

## Effects of *FABP4* variation on milk fatty-acid composition in dairy cattle grazed on pasture in late lactation

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### Supplementary material and methods

After individual measurement, the FAs detected were divided into various groups as follows: short chain FAs (SCFA) = C4:0 + C6:0 + C8:0; medium chain FAs (MCFA) = C10:0 + C12:0 + C14:0; long chain FAs (LCFA) = C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C24:0; omega 3 FAs = C18:3 *cis*-9,12,15 + C20:5 *cis*-5,8,11,14,17 + C22:5 *cis*-7,10,13,16,19; omega 6 FAs = C18:2 *cis*-9,12 + C18:3 *cis*-6,9,12 + C20:3 *cis*-8,11,14 + C20:4 *cis*-5,8,11,14; monounsaturated FAs (MUFA) = C10:1 + C12:1 + C14:1 *cis*-9 + C15:1 + C16:1 *cis*-9 + C17:1 + C18:1 *trans*-11 + C18:1 *cis*-9 + C18:1 *cis*-(10 to 15) + C20:1 *cis*-5 + C20:1 *cis*-9 + C20:1 *cis*-11; polyunsaturated FAs (PUFA) = C18:2 *trans*-9,12 + C18:2 *cis*-9,*trans*-13 + C18:2 *cis*-9,*trans*-12 + C18:2 *trans*-9,*cis*-12 + C18:2 *cis*-9,12 + C18:3 *cis*-6,9,12 + C18:3 *cis*-9,12,15 + CLA + C20:3 *cis*-8,11,14 + C20:4 *cis*-5,8,11,14 + C20:5 *cis*-5,8,11,14,17 + C22:5 *cis*-7,10,13,16,19; total branched FA= C13:0 *iso* + C13:0 *anteiso* + C15:0 *iso* + C15:0 *anteiso* + C17:0 *iso*; total UFA = MUFA + PUFA; and total SFA = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0.

Unsaturated FA ratios were also calculated as: total index (total UFA divided by the sum of total SFA and total UFA); MUFA index (MUFA divided by the sum of MUFA, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0); C10:1 index (C10:1 divided by the sum of C10:0 and C10:1); C12:1 index (C12:1 divided by the sum of C12:0 and C12:1); C14:1 index (C14:1 *cis*-9 divided by the sum of C14:0 and C14:1 *cis*-9); C16:1 index (C16:1 *cis*-9 divided by the sum of C16:0 and C16:1 *cis*-9); C18:1 index (C18:1 *cis*-9 divided by the sum of C18:0 and C18:1 *cis*-9);

and CLA index (CLA divided by the sum of CLA and C18:1 *trans*-11).

**Supplementary Table S1.** Average quantity of individual milk fatty acid methyl esters (FAME) (means  $\pm$  SE) in late lactation, in mixed-age New Zealand (NZ) HF $\times$ J - cross cows grazing on pasture.

