Sustainable intensification? Increased production diminishes omega-3 content of sheep milk

Article

Supplemental Material

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Table S1. monthly temperature and rainfall during the study. (mean values ±sem).

	December	January	February	March	April	May	June	July	August
					Month				
					Variable[1]				
					Temperature (°C)				
Year 1	16.2 ±2.0	15.7 ±2.9	11.2 ±2.7	14.7 ±2.0	14.1 ±2.2	<u>16.3 ±2.0</u>	17.3 ±5.2	23.7 ±2.7	25.2 ±2.5
Year 2	16.2 ±3.6	12.9 ±4.1	11.2 ±2.5	12.2 ±2.8	14.6 ±2.6	<u>18.6 ±2.7</u>	22.0 ±2.7	24.4 ±1.8	26.0 ±1.2
					Rainfall (mm)				
Year 1	4.1 ±1.4	2.1 ±1.4	5.7 ±3.4	1.0 ±0.8	0.4 ±0.5	0.3 ±0.1	0.9 ±0.9	0.0 ±0.0	0.0 ±0.0
Year 2	2.7 ±1.8	3.8 ±1.6	5.0 ±1.7	4.7 ±2.9	1.7 ±0.7	1.3 ±0.7	0.8 ±0.6	1.4 ±5.7	0.0 ±0.0

[1]Average values from onsite records

Table S2. Grazing regimes, supplementary concentrate, and conserved forages used in different management systems (production intensity),by sampling months and year. (mean values ±sem).

Factors	grazing time on pasture: hours/day		Concentrate feeds g/ewe/day	conserved forage used: g/ewe /day		
	natural	improved		total	oat	alfalfa
Management System						
Semi-intensive	3.5 ±0.4	2.6 ±0.1	934 ±31	183 ±21	68 ±13	115 ±17
Extensive	6.6 ±0.4	0.4 ±0.1	712 ±40	267 ±29	25 ±9	242 ±28
Sampling Months						
January	2.0 ±0.4[e]	1.2 ±0.2[cd]	1036 ±40[a]	473 ±37[a]	126 ±27[a]	347 ±45[a]
February	2.4 ±0.5[de]	1.7 ±0.3[abc]	1017 ±42[a]	423 ±4 [a]	104 ±26[a]	319 ±45[ab]
March	3.4 ±0.5[d]	2.2 ±0.3 [a]	942 ±52[a]	263 ±39[b]	23 ±17[b]	241 ±38[b]
April	5.5 ±0.6[c]	2.0 ±0.3[ab]	738 ±61[b]	103 ±26[c]	21 ±13[b]	82 ±24[c]
May	7.7 ±0.6[b]	1.3 ±0.3[bcd]	584 ±68[c]	58 ±34[d]	0 ±0[b]	58 ±34[c]
June	9.6 ±0.6[a]	0.6 ±0.2[d]	551 ±66[c]	10 ±7[e]	3 ±3[b]	7 ±7[c]
Year						
First	4.6 ±0.4	1.5 ±0.2	952 ±34	290 ±26	79 ±14	212 ±25
Second	5.5 ±0.4	1.5 ±0.2	698 ±36	159 ±23	14 ±6	144 ±22
ANOVA (*P*-values)						
Main Effects						
Management systems (MS)	***	***	***	**	***	***
Sampling Months (SM)	***	***	***	***	***	***
Year (Y)	**	ns	***	***	***	**
Interactions						
MS x SM	***	*	***[1]	ns	** [1]	**[1]
MS x Y	ns	ns	ns	ns	* [2]	ns
SM x Y	ns	ns	ns	ns	***[3]	* [3]
MS x SM x Y	ns	ns	ns	ns	*	ns

[a-e] Means within a column with different superscripts are significantly different(*P*-values < 0.05).

[1] see **Figure1** for interaction means/SE

[2] **Table S4** reports interaction means/SE.

[3] **Table S5** reports interaction means/SE.

Table S3. Interaction means±SEs for milk lactose content for the management systems and sampling months.

Sampling Months	January	February	March	April	May	June
Factors						
			lactose (g/100ml milk)			
semi-intensive system	4.77±0.04A[ab]	4.79±0.03A[ab]	4.72±0.07A[b]	4.82±0.05A[ab]	4.91±0.04A[a]	4.85±0.04A[ab]
extensive system	4.86±0.05A[a]	4.83±0.05A[a]	4.78±0.06A[ab]	4.74±0.08A[ab]	4.65±0.05B[b]	4.25±0.15B[c]

[a-c] Means within a row with different superscripts are significantly different(*P*-values < 0.05).
A-B Means within a column with different superscripts are significantly different (*P*-values < 0.05).

