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Effect of DGAT1 variant (K232A) on milk traits and milk fat composition in outdoor pasture-grazed dairy cattle

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Abstract: Milk fat production is important in the New Zealand (NZ) dairy industry. Elsewhere, an amino acid substitution (K232A) in the enzyme diacylglycerol acyl-CoA acyltransferase (DGAT1) has been reported to explain variation in some milk traits, including variation in milk fatty acid (FA) profile. In this study, associations between K232A, and milk traits including milk FA composition, were studied in wholly pasture-fed NZ Holstein-Friesian × Jersey (HF×J) cross-bred dairy cows. With a high frequency of K variant (61.9%), the KK cows produced more milk fat than the AA cows (5.41 ± 0.04 % vs 4.42 ± 0.05 %). The milk volume, fat concentration and protein concentration of AK cows were between the genotypes, AA and KK. More C16:0, CLA and C18:3 cis-9, 12, 15 FA were found in the milk of Kiwi-cross cows feed in outdoor pasture grazing system., and the influence of DGAT1 K232A, on these FAs from mid- and late lactation stages were significant. The AA cows produced ($P < 0.001$) more CLA and C18:3 cis-9, 12, 15, but less C16:0 (1.137 ± 0.047, 0.855 ± 0.015 and 35.170 ± 0.355) than the KK cows (0.934 ± 0.025, 0.778 ± 0.009 and 38.010 ± 0.250).

Keywords: diacylglycerol acyl-CoA acyltransferase (DGAT1), K232A, milk traits, milk fatty acid

1. Introduction

New Zealand (NZ) is a major exporter of dairy products, with only a small domestic market for whole milk and milk products. The milk produced is therefore largely processed and manufactured into products, with payment to farmers being based on milk solid (MS) levels (mainly fat and protein), and not milk volume.

Most dairy production in NZ is seasonal and pasture-based, with minimal use of supplements, although this is changing. In the 2016-2107 season the DairyNZ (a farmer-good, levy-collecting organisation) Economic Survey [1] reported that pasture (including hay and silage/baleage) accounted for approximately 82 % of total feed dry matter (DM) eaten, with palm kernel extract accounting for 6% and fodder beet 4%. Further statistics [2] produced by DairyNZ and the Livestock Improvement Corporation Ltd (LIC, Hamilton, NZ) for the 2016-2017 season, suggest the average NZ dairy cow is now producing 381 kg of MS annually, and that these cows are predominantly (48%) KiwicrossTM cows, a Holstein-Friesian x Jersey cross, of variable proportion.

Breeding for dairy production in NZ is strongly influenced using a breeding index system that is administered by NZ Animal Evaluations Ltd (NZAEL), a subsidiary of DairyNZ (www.dairynz.co.nz/animal/animal-evaluation/). This is known as the Breeding Worth (BW) index, and it includes estimates of an animal's genetic merit (estimated breeding values, ebvs) for eight traits that are of value to the NZ industry. These traits are: milkfat production, milk protein production, milk volume, cow live-weight, somatic cell score, fertility, body condition score and residual survival; each having a distinctive assessment regime.

The genes that underpin key dairy traits are of ongoing interest in dairy production. Key among these genes is the diacylglycerol acyl-CoA acyltransferase 1 (DGAT1) gene (DGAT1). This is located on bovine chromosome 14, in proximity to where a milk fat QTL was historically positioned [3]. Sequence variation in DGAT1 has been described, and a well-studied polymorphism results in the substitution of lysine (K) with alanine (A) at position 232 of the amino acid sequence (known as K232A). This was first described by Grisart, Coppieters [4] where it was associated with various milk traits. Subsequently, analysis of DGAT1 variation in NZ dairy cows [5] suggested that the average allele substitution effects were 2-3 kg of protein and 120-130 L of milk for both the Jersey and Holstein Friesian breeds, with a substitution effect of 6 kg of milk fat for Holstein-Friesians and 3 kg for Jersey cows. The effect of K232A in the KiwicrossTM cow could not be assessed, as these genetics was not released by LIC until 2005.