FAME (n = 455)	g/100 g Total FAs	FAME (n = 455)	g/100 g Total FAs
C4:0	1.273 $\pm$ 0.006	C18:1 <i>trans</i> 11	2.677 $\pm$ 0.041
C6:0	1.564 $\pm$ 0.005	C18:1 <i>cis</i> -9	13.052 $\pm$ 0.076
C8:0	1.183 $\pm$ 0.004	C18:1 <i>cis</i> (10 to 12)	0.505 $\pm$ 0.003
C10:0	3.231 $\pm$ 0.017	C18:2 <i>trans</i> -9,12	0.415 $\pm$ 0.002
C10:1	0.281 $\pm$ 0.002	C18:2 <i>cis</i> -9, <i>trans</i> -13	0.287 $\pm$ 0.002
C11:0	0.057 $\pm$ 0.001	C18:2 <i>cis</i> -9, <i>trans</i> -12	0.072 $\pm$ 0.001
C12:0	3.909 $\pm$ 0.024	C18:2 <i>trans</i> -9, <i>cis</i> -12	0.468 $\pm$ 0.006
C13:0 <i>iso</i>	0.078 $\pm$ 0.001	C18:2 <i>cis</i> -9,12	0.703 $\pm$ 0.004
C12:1	0.089 $\pm$ 0.001	C19:0	0.143 $\pm$ 0.001
C13:0 <i>anteiso</i>	0.037 $\pm$ 0.000	C18:3 <i>cis</i> -6,9,12	0.076 $\pm$ 0.001
C13:0	0.118 $\pm$ 0.001	C18:3 <i>cis</i> -9,12,15	0.817 $\pm$ 0.005
C14:0	12.447 $\pm$ 0.042	CLA <sup>1</sup>	0.968 $\pm$ 0.016
C14:1 <i>cis</i> -9	0.946 $\pm$ 0.010	C20:0	0.132 $\pm$ 0.001
C15:0 <i>iso</i>	0.292 $\pm$ 0.001	C20:1 <i>cis</i> -5	0.059 $\pm$ 0.001
C15:0 <i>anteiso</i>	0.635 $\pm$ 0.005	C20:1 <i>cis</i> -9	0.151 $\pm$ 0.001
C15:0	1.492 $\pm$ 0.008	C20:1 <i>cis</i> -11	0.076 $\pm$ 0.001
C15:1	0.284 $\pm$ 0.002	C20:3 <i>cis</i> -8,11,14	0.030 $\pm$ 0.000
C16:0	37.623 $\pm$ 0.160	C20:4 <i>cis</i> -5,8,11,14	0.035 $\pm$ 0.000
C16:1 <i>cis</i> -9	1.265 $\pm$ 0.012	C22:0	0.065 $\pm$ 0.001
C17:0 <i>iso</i>	0.563 $\pm$ 0.003	C20:5 <i>cis</i> -5,8,11,14,17	0.088 $\pm$ 0.001
C17:0	0.889 $\pm$ 0.004	C24:0	0.045 $\pm$ 0.000
C17:1	0.200 $\pm$ 0.001	C22:5 <i>cis</i> -7,10,13,16,19	0.125 $\pm$ 0.001
C18:0	8.650 $\pm$ 0.061		

<sup>1</sup>CLA = conjugated linoleic acid 'C18:2 *cis*-9, *trans*-11'.

**Supplementary Table S2.** Average quantity of grouped milk fatty acid methyl esters (FAME) (means  $\pm$  SE) and unsaturated ratio in late-lactation, mixed-age New Zealand (NZ) HF $\times$ J - cross cows grazing on pasture.

Grouped FAME (n = 455)	g/100 g Total FAs	Desaturation index	%
SCFA	4.020 $\pm$ 0.013	Total index	25.750 $\pm$ 0.130
MCFA	19.588 $\pm$ 0.076	C10:1 index	8.062 $\pm$ 0.067
LCFA	49.039 $\pm$ 0.139	C12:1 index	2.224 $\pm$ 0.017
Total C18:1	16.524 $\pm$ 0.097	C14:1 index	7.072 $\pm$ 0.073
Total C18:2	2.915 $\pm$ 0.024	C16:1 index	3.253 $\pm$ 0.027
Total C18:3	0.893 $\pm$ 0.005	C18:1 index	65.647 $\pm$ 0.162
Omega 3	1.044 $\pm$ 0.006	CLA index	26.419 $\pm$ 0.136
Omega 6	0.845 $\pm$ 0.004		
MUFA	19.79 $\pm$ 0.099		
PUFA	4.101 $\pm$ 0.025		
Total branched FA	1.605 $\pm$ 0.007		
Total UFA	23.891 $\pm$ 0.118		
Total SFA	68.912 $\pm$ 0.131		

**Supplementary Table S3.** Association between individual fatty acid levels and *FABP4* variation corrected for *DGAT1* genotype

