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Genetic Variation of the *DGAT1* gene in Dual-Purpose Dairy Cows and its Influence on Economically Important Breeding Traits

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Abstract

Background

Over the last 10 years, the discovery of individual, or candidate, genes that influence economically important traits has revolutionised how the dairy industry selects cows for breeding programmes. One such gene, the diacylglycerol O-acyltransferase 1 (*DGAT1*) gene is significant in dairy production as it is associated with the synthesis of fat in milk, and polymorphisms within the gene can be used for selection purposes. In this study, the variants (genotypes) of the K232A polymorphism of the *DGAT1* gene, within a dual-purpose dairy herd, bred for both dairy and beef production, were determined, and compared to the Economic Breeding Index (EBI) and EBI sub-indexes to ascertain if any statistically significant relationships existed.

Methods

DNA was extracted from hair samples, and the genotypes of the *DGAT1* gene were determined using a combination of conventional PCR, restriction fragment length polymorphism (RFLP), gel electrophoresis, DNA sequencing and novel real-time PCR assays.

Results

The three genotypes within the herd were: KK (n = 5), AA (n = 10), KA (n = 8). KK denoted a homologous Lysine allele, AA denoted a homologous Alanine allele, and KA denoted a heterologous Lysine allele. There was no association found between the milk production traits and the *DGAT1* K232A genotype ($P = 0.178$). However, there was a significant association found between the K232A polymorphism and other EBI traits including 'Beef' and 'Management' which had P values of 0.035 and 0.023 respectively. The AA genotype was associated with higher beef traits (higher carcass values) and lower management traits (reduced milking time and good temperament). The KK genotype was associated with higher management traits and lower beef traits. These results were not always consistent with those in the literature.

Conclusions

This study showed that animals with the A allele of the *DGAT1* gene polymorphism may be better suited for dual-purpose dairy herds. It is also possible that different breeding goals and farm environments may influence the results suggesting that these types of studies should be conducted on a variety of herds and include larger samples sizes.

Keywords: *DGAT1*, Real-Time PCR, PCR, Animal Breeding, Dairy Science

1. Introduction

The Economic Breeding Index (EBI) is a profit index aimed at helping dairy farmers identify the most profitable animals in the herd (Berry, *et al*., 2005). It consists of information on seven sub-indexes related to profitable milk production, all based on genetic heritability. The EBI is a Euro rating assigned to each of these indexes, and provides each cow with an overall EBI value. The indexes include: milk production, fertility, calving performance, beef carcass, cow maintenance, cow management and health. The EBI value is based on the amount of genetic benefit the dam's offspring will have compared to the 'base cow'. A higher overall EBI figure will represent a more productive and economic animal, as has been seen in recent studies (O'Sullivan, *et al*., 2019). The EBI system is managed by the Irish Cattle Breeding Federation (ICBF), a non-profit organisation that provides cattle breeding information to the Irish dairy and beef industries www.icbf.com.

The integration of state-of-the-art genetic and genomic selection tools into animal breeding programmes accelerates the rate of genetic gain. Furthermore, different dairy systems such as dairy-beef enterprises, grass-based systems and non-grazing systems each require tailored breeding programmes, and individual based evaluations to understand the relationships between the various traits and genotypes, and how they interact in different environments (Dillon, 2018).

One such gene targeted in genetic selection studies is the diacylglycerol O-acyltransferase 1 (*DGAT1*) gene, with the gene encoding for an enzyme that plays a vital role in the synthesis of triacyclglycerols. DGAT initiates the final step in triacylglycerol production by using fatty acyl CoAs and diacylglycerol as substrates. The DGAT enzyme is encoded by the *DGAT1* gene (Cases, *et al*., 1998). The *DGAT1* gene is found on exon eight of chromosome 14 in the *Taurus bos* genome. Polymorphisms at K232A in the *DGAT1* gene are linked to a quantitative trait locus (QTL) for milk fat content (Kühn, *et al.,* 2004). The alanine allele (A) is represented by the guanine/cytosine nucleotides and the lysine allele (K) is represented by the adenine/adenine nucleotides. The GC/AA polymorphism is located at positions 10,433 and 10,434 of the *DGAT1* gene (Winter, *et al*., 2002).

Different genetic variations within the *DGAT1* gene can have major effects on milk fat percentage, protein percentage, and overall milk volume (Littlejohn, *et al.*, 2014). Shennick and co-authors (2007) found that the *DGAT1* A allele was associated with a greater unsaturated Fatty Acid (FA) level in milk, and suggested that selective breeding focusing on the A allele would give milk with a more sought after FA composition. However, Juhlin and co-authors (2012) determined that milk produced from dairy cows with the A allele had an increased vulnerability to form an oxidized milk flavour due to the higher concentration of long-chain FA.