In 2007, Schennink, Stoop [6] reported how the K232A variation in DGAT1 affected milk fatty acid (FA) composition, with the K allele not only being associated with a decreased milk yield and increased milk fat content, but also with a higher concentration of saturated fat and C16:0 FA, and a lower concentration of C14:0, unsaturated c18 and Conjugated Linoleic Acid (CLA). They concluded that "selective breeding can make a significant contribution to change the fat composition of cow's milk".

While K232A has been investigated in pasture-fed NZ Holstein-Friesian and Jersey cows [5], its effect on milk FA component composition, and specifically in the now dominant KiwicrossTM cow has not been studied, and this is therefore the basis of this investigation.

2. Materials and Methods

2.1. Cattle and milk sample collection

This research was approved by the Lincoln University Animal Ethics Committee (AEC Number 521) under the provisions of the Animal Welfare Act 1999 (NZ Government).

A total of 395 Holstein-Friesian × Jersey (HF × J)-cross dairy cows (KiwicrossTM cows), of variable and unknown breed proportion, and of 3 to 10 years of age were studied. These were obtained from two herds: 113 cows in herd 1, and 282 cows in herd 2. All the cows were grazed on pasture (a mixture of perennial ryegrass and white clover) on the Lincoln University Dairy Farm (LUDF; Canterbury, NZ). All the cows calved over the months August-September and they were milked twice a day.

Samples for milk trait analyses were collected once a month from September to February. The daily milk yield in litres was recorded using Tru-test milk meters (Tru-test Ltd, Auckland, NZ). These samples were analysed for fat percentage (%) and protein percentage (%) using Fourier-Transform Infra-Red Spectroscopy (MilkoScan FT 120 Foss, Hillerød, Denmark). The milk samples for FA analysis were collected from each cow in a single afternoon milking in mid-January (days in milk (DIM) = 148 ± 19 days). These were frozen at -20 ℃, and then freeze-dried, prior to being individually ground to a fine powder for component analysis.

2.2. Gas Chromatography of the Fatty Acids in the Milk Samples

The milk FAs were methylated and extracted in n-heptane, before being analysed by Gas Chromatography (GC) as FA methyl esters (FAMEs). The methylation reactions for ester formation were performed in 10-mL Kimax tubes. Individual powdered milk samples (0.17 g), were dissolved in 900 μL of n-heptane (100%, AR grade), before 100 μL of internal standard (5 mg/ml of C21:0 methyl ester in n-heptane) and 4.0 mL of 0.5 M NaOH (in 100% anhydrous methanol) were added. The tubes were vortexed then incubated in a block heater (Ratek Instruments, Australia) at 50℃ for 15 min. After cooling to room temperature, another 2.0 mL of n-heptane and 2.0 mL of deionized water was added to each tube. After vortexing, the tubes were centrifuged for 5 min. at 1500g (Megafuge 1.0R, Heraeus, Germany). The top layer of n-heptane was transferred with a Pasteur pipette into a second Kimax tube, and another 2.0 mL of n-heptane was added to each of the original tubes. The extraction was repeated

and the n-heptane aspirates were then pooled. Finally, anhydrous sodium sulphate (10 mg) was added to the n-heptane extracts, to remove any residual water.

The GC analysis was carried out using a Shimadzu GC-2010 Gas Chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and an AOC-20i auto sampler. The output was analysed with GC Solution Software (Shimadzu). For analysis, 1 µL of the n-heptane sample extract was injected into an 100 metre GC column (250 µm × 0.25 µm capillary column, CP-Select, Varian) with a 1:60 split ratio. The separation was undertaken with a pure helium carrier gas and was run for 92 min. The temperature of both the injector and detector were set at 250℃ and the thermal profile of the column consisted of 45℃ for 4 min., followed by 27 min. at 175℃ (ramped at 13 ℃/min.), 35 min. at 215℃ (ramped at 4 ℃/min.), and a final 'bake-off' at 250℃ for 5 min. (ramped at 25 ℃/min.). The individual FAMEs were identified by the peak retention time compared to commercially obtained external standards (ME61, ME93, BR3, BR2, ME100, GLC411 and GLC463; Laroden AB, Sweden). Quantification of the individual FAMEs was based on peak area assessment and comparison with the internal and external standards. The threshold for peak area determination on the chromatogram was a 500-unit count, with peaks that were under 500-unit count, being ignored. The calculated minimum component of an individual FAME was therefore 0.01 g per100 gram of total FA.