FAME	Haplotype	Other haplotype in model	Mean FAME level $\pm$ SE <sup>1</sup> (g/100 g milk FA) associated with <i>FABP4</i> haplotypes				<i>P</i>	
			Absent	n	Present	n	<i>DGAT1</i>	<i>FABP4</i>
<b>C10:0</b>	<i>A</i>	none	3.256 $\pm$ 0.046	100	3.277 $\pm$ 0.034	307	n.s.	n.s.
	<i>B</i>	none	3.235 $\pm$ 0.035	280	3.339 $\pm$ 0.042	127	n.s.	< <b>0.01</b> <sup>2</sup>
	<i>C</i>	none	3.296 $\pm$ 0.038	182	3.250 $\pm$ 0.036	225	n.s.	n.s.
<b>C10:1</b>	<i>A</i>	none	0.273 $\pm$ 0.005	100	0.274 $\pm$ 0.004	307	<i>n.s.</i> <sup>3</sup>	n.s.
	<i>B</i>	none	0.271 $\pm$ 0.004	280	0.279 $\pm$ 0.005	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	none	0.280 $\pm$ 0.004	182	0.269 $\pm$ 0.004	225	<i>n.s.</i>	< <b>0.05</b>
	<i>B</i>	<i>C</i>	0.272 $\pm$ 0.006	280	0.278 $\pm$ 0.007	127	<i>n.s.</i>	n.s.
	<i>C</i>	<i>B</i>	0.280 $\pm$ 0.005	182	0.269 $\pm$ 0.004	225	<i>n.s.</i>	< <b>0.05</b>
<b>C12:0</b>	<i>A</i>	none	3.933 $\pm$ 0.061	100	3.963 $\pm$ 0.045	307	n.s.	n.s.
	<i>B</i>	none	3.905 $\pm$ 0.046	280	4.051 $\pm$ 0.055	127	n.s.	< <b>0.01</b>
	<i>C</i>	none	3.996 $\pm$ 0.050	182	3.921 $\pm$ 0.048	225	n.s.	<i>n.s.</i>
	<i>B</i>	<i>C</i>	3.905 $\pm$ 0.047	280	4.051 $\pm$ 0.056	127	n.s.	< <b>0.01</b>
	<i>C</i>	<i>B</i>	3.995 $\pm$ 0.079	182	3.951 $\pm$ 0.079	225	n.s.	n.s.
<b>C12:1</b>	<i>A</i>	none	0.088 $\pm$ 0.002	100	0.089 $\pm$ 0.002	307	n.s.	n.s.
	<i>B</i>	none	0.087 $\pm$ 0.002	280	0.092 $\pm$ 0.002	127	<i>n.s.</i>	< <b>0.01</b>
	<i>C</i>	none	0.091 $\pm$ 0.002	182	0.087 $\pm$ 0.002	225	n.s.	< <b>0.05</b>
	<i>B</i>	<i>C</i>	0.087 $\pm$ 0.002	280	0.092 $\pm$ 0.003	127	<i>n.s.</i>	< <b>0.05</b>
	<i>C</i>	<i>B</i>	0.091 $\pm$ 0.003	182	0.087 $\pm$ 0.003	225	n.s.	<i>n.s.</i>
<b>C14:0</b>	<i>A</i>	none	12.411 $\pm$ 0.103	100	12.509 $\pm$ 0.075	307	< <b>0.001</b>	n.s.