Table S4. Interaction means/SEs for total PUFA, omega-3 PUFA, lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1 *cis 9*), a-Linolenic acid (C18:3*cis9.cis12.cis15*), and eicosapentaenoic acid (EPA) in milk fat from two management systems and two years.

	Year 1		Year 2	
Parameter assessed	Semi intensive	Extensive	Semi intensive	Extensive
Wholecrop oat (g/ewe/day)	112 ±22[a]	45 ±16[b]	24 ±10[b]	4 ±4[b]
		FA (g/100g total FA)		
PUFA	5.13 ±0.11[c]	5.00 ±0.13[c]	6.33 ±0.09[b]	6.69 ±0.19[a]
n-3PUFA	0.74 ±0.04[c]	0.83 ±0.07[c]	1.20 ±0.04[b]	1.50 ±0.09[a]
C12:0	5.70 ±0.15[a]	4.62 ±0.18[b]	4.15 ±0.16[c]	3.55 ±0.13[d]
C16:0	28.28 ±0.26[b]	29.24 ±0.31[a]	26.06 ±0.24[c]	26.14 ±0.22[c]
C18:1 *cis 9*	17.69 ±0.32[c]	20.41 ±0.53[b]	20.87 ±0.51[ab]	22.08 ±0.44[a]
C18:3*cis9.cis12.cis15*	0.48 ±0.03[d]	0.59 ±0.06[c]	0.61 ±0.03[b]	0.89 ±0.07[a]
EPA	0.03 ±0.00[b]	0.06 ±0.00[a]	0.06 ±0.00[a]	0.07 ±0.00[a]

[a-c] Means within a row with different superscripts are significantly different(*P*-values < 0.05).

Table S5. Interaction mean values±SEs for whole-crop oat and alfalfa hay fed and palmitic acid and conjugated linoleic acid (CLA) concentrations in milk fat from two years and sampling months.

Sampling Months	January	February	March	April	May	June
Factors						
Oat hay (g /ewe/day)						
Year 1	196 ±40A[a]	183 ±45A[a]	45 ±33A[b]	43 ±25A[b]	0 ±0B[c]	6 ±6B[c]
Year 2	55 ±27B[a]	30 ±17B[a]	0 ±0B[b]	0 ±0B[b]	0 ±0B[b]	0 ±0B[b]
Alfalfa hay(g/ewe/day)						
Year 1	326 ±60A[a]	299 ±66A[a]	353 ±56A[a]	148 ±43A[b]	115 ±67A[bc]	14 ±14A[c]
Year 2	369 ±69A[a]	338 ±63A[a]	129 ±38B[b]	15 ±10B[c]	0 ±0B[c]	0 ±0A[c]
C16:0 (g/100g total FA)						
Year 1	27.67 ±0.32A[b]	27.66 ±0.41A[b]	28.24 ±0.32A[b]	27.97 ±0.31A[b]	30.43 ±0.66A[a]	30.87 ±0.37A[a]
Year 2	26.14 ±0.36B[ab]	25.65 ±0.50B[ab]	25.87 ±0.34B[ab]	25.55 ±0.52B[b]	26.74 ±0.31B[a]	26.68 ±0.24B[a]
CLA*c9.t11* (g/100g total FA)						
Year 1	0.79 ±0.06B[c]	0.79 ±0.06B[c]	0.96 ±0.05B[ab]	0.97 ±0.06B[ab]	1.06 ±0.10A[a]	0.86 ±0.04B[bc]
Year 2	0.95 ±0.07A[c]	1.17 ±0.07A[ab]	1.17 ±0.06A[ab]	1.29 ±0.07A[a]	1.09 ±0.05A[bc]	1.06 ±0.05A[bc]

[a-c] Means within a row with different superscripts are significantly different(P-values < 0.05).

[A-B] Means within a column with different superscripts are significantly different (P-values < 0.05).

Table S6. Effect of management systems (production intensity), sampling month, and year on concentrations of odd chain fatty acids in milk fat. (mean values ±sem).