After individual FA measurement (Table 1), the FAs were arranged into various groups and indices (Table 2), and the mean levels in the 395 cows calculated. These groups were, 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 + C20:0 + C22:0 + 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 + C22:1 trans-13; 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 and indices were also calculated as follows: 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).

2. 3. PCR-SSCP analysis and Genotyping

A blood sample from each cow was collected onto FTA cards and air dried. Genomic DNA was purified from a 1.2-mm punch of the dried blood spot, using a two-step washing procedure, as described by Zhou, Hickford [7]. PCR amplification was performed in a 15-µL reaction containing the genomic DNA (punch of FTA paper), 0.25 µM of each designed primer, 150 µM of each dNTP (Bioline, London, UK), 2.5 mM of Mg2+, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× the reaction buffer supplied with the polymerase enzyme. Specific primers (Forward: 5'-

CCACTGGGCTGCCACTTG-3′and Reverse: 5′-GAAGCAAGCGGACAGTGAG-3′) were designed on the basis of bovine DGAT1 sequence (AJ318490) at GenBank (Figure 1).

Figure 1. Positions of diacylglycerol acyl-CoA acyltransferase (DGAT1) primers. Bold type shows the DGAT1 gene eighth exon1.

Amplification was undertaken using S1000 thermal cyclers (Bio-Red, Hercules, CA, USA) and the thermal profile included an initial denaturation for 2 min at 94 ℃; followed by 35 cycles of 30 s at 94 ℃, 30 s at 58 ℃ and 30 s at 72 ℃; with a final extension for 5 min at 72 ℃. Following amplification, a 0.7-μL aliquot of the PCR products was mixed with 7 μL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 95 ℃ for 5 min and rapid cooling on wet ice, the samples were loaded on 16 cm × 18 cm, 12% acrylamide: bisacrylamide (37.5: 1) (Bio-Rad) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad), at 300 V for 19 h at 26 ℃ in 0.5× TBE buffer. The method of Byun, Fang [8] was used to silver-stain the gels.

2. 4. Statistical analysis

Hardy-Weinberg equilibrium (HWE) for the DGAT1 genotypes was analysed using an online chi-square calculator (http://www.oege.org/software/hwe-mr-calc.shtml).

All other statistical analysis was carried out using IBM SPSS version 22 (IBM, NY, USA), with associations between K232A genotypes and variation in milk traits and milk FA component levels being tested using General Linear Mixed-effects Models (GLMMs). First, a GLMM (fixed effect: genotype, days in milk, age and herd) and multiple pair-wise comparisons with Bonferroni corrections were used to ascertain the effect of the different genotypes on milk production traits. Days in milk (DIM) was counted from the day of calving. Next, a GLMM (fixed effect: genotype, DIM, age and herd) and multiple pair-wise comparisons with Bonferroni corrections were used to ascertain the effect of genotypes on milk FA component levels.

The effect of sire of cow could not be included in the GLMMs. Some semen straws (sire genetics) used in NZ dairy cattle artificial insemination-based breeding approaches, contain mixed-sire semen purchased from commercial semen producers. In these cases, individual sire identity is impossible to ascertain, but because the straws were mixed-semen straws and because different sires are used for

different inseminations, in different years, it is unlikely that sire was a strongly confounding effect. Cow age and herd might also be confounded with sire, but this cannot be confirmed.

3. Results

3. 1. Milk production traits and variant K232A

In the cattle analysed DGAT1 genotypes AA, AK, and KK were found, with frequencies of 14.9%, 46.3% and 38.7% respectively. The most frequent variant was K (61.9%) and the frequency of A was 38.1%. The P-value for the chi-square for deviation from HWE was 0.724, suggesting the population was at equilibrium.