	<i>B</i>	none	12.396 ± 0.078	280	12.653 ± 0.092	127	< <b>0.001</b>	< <b>0.01</b>
	<i>C</i>	none	12.566 ± 0.084	182	12.416 ± 0.081	225	< <b>0.001</b>	<i>n.s.</i>
	<i>B</i>	<i>C</i>	12.396 ± 0.078	280	12.653 ± 0.093	127	< <b>0.001</b>	< <b>0.01</b>
	<i>C</i>	<i>B</i>	12.564 ± 0.134	182	12.469 ± 0.134	225	< <b>0.001</b>	<i>n.s.</i>
<b>C14:1 <i>cis</i>-9</b>	<i>A</i>	none	0.914 ± 0.026	100	0.922 ± 0.019	307	<i>n.s.</i>	<i>n.s.</i>
	<i>B</i>	none	0.908 ± 0.019	280	0.943 ± 0.024	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	none	0.945 ± 0.021	182	0.898 ± 0.020	225	<i>n.s.</i>	< <b>0.05</b>
	<i>B</i>	<i>C</i>	0.912 ± 0.027	280	0.937 ± 0.030	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	<i>B</i>	0.945 ± 0.022	182	0.898 ± 0.020	225	<i>n.s.</i>	< <b>0.05</b>
<b>C18:0</b>	<i>A</i>	none	8.709 ± 0.161	100	8.973 ± 0.121	307	<i>n.s.</i>	<i>n.s.</i>
	<i>B</i>	none	8.980 ± 0.127	280	8.793 ± 0.145	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	none	8.892 ± 0.135	182	8.932 ± 0.131	225	<i>n.s.</i>	<i>n.s.</i>
<b>C18:1 <i>trans</i>-11</b>	<i>A</i>	none	2.906 ± 0.103	100	2.724 ± 0.070	307	<i>n.s.</i>	< <b>0.05</b>
	<i>B</i>	none	2.751 ± 0.075	280	2.787 ± 0.089	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	none	2.723 ± 0.079	182	2.800 ± 0.079	225	<i>n.s.</i>	<i>n.s.</i>
<b>CLA</b>	<i>A</i>	none	1.041 ± 0.041	100	0.973 ± 0.028	307	< <b>0.01</b>	<i>n.s.</i>
	<i>B</i>	none	0.979 ± 0.030	280	1.006 ± 0.036	127	< <b>0.001</b>	<i>n.s.</i>
	<i>C</i>	none	1.009 ± 0.026	182	1.016 ± 0.024	225	< <b>0.001</b>	<i>n.s.</i>
<b>C20:0</b>	<i>A</i>	none	0.123 ± 0.002	100	0.128 ± 0.001	307	<i>n.s.</i>	< <b>0.05</b>
	<i>B</i>	none	0.127 ± 0.002	280	0.126 ± 0.002	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	none	0.127 ± 0.002	182	0.126 ± 0.002	225	<i>n.s.</i>	<i>n.s.</i>
<b>C22:0</b>	<i>A</i>	none	0.061 ± 0.002	100	0.066 ± 0.001	307	<i>n.s.</i>	< <b>0.01</b>
	<i>B</i>	none	0.066 ± 0.001	280	0.062 ± 0.001	127	<i>n.s.</i>	< <b>0.05</b>
	<i>C</i>	none	0.065 ± 0.001	182	0.064 ± 0.001	225	<i>n.s.</i>	<i>n.s.</i>
	<i>A</i>	<i>B</i>	0.061 ± 0.002	100	0.065 ± 0.001	307	<i>n.s.</i>	< <b>0.01</b>
	<i>B</i>	<i>A</i>	0.064 ± 0.002	280	0.062 ± 0.002	127	<i>n.s.</i>	<i>n.s.</i>