	decapentanoic acid C15:0	heptapentanoic acid C17:0	Total odd chain fatty acids	C15:0 : C17:0 ratio
Factors	g/100 g total FA			
Management System				
Semi-intensive	1.05 ±0.02	0.50 ±0.01	2.31 ±0.04	0.004 ±0.0003
Extensive	0.97 ±0.02	0.49 ±0.01	2.12 ±0.03	0.006 ±0.0004
Sampling Months				
January	0.92 ±0.03[ab]	0.48 ±0.02[ab]	2.16 ±0.06[ab]	0.005 ±0.001[ab]
February	0.95 ±0.03[b]	0.49 ±0.02[ab]	2.21 ±0.06[ab]	0.004 ±0.001[a]
March	1.03 ±0.03[c]	0.46 ±0.02[b]	2.30 ±0.06[a]	0.004 ±0.001[a]
April	1.06 ±0.03[c]	0.49 ±0.02[ab]	2.27 ±0.06[a]	0.006 ±0.001[b]
May	1.08 ±0.03[c]	0.52 ±0.03[a]	2.25 ±0.06[ab]	0.005 ±0.001[a]
June	1.01 ±0.04[cb]	0.53 ±0.03[a]	2.09 ±0.07[b]	0.004 ±0.000[a]
Milking Season				
First	1.02 ±0.02	0.40 ±0.01	2.15 ±0.04	0.005 ±0.0003
Second	1.00 ±0.02	0.58 ±0.01	2.28 ±0.03	0.005 ±0.003
ANOVA (P-values)[1]				
Main effects				
Management (MS)	***	ns	***	**
Sampling months (SM)	***	*	ns	ns
Milking year (Y)	ns	***	**	ns
Interactions				
MS x SM	**	*	*	ns
MS x Y	ns	ns	ns	ns
SM x Y	**	***	*	*
MS x SM x Y	ns	ns	ns	ns

[a-e] Means within a column with different superscripts are significantly different according to Tukey's honestly significant difference test   (*P*-values < 0.05).