For the GLMMs assessing the effect of DGAT1 K232A on gross milk traits, associations were found between the three genotypes and average daily milk yield, and fat and protein percentage levels (Table 1). Genotype AA was associated with a lower content of milk fat and protein compared to the cows of genotype AK and KK. Genotype AA cows had a higher ($P < 0.001$) milk production (23.70 ± 0.45 L/day) than AK cows (22.45 ± 0.27 L/day) and KK cows (20.82 ± 0.30 L/day).

Table 1. Associations between *DGAT1* K232A genotypes and gross milk production traits

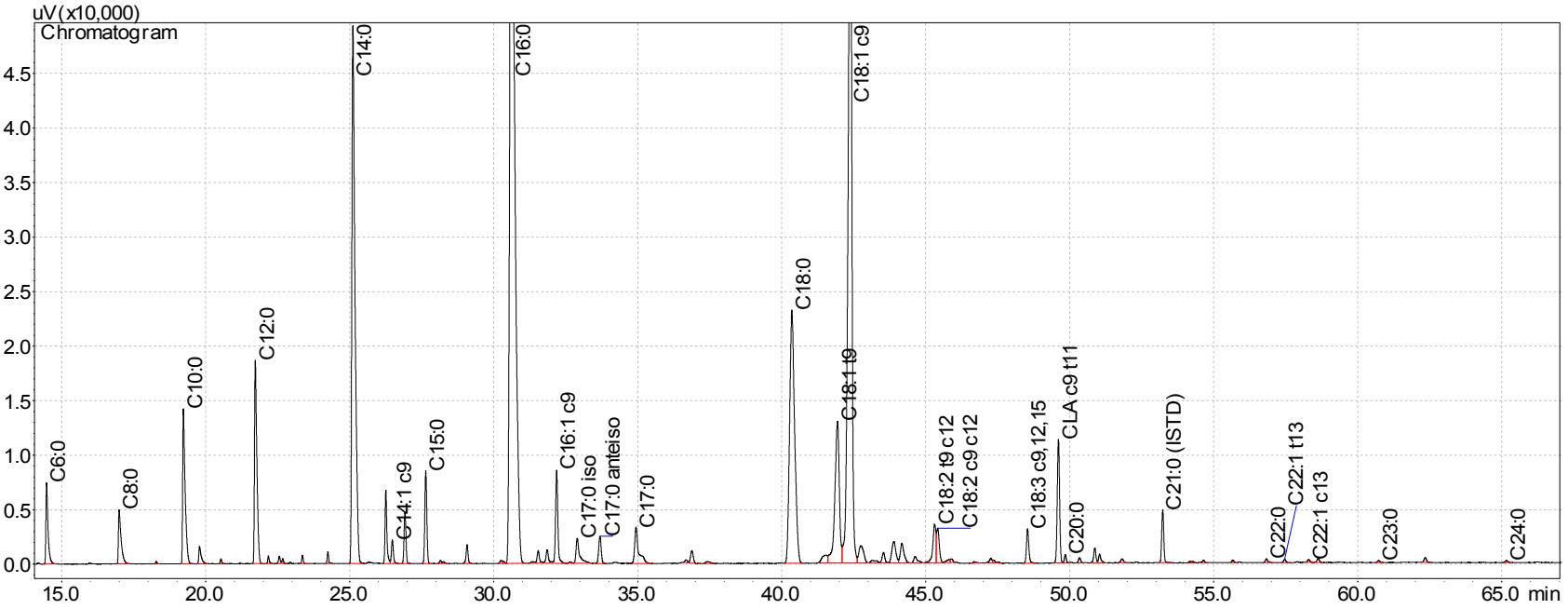
	Mean ± SE[1]			
	AA	**AK**	**KK**	***P***
	n = 59	n = 183	n = 153	
Average milk volume (L)	23.65 ± 0.45[a]	22.45 ± 0.27[b]	20.82 ± 0.29[c]	< 0.001
Fat (%)	4.43 ± 0.06[c]	4.98 ± 0.03[b]	5.41 ± 0.04[a]	< 0.001
Protein (%)	3.99 ± 0.04[c]	4.11 ± 0.02[b]	4.26 ± 0.02[a]	< 0.001

[1]Predicted means and standard error of those means were derived from the GLMMs. 'Cow age', 'days in milk (DIM)' and 'herd' were fitted as fixed effects.