<b>C24:0</b>	A	none	0.042 ± 0.001	100	0.046 ± 0.001	307	<i>n.s.</i>	<b>&lt;0.001</b>
	B	none	0.045 ± 0.001	280	0.044 ± 0.001	127	<i>n.s.</i>	<i>n.s.</i>
	C	none	0.045 ± 0.001	182	0.044 ± 0.001	225	<i>n.s.</i>	<i>n.s.</i>
<b>MCFA</b>	A	B	0.042 ± 0.001	100	0.046 ± 0.001	307	<i>n.s.</i>	<b>&lt;0.001</b>
	B	A	0.044 ± 0.002	280	0.043 ± 0.002	127	<i>n.s.</i>	<i>n.s.</i>
	A	none	19.604 ± 0.197	100	19.754 ± 0.144	307	<b>&lt; 0.001</b>	<i>n.s.</i>
	B	none	19.542 ± 0.147	280	20.047 ± 0.176	127	<b>&lt; 0.001</b>	<b>&lt; 0.01</b>
	C	none	19.864 ± 0.161	182	19.592 ± 0.155	225	<b>&lt; 0.001</b>	<i>n.s.</i>
<b>LCFA</b>	B	C	19.542 ± 0.149	280	20.046 ± 0.178	127	<b>&lt; 0.001</b>	<b>&lt; 0.01</b>
	C	B	19.859 ± 0.266	182	19.700 ± 0.265	225	<b>&lt; 0.001</b>	<i>n.s.</i>
	A	none	48.802 ± 0.308	100	47.691 ± 0.224	307	<b>&lt; 0.001</b>	<i>n.s.</i>
	B	none	47.893 ± 0.234	280	47.395 ± 0.270	127	<b>&lt; 0.001</b>	<i>n.s.</i>
	C	none	47.502 ± 0.248	182	47.908 ± 0.243	225	<b>&lt; 0.001</b>	<i>n.s.</i>
<b>Total C18:3</b>	A	none	0.882 ± 0.014	100	0.887 ± 0.010	307	<b>&lt; 0.001</b>	<i>n.s.</i>
	B	none	0.891 ± 0.011	280	0.875 ± 0.012	127	<b>&lt; 0.001</b>	<i>n.s.</i>
	C	none	0.882 ± 0.011	182	0.889 ± 0.011	225	<b>&lt; 0.001</b>	<i>n.s.</i>
<b>PUFA</b>	A	none	4.147 ± 0.059	100	4.076 ± 0.042	307	<b>&lt; 0.001</b>	<i>n.s.</i>
	B	none	4.096 ± 0.044	280	4.085 ± 0.052	127	<b>&lt; 0.001</b>	<i>n.s.</i>
	C	none	4.081 ± 0.047	182	4.103 ± 0.046	225	<b>&lt; 0.001</b>	<i>n.s.</i>
<b>C10:1 index</b>	A	none	7.778 ± 0.171	100	7.783 ± 0.125	307	<i>n.s.</i>	<i>n.s.</i>
	B	none	7.786 ± 0.131	280	7.775 ± 0.153	127	<i>n.s.</i>	<i>n.s.</i>
	C	none	7.885 ± 0.142	182	7.692 ± 0.134	225	<i>n.s.</i>	<i>n.s.</i>
<b>C12:1 index</b>	A	none	2.192 ± 0.044	100	2.189 ± 0.032	307	<i>n.s.</i>	<i>n.s.</i>
	B	none	2.171 ± 0.033	280	2.225 ± 0.039	127	<i>n.s.</i>	<i>n.s.</i>
	C	none	2.229 ± 0.036	182	2.155 ± 0.034	225	<i>n.s.</i>	<b>&lt; 0.05</b>
<b>C14:1 index</b>	A	none	6.854 ± 0.187	100	6.868 ± 0.137	307	<i>n.s.</i>	<i>n.s.</i>

	<i>B</i>	none	6.823 ± 0.142	280	6.943 ± 0.169	127	<i>n.s.</i>	<i>n.s.</i>
	<i>C</i>	none	7.004 ± 0.156	182	6.743 ± 0.145	225	<i>n.s.</i>	<i>n.s.</i>
<b>C18:1 index</b>	<i>A</i>	none	66.415 ± 0.442	100	65.823 ± 0.320	307	<b>&lt; 0.001</b>	<i>n.s.</i>
	<i>B</i>	none	65.860 ± 0.335	280	66.131 ± 0.392	127	<b>&lt; 0.001</b>	<i>n.s.</i>
	<i>C</i>	none	66.056 ± 0.360	182	65.870 ± 0.347	225	<b>&lt; 0.001</b>	<i>n.s.</i>
<b>CLA index</b>	<i>A</i>	none	26.282 ± 0.343	100	26.249 ± 0.250	307	<b>&lt; 0.001</b>	<i>n.s.</i>
	<i>B</i>	none	26.168 ± 0.260	280	26.419 ± 0.307	127	<b>&lt; 0.001</b>	<i>n.s.</i>
	<i>C</i>	none	26.524 ± 0.282	182	26.022 ± 0.268	225	<b>&lt; 0.001</b>	<i>n.s.</i>

<sup>1</sup>Predicted means and standard error of those means derived from GLMM. ‘Cow age’, ‘days in milk (DIM)’, ‘herd’ and ‘*DGATI* p.K232A’ were fitted to the models as fixed effects.

<sup>2</sup>Significant results are in bold type, trends are in italic type and non-significant results are shown as ‘n.s.’