[1]   ***: p < 0.001, **: p< 0.01; *: p< 0.05, t: p <0.1, ns: p > 0.1.
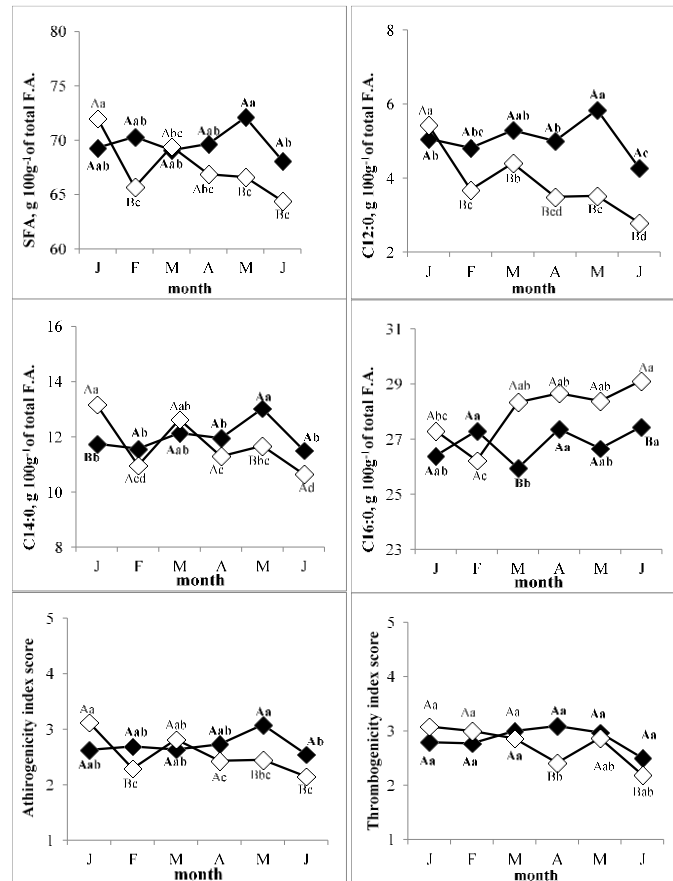
Figure S1. Interaction means for the concentration of saturated fatty acids (SFA), Lauric acid (C12:0), Myristic acid (C14:0), Palmitic acid (C16:0) in milk fat and the respective Athirogenicity (AI) and Thrombogenicity (TI) indices of milk for from different management systems and sampling months. Semi-intensive management system is represented by (♦) and extensive management system by (◊). J:January, F:February, M:March, A:April, M:May, J:June.Values with different,capitilized letters represent statistically significant differences between the two management systems (*P*-value<0.05). Values with differernet lowercase represent statistically significant differences between months within the same system.
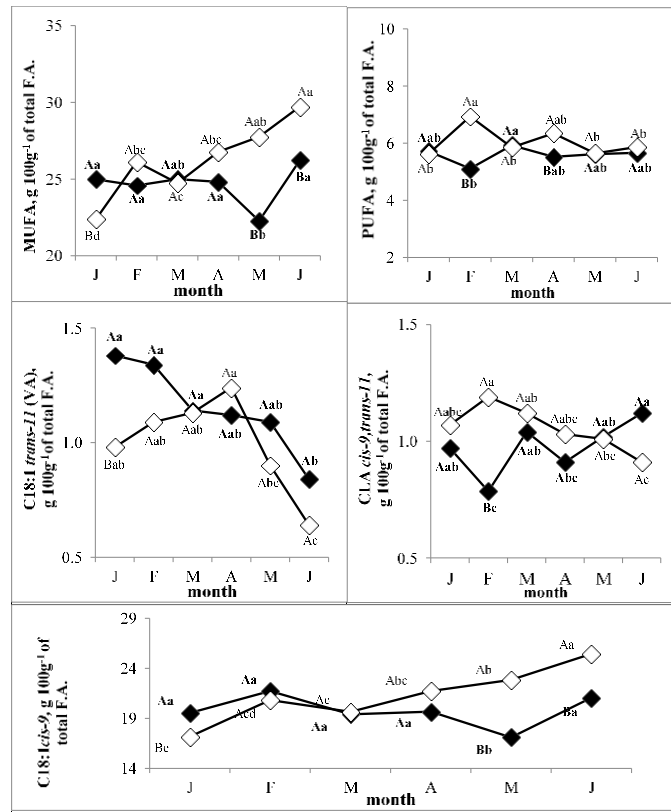
Figure S2. Interaction means the concertation of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), Vaccenic acid (C18:1*trans-11*) and CLA*cis9trans-11*in milk and oleic acid (C18:1 cis9) fat for the different management systems and sampling months. Semi-intensive management system is represented by (◆) and extensive management system by (◊). J:January, F:February, M:March, A:April, M:May, J:June.Values with different capitilized letters represent statistically significant differences between the two management systems (*P*-value<0.05). Values with differernet lowercase represent statistically significant differences between months within the same system**.**
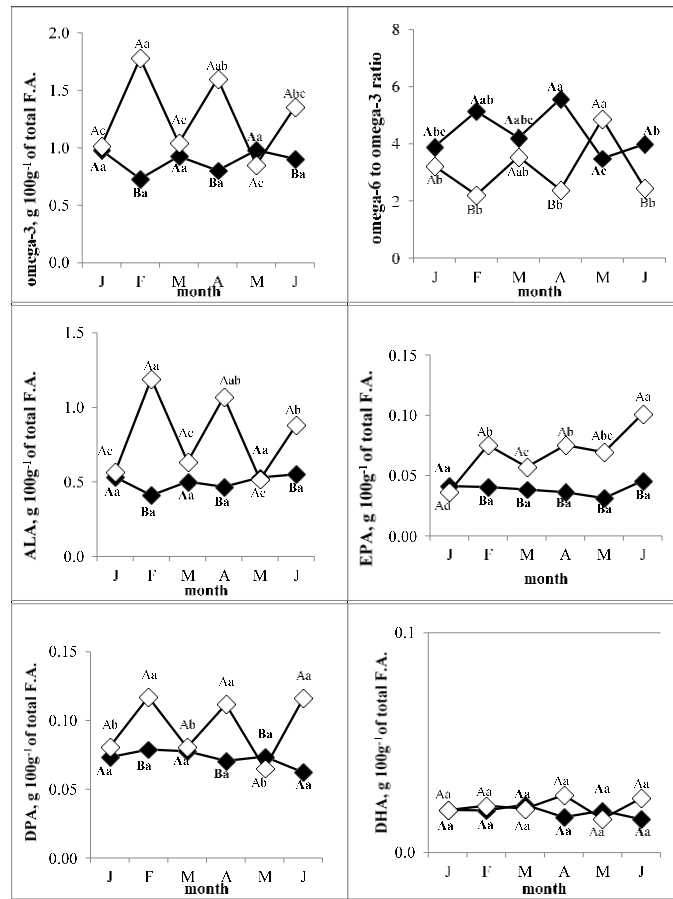
Figure S3. Interaction means for omega-3 PUFA (omega-3), omega-3 to omega-6 ratio, a-Linoleic acid (C18:3cis9.cis12.cis15; ALA), Eicosapentaenoic acid (C20:5*omega-3*; EPA), Docosapentaenoic acid (C22:5*omega-3*; DPA) and Docosahexaenoic acid (C22:6*omega-3*; DHA) for the different management systems and sampling months. Semi-intensive management system is represented by (♦) and extensive management system by (◊). J:January, F:February, M:March, A:April, M:May, J:June. Values with different capitilized letters represent statistically significant differences between the two management systems (*P*-value<0.05). Values with differernet lowercase represent statistically significant differences between months within the same system.