[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

3. 2. The Fatty Acid Composition of Milk

Forty-six FAMEs were detected at levels over the threshold value and a sample output from the GC is shown in Figure 2. Table 2 summarises the average FA composition of the milk samples analysed in this investigation. The least abundant FAME was C20:3 cis-8, 11, 14 (0.030 ± 0.000%), and the most abundant was C16:0 (37.623 ± 0.160%). The average abundance of the grouped FA and indices are summarised in Table 3.



184 **Figure 2.** Sample GC output of milk fatty acid methyl ester (FAME)1 profile from late lactation New Zealand (NZ) HF × J-cross cows grazing on pasture. [1]The individual FAME

185 profile was identified by the peak retention time compared with external standards. Quantification of the peak areas was based on comparison with the internal standard

186 (ISTD) C21:0.

187

188 **Table 2.** Average quantity of individual milk fatty acid methyl ester (FAME) (means ± SE) in late
189 lactation, mixed-age New Zealand (NZ) HF × 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 ± 0.006	C18:1 *trans*-11	2.677 ± 0.041
C6:0	1.564 ± 0.005	C18:1 *cis*-9	13.052 ± 0.076
C8:0	1.183 ± 0.004	C18:1 *cis* (10 to 12)	0.505 ± 0.003
C10:0	3.231 ± 0.017	C18:2 *trans*-9,12	0.415 ± 0.002
C10:1	0.281 ± 0.002	C18:2 *cis*-9, *trans*-13	0.287 ± 0.002
C11:0	0.057 ± 0.001	C18:2 *cis*-9, *trans*-12	0.072 ± 0.001
C12:0	3.909 ± 0.024	C18:2 *trans*-9, *cis*-12	0.468 ± 0.006
C12:1	0.089 ± 0.001	C18:2 *cis*-9,12	0.703 ± 0.004
C13:0 *iso*	0.078 ± 0.001	C19:0	0.143 ± 0.001
C13:0 *anteiso*	0.037 ± 0.000	C18:3 *cis*-6,9,12	0.076 ± 0.001
C13:0	0.118 ± 0.001	C18:3 *cis*-9,12,15	0.817 ± 0.005
C14:0	12.447 ± 0.042	CLA[1]	0.968 ± 0.016
C14:1 *cis*-9	0.946 ± 0.010	C20:0	0.132 ± 0.001
C15:0 *iso*	0.292 ± 0.001	C20:1 *cis*-5	0.059 ± 0.001
C15:0 *anteiso*	0.635 ± 0.005	C20:1 *cis*-9	0.151 ± 0.001
C15:0	1.492 ± 0.008	C20:1 *cis*-11	0.076 ± 0.001
C15:1	0.284 ± 0.002	C20:3 *cis*-8,11,14	0.030 ± 0.000
C16:0	37.623 ± 0.160	C20:4 *cis*-5,8,11,14	0.035 ± 0.000
C16:1 *cis*-9	1.265 ± 0.012	C22:0	0.065 ± 0.001
C17:0 *iso*	0.563 ± 0.003	C22:1 *trans*-13	0.066 ± 0.001
C17:0	0.889 ± 0.004	C20:5 *cis*-5,8,11,14,17	0.088 ± 0.001
C17:1	0.200 ± 0.001	C24:0	0.045 ± 0.000
C18:0	8.650 ± 0.061	C22:5 *cis*-7,10,13,16,19	0.125 ± 0.001

190 [1]CLA = conjugated linoleic acid (C18:2 cis-9, trans-11).

191 **Table 3.** Average quantity of grouped milk fatty acid methyl esters (FAMEs) (means ± SE) and
192 various FA indexes in mixed age New Zealand (NZ) HF × J-cross cows grazing on pasture.

