0004	0.1554
DI C18:1	80.43	77.93	79.94	80.00	77.95	82.69	81.06	81.28	0.2057	0.4639
DI CLA	52.81	49.25	50.85	52.43	51.59	54.18	49.96	51.32	0.7001	0.6582

ΣFAs = sum of all FAs; FAME = fatty acid methyl ester; SA = stearic acid; LA = linoleic acid; ALA =

linolenic acid; ARA=arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic

acid; DHA = docosahexaenoic acid. SFA = sum of the individual saturated fatty acids; UFA = sum of the individual unsaturated fatty

acids; MUFA = sum of the individual monounsaturated fatty acids; PUFA = sum of the individual

polyunsaturated fatty acids; OCFA = odd-chain fatty acids; BCFA = branched-chain fatty acids, sum of iso- and anteiso-FA; OBCFA

= odd- and branched-chain fatty acids, sum of odd-, iso-, and anteiso-FA; SCFA, short-chain fatty acids (sum of individual fatty acids

from C4:0 to C10:0); MCFA = medium-chain fatty acids, sum of the individual fatty acids from C11:0 to C17:0; LCFA = long-chain

fatty acids, sum of the individual fatty acids from C18:0 to DHA; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids,

respectively; CLA = sum of individual conjugated linoleic acids; TI = thrombogenic index; AI = atherogenic index; h:H =

hypcholesterolemic to hypercholesterolemic ratio.

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