The K232A genotype of the *DGAT1* gene has a direct influence on the animal's metabolism. The polymorphism influences milk production traits that impact the total energy output in milk, and subsequently, the overall energy balance of cows. A negative energy balance post-partum in high-yielding dairy cows can result in reproductive and metabolic disorders. Cows with the KA genotype had significantly higher values for milk energy output post-partum compared to AA or KK cows (Bovenhuis, *et al.*, 2015). Therefore, the *DGAT1* gene can be associated with several economically important selection traits.

The aim of this study was to determine the genetic variants of the of *DGAT1* gene from a dual-purpose dairy herd using a combination PCR and RFLP analysis, and novel real-time PCR assays to genotype DNA extracted from hair. The secondary aim was to determine if genetic variants of the *DGAT1* gene were associated with the various EBI traits of the animals, which could be used to inform future breeding decisions on the farm.

2. Materials and Methods

Animal Selection and Milk Traits

Twenty-three animals were selected at random from a herd of 140 dual-purpose dairy cows based in Borrisokane in North Tipperary, Ireland. The herd has a variety of breeds including Holstein, Friesian, Norwegian Red, Montbeliarde, Rotbunte, and various crosses of the breeds. The herd has a wide variation in EBI and EBI sub-indexes as the breeding goals for the herd is to produce animals that can provide an income from both milk and beef (Table 1). In 2017, the herd produced 631,978 litres of milk, and 50,225 kg of milk solids. The milk contained 4.16 % fat, and 3.56 % protein. All cows analysed in this study were part of this dataset. The herd is served via Artificial Insemination (AI) using various stock bulls offered by Dovea Genetics, Thurles, Co. Tipperary. All breeding decisions are made in early January. The herd had not been previously genotyped, and milk recording does not take place.

Sample Collection

Twenty-three animals were removed from the herd after milking and held in a collecting yard until the milking was finished. Hair samples were taken from the tail of the animal with a sterile scissors and the samples were placed in a small plastic sample bag that was labelled with the jumbo tag number and the sample number of the animal. The scissors were sterilised in between samples with an alcohol wipe that removed any hair present, and they were placed in a tub of methylated spirits when not in use. There was no focus made on the collection of hair follicles, which is often considered important in genotyping studies when using DNA extracted from hair. The samples were frozen at -20 °C before DNA extraction. All associated EBI data (Table 1) was downloaded from ICBF.

DNA Extraction

DNA was extracted using the ZR Genomic DNA™ II – Tissue MiniPrep Kit (Zymo Research). Hair was cut and transferred into an Eppendorf tube, and 90 µl of molecular grade water, 90 µl of 2X Digestion Buffer, 10 µl of Proteinase K and 10 µl of 1M DTT was added to each of the Eppendorf tubes, and the samples were digested for a minimum of one hour at 56 °C. Darker hair was generally thicker and took longer to digest, and was digested for a maximum of three hours. The samples were vortexed intermittently to mix the samples and to aid hair digestion. The samples were purified according to the manufacturer's instructions, and the DNA was eluted in 100 µl of molecular grade H_2O. The extracted DNA was stored at -20 °C.

Genotyping

A 411-bp fragment of the *DGAT1* gene that contained the K232A mutation was amplified, via conventional PCR, using the primers developed by Winter and co-authors (2002). The primer forward and reverse primer sequences were: Forward 5`-GCACCATCCTCTTCCTCAAG-3` and Reverse 5`-GGAAGCGCTTTCGGATG-3`. The primers were purchased from Eurofins Genomics, Germany in a lyophilised state and were re-suspended in molecular grade H_2O to a stock concentration of 100 pmol/µl and diluted to a working concentration of 5 pmol/µl (5 µM), containing both the forward and reverse primers. The 10 µl PCR reaction contained 5 µl of GoTaq Hot Start Green Master Mix (Promega), 1 µl of the primer mix containing the forward and reverse primers, 2 µl of molecular grade H_2O and 2 µl of DNA. For negative controls, 2 µl of molecular grade H_2O was instead of the DNA. A beef tissue DNA extract (2 µl), standardised to 2 ng per µl was used as a positive control. The PCR conditions were set to 15 min at 95 °C; 35 cycles of 60

seconds at 94 °C, 60 seconds at 60 °C, 60 seconds at 72 °C; a final 7-minute extension at 72 °C, and then held a 4 °C, using a 2720 Thermal Cycler (Applied Biosystems).
Gel electrophoresis was used to visualise the PCR products, and to determine which samples were suitable for RFLP analysis. A 2% (w/v) agarose gel was used, and 1 µl of a 50-bp DNA ladder (Zymo Research) was placed in the first well of the gel, followed by 2 µl of each of the PCR products in the subsequent wells. The gel was visualised by exposing it to UV light using the SynGene Bio-imaging Software. The samples that contained positive bands were deemed to contain suitable levels of DNA and were taken forward for RFLP analysis. The remaining samples which were likely to have lower quantity and quality DNA were tested using real-time PCR.
For the RFLP analysis, the PCR products (5 µl) were digested with 2 µl of *EaeI* restriction enzyme (New England Biolabs), 1.5 µl of the 10 X buffer and 15 µl of molecular grade H_2O. The samples were digested at 37 °C for one hour on a heating block. The digested PCR products were separated and visualised along with a 50-bp ladder via gel electrophoresis and exposure to UV light as before. The K232A polymorphisms were identified by analysing the differential migration of the DNA fragments.