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

193

194 *3. 3. Milk fatty acid components and K232A variation*

195 Table 4 summarises the associations revealed between DGAT1 K232A variation, and 15 of the
196 individual FAs and 15 of the grouped FAs. Of the FAs detected in this study, results were not presented
197 if an association was not found.
198 Compared with variant K, variant A was associated with lower Total saturated FA content and
199 higher Total unsaturated FA content. Variant A was also associated with lower levels of C6:0, C11:0,
200 C13:0, C16:0, C16:1 and Total LCFA, but increased C13:0 anteiso C14:0, C15:0 iso, C15:0 anteiso, C15:1,
201 C17:1, C18:1 c9, CLA, MCFA, Total C18:1, Total C18:2, Total C18:3, Omega 3, Omega 6, MUFA, PUFA,
202 branched FA, Total UFA, Total index, C18 index and CLA index.
203
204 **Table 4.** Association between milk fatty acid levels and DGAT1 K232A genotypes

FAME	Mean FAME level ± SE[1] (g/100 g milk FA)			*P*
	AA	AK	KK	
	n = 59	n = 183	n = 153	
C6:0	1.519 ± 0.014[b]	1.555 ± 0.009[ab]	1.574 ± 0.009[a]	0.004
C8:0	1.161 ± 0.012	1.193 ± 0.007	1.194 ± 0.008	0.040
C11:0	0.050 ± 0.002[c]	0.058 ± 0.001[b]	0.065 ± 0.002[a]	<0.001
C13:0 anteiso	0.040 ± 0.001[a]	0.039 ± 0.000[b]	0.037 ± 0.000[c]	<0.001
C13:0	0.112 ± 0.003[c]	0.119 ± 0.002[b]	0.126 ± 0.002[a]	<0.001
C14:0	12.946 ± 0.112[a]	12.602 ± 0.066[b]	12.089 ± 0.068[c]	<0.001
C15:0 iso	0.303 ± 0.004[a]	0.298 ± 0.002[a]	0.288 ± 0.002[b]	<0.001
C15:0 anteiso	0.673 ± 0.013[a]	0.649 ± 0.008[a]	0.621 ± 0.008[b]	0.001
C15:1	0.294 ± 0.004[a]	0.287 ± 0.002[a]	0.278 ± 0.003[b]	0.001
C16:0	35.170 ± 0.355[c]	36.697 ± 0.223[b]	38.010 ± 0.250[a]	<0.001
C16:1 *cis*-9	1.163 ± 0.029[c]	1.247 ± 0.019[b]	1.317 ± 0.021[a]	<0.001
C17:0	0.707 ± 0.008[a]	0.684 ± 0.005[b]	0.688 ± 0.005[ab]	0.047
C18:1 c9	14.213 ± 0.214[a]	13.124 ± 0.120[b]	12.634 ± 0.124[c]	<0.001
C18:3 *cis*-9,12,15	0.855 ± 0.015	0.808 ± 0.008	0.778 ± 0.009	<0.001
CLA	1.137 ± 0.047[a]	1.024 ± 0.026[b]	0.934 ± 0.025[c]	<0.001
MCFA	20.104 ± 0.208[a]	19.886 ± 0.124[b]	19.268 ± 0.130[c]	<0.001
LCFA	46.422 ± 0.312[c]	48.016 ± 0.194[b]	49.428 ± 0.216[a]	<0.001
Total C18:1	17.882 ± 0.250[a]	16.692 ± 0.141[b]	16.073 ± 0.146[c]	<0.001
Total C18:2	3.177 ± 0.063[a]	2.960 ± 0.035[b]	2.794 ± 0.036[c]	<0.001
Total C18:3	0.935 ± 0.015[a]	0.882 ± 0.009[b]	0.849 ± 0.009[c]	<0.001
Omega 3	1.066 ± 0.015[a]	1.019 ± 0.009[b]	0.993 ± 0.009[c]	<0.001
Omega 6	0.892 ± 0.011[a]	0.838 ± 0.006[b]	0.809 ± 0.007[c]	<0.001
MUFA	21.190 ± 0.256[a]	20.139 ± 0.147[b]	19.521 ± 0.153[c]	<0.001
PUFA	4.396 ± 0.064[a]	4.120 ± 0.037[b]	3.928 ± 0.037[c]	<0.001
Branched FA	1.642 ± 0.020[a]	1.626 ± 0.012[a]	1.570 ± 0.013[b]	0.001
Total UFA	25.588 ± 0.301[a]	24.260 ± 0.173[b]	23.450 ± 0.179[c]	<0.001
Total SFA	70.634 ± 0.301[c]	72.083 ± 0.185[b]	72.941 ± 0.203[a]	<0.001
Total index	26.583 ± 0.319[a]	25.177 ± 0.183[b]	24.314 ± 0.189[c]	<0.001
C18 index	67.344 ± 0.464[a]	65.629 ± 0.273[b]	64.565 ± 0.289[c]	<0.001
CLA index	28.239 ± 0.385[a]	26.581 ± 0.219[b]	25.558 ± 0.226[c]	<0.001

205 [1]Predicted means and standard error of those means were derived from GLMM. 'Cow age', 'days in milk (DIM)'
206 and 'herd' were fitted as fixed effects.
207 a,bValues within a row with different superscripts differ significantly at $P < 0.05$
208

4. Discussion

The NZ dairy industry produced approximately 33% of the cross-border international milk trade and the main export milk product was milk solids (MS). In a pasture-based system, the ideal cow would therefore be an efficient converter of pasture to milk that contains high levels of these solids. Prior to 2005 pure-bred Jersey and Holstein-Friesian cows were the dominant cattle in the dairy industry, but since then the Holstein-Friesian × Jersey cross-bred has been developed. With the exception of whole milk production levels, Rowarth [9] describes this cross as having the best attributes of both Holstein-Friesian and Jersey cows, such as having lower cow size (a lower maintenance cost, and impact on soil), being more fertile, having improved calving ease and being longer-lived. As a consequence, data (LIC) from the 2016-2017 season reveals that 48% of cows in NZ are now crosses of varying proportion.

It has been suggested previously that DGAT1 variation affects the milk fat levels and milk yield in different breeds of dairy cattle. For example, as separate breeds, both Holstein-Friesian and Jersey cows with variant K produce more milk fat [4, 10-13]. The frequency of K in Kiwi-cross cows described here (0.619) was closer to the high end of the previously reported frequency in NZ Holstein-Friesian cows (0.36 to 0.60) [4, 5], but not as high as reported previously for NZ Jersey cattle (0.88) [5]. Based on New Zealand Breeding Worth (BW) system, Spelman, Ford [5] reported that their Q allele (equal to K) provided a selective advantage in NZ cows, while [4] suggested the K variant could be rapidly incorporated into cattle using artificial insemination. Given the finding here that DGAT1 K was associated with increased milk protein and fat content, but reduced milk volume, the benefit of breeding for increased occurrence of K would therefore seem sensible, esepcially given the positive weighting placed on milk fat percentage and protein percentage traits in the BW evaluation system, and the negative weighting placed on milk volume.

Tabaran, Balteanu [13] have suggested that for Romanian Holstein and Buffalo cows over days 63 to 263 of milking (63 – 263 DIM), higher SFA concentrations in milk, such as for C10:0, C12:0, C15:0, C16:0 and C18:0, could be related to fixation of the K allele. Equally, Duchemin, Bovenhuis [14] identified that Dutch Holstein-Friesian cows with the K variant produce more C6:0, C8:0 and C16:0 in winter (DIM = 63 to 282) and summer (DIM = 97 to 335), and that the interaction between DGAT1 genotype and season was not significant for C16:0 levels. Bovenhuis, Visker [15] investigated the effects of K232A on milk fatty acid composition in late lactation with Danish Holstein-Friesian cattle (DIM = 129 to 229), Danish Jersey cattle (DIM = 130 to 252) and Dutch Holstein-Friesian cattle (DIM = 63 to 282), with the effect of K232A on milk saturated FA levels being different in these three breeds, but all cows with the K variant contain more C16:0 in milk fat. These studies are comparable with what was observed here for KiwicrossTM cows at day DIM = 148 ± 19 days, with there being more C6:0, C11:0, C13:0 and C16:0 in AK and KK cows.

Within pasture grazing systems, it has been suggested that dairy cattle produce more unsaturated FA, but less saturated FA. For example Villeneuve, Lebeuf [16] report that Holstein cows produce less C16:0 but more C18:1, C18:2 and C18:3 when fed fresh pasture, compared to cows fed hay or silage. Capuano, van der Veer [17] investigated milk FA composition in Netherland cows to compare the influence of pasture grazing compared to cattle housed indoors with no fresh grass. They also found that the cows produced less C16:0 but more trans C18:1, cis-9, C18:1, cis-9, trans-11, CLA and C18:3 n-3 (equal to C18:3 cis-9, 12, 15 in our study) in outdoor pasture grazing system. The short and medium chain saturated FA levels (C4:0 to C15:0) weren't affected by different diets in their cows. All the cows in this study were grazing mixed perennial perennial ryegrass and white clover pasture. Table 2 reveals that the cows investigated here produced more cis-9, trans-11 CLA (0.968 ± 0.016 g/100 g) and C18:3 cis-9, 12, 15 FA (0.817 ± 0.005 g/100 g) than the cows described by Villeneuve, Lebeuf [16] (CLA = 0.837 ± 0.016 g/100 g, C18:3 n-3 = 0.568 ± 0.024 g/100 g) and Capuano, van der Veer [17] (CLA = 0.76 g/100 g, C18:3 n-3 = 0.68 g/100 g). The content of CLA (0.46 ± 0.11 g/100 g, 0.62 ± 0.16 g/100 g and 0.39 ± 0.11 g/100 g) and C18:3 cis-9, 12, 15 (0.41 ± 0.08 g/100 g, 0.49 ± 0.10 g/100 g and 0.41 ± 0.11 g/100 g) in Danish Jersey, Danish Holstein and Dutch Holstein cows respectively, reported by Bovenhuis, Visker [15], were also lower than the levels found in the milk of the KiwicrossTM cattle. Their three breeds were feed with mainly grass silage, but minor amounts of whole crop silage, hay, and straw. The difference

in diet may have led to the higher levels of cis-9, trans-11 CLA and C18:3 cis-9, 12, 15 in our cows, but the effects of K232A described by Bovenhuis, Visker [15] were similar to those described here, with cows with the K variant producing less CLA and C18:3 cis-9, 12, 15, but more C16:0 than the cows with the A variant.

In general, the associations between DGAT1 K232A and the levels of milk FAs, especially for C16:0, CLA and C18:3 cis-9, 12, 15, were consistent with what has been reported in other breeds, but some exceptions were found. For example, Juhlin, Fikse [18] reported that there were no significant difference on the most milk FAs (except C16:0 and CLA levels) between their AA and AK cows. In addition, Carvajal, Huircan [19] didn't find any associations between K232A and C16:0, C16:1, C18:1, CLA and MUFA levels. All the cows Carvajal, Huircan [19] investigated were fed predominantly pasture (supplemented with conserved forage such as silage, hay, and maize during autumn and winter). One possible reason to explain the inconsistency with the results of Carvajal, Huircan [19] and Juhlin, Fikse [18], was that the samples they investigated were collected from over a whole lactation period (about 1 year), when the effects of K232A on milk FAs identified by Duchemin, Bovenhuis [14], Tabaran, Balteanu [13], Bovenhuis, Visker [15] and us were from the cows sampled after the 60th day in milk. Except the influences of diet and animal genetic, the animal body fat mobilization also affected milk fat in early lactation stage [20]. As 90% of FAs in adipose tissue were C18:1cis-9, C16:0, and C18:0 , Garnsworthy, Masson [21] suggested that the body fat mobilization would increase the transportation of these FAs into milk fat directly. After investigated the milk fatty acid compositions in cows with same diets but different lactation stages (day in milk = 13, 130 and 283), Garnsworthy, Masson [21] found that the milk FA compositions in early lactation stage were different with the compositions in other stages significantly on C10:0, C12:0, C14:0, C16:0, C14:1, C16:1, C18:1 and C18:2 cis-9, trans-11 levels.

5. Conclusion

This study described the associations between DGAT1 K232A and milk traits and milk FA composition in NZ pasture-fed HF × J-cross cows. The effect of K232A on milk FA component levels, especially on C16:0, CLA and C18:3 cis-9, 12, 15 levels, was notable in late lactation.

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