DNA Sequencing
One sample of each of the identified K232A variants, or genotypes, was used for DNA sequencing. PCR samples were purified using the Clean and Concentrator-25TM kit (Zymo Research), as per the manufacturer's instructions. The quantity and quality of the DNA was measured using the NanoDrop® ND-1000UV-Vis Spectrophotometer (ThermoFisher). The purified PCR product was mixed, at a concentration of 20-80 ng/µl, with 5 µl of the forward primer used above. Molecular grade H_2O was added to make a final volume of 10 µl. The samples were sequenced in one direction by GATC – Biotech, Germany using the Lightrun service.

Real-Time PCR
Primers for real-time PCR were designed based on the resulting *DGAT1* sequences. The forward primer GC-5`- CGTAGCTTTGGCAGGTAAGGC-3`was designed for the amplification of the A allele (lysine) polymorphism, and the forward primer AA-5`-CTCGTAGCTTTGGCAGGTAAGAA-3` was designed to amplify the K allele (lysine). The primers were designed with the specific polymorphism on the 3' end of the primer, as underlined in the primer sequences. The same reverse primer AA/GC-5`-AGGTCAGGTTGTCGGGGTAG-3`was used to amplify both variants, generating a 74-bp product for the A allele and a 76-bp product for the K allele. Equal amplification by both the GC and AA forward primers would detect the GC and AA variant (KA), a heterozygote animal. Whereas, a higher Ct value would be expected to be obtained in the absence of either the K or A allele variant indicating a homozygote animal for the respective allele with the lower Ct value. Primers were designed using the Primer Design Tool on the NCBI website https://www.ncbi.nlm.nih.gov/tools/primer-blast (Ye, *et al.*, 2012). Primers were ordered from Eurofins Genomics, Germany and reconstituted with molecular grade water as described previously.
The hair samples were serially diluted to test the efficiency of the real-time PCR assays. A serial dilution of each of the three known gene variants (determined using RFLP analysis and DNA sequencing), KK, KA and AA were prepared by serially diluting the hair DNAs from 10^{-1} to 10^{-5}. Each variant was tested with the GC and the AA primer in triplicate to produce a standard curve for each variant. Real-time PCR was performed in a 10 µl volume consisting of 5 µl of FastStart SYBR Green Master mix (Roche), 0.4 µl of primer mix (prepared as before), 2 µl of DNA and 2.6 µl of molecular grade H_2O. The negative controls

were prepared by adding 5 µl of FastStart SYBR Green Master mix, 0.4 µl of the primer mix and 4.6 µl of molecular grade H_2O. Each of the remaining DNA samples was subsequently amplified in duplicate using both the GC and AA primer pairs. Negative controls were as described above, and a hair DNA sample of each genetic variant was used as a positive control (2 µl). It was not possible to standardise the DNA for the positive controls as the NanoDrop cannot be used quantify DNA extracted from hair. However, during the initial testing, the DNAs were not found to be inhibited and were deemed as suitable positive controls for detection of the polymorphisms. The instrument used for the amplification was the Applied Biosystems 7300 Real-time PCR System, using the default settings. The PCR reaction conditions were: 10 min at 95 °C, 50 cycles of 60 s at 95 °C, 60s at 60 °C 60s at 72 °C and a final extension in 7 min at 72 °C. A dissociation step of 15 s at 95 °C, 30 s at 60 °C and 15 s at 95 °C was added for melt curve analysis to confirm specific amplification. The dissociation curve identified the melting temperature of the DNA (dissociation of the double strand of DNA). The dissociation step gradually increases the temperature of the plate, and as the amplicon melts, the decrease in the SYBR Green dye is recorded.

The results were recorded in Microsoft Excel and interpreted using Minitab. The animals were divided according to their genotype of the *DGAT1*. Their corresponding EBI and EBI sub traits were statistically compared based on the *DGAT1* genotypes. The traits analysed included EBI, milk production, fertility, calving difficulty, beef, carcass, maintenance, health, and management (Table 1). One-way ANOVA functions were used to determine if the EBI index and sub index data differed significantly by genotype. Any trait that had significant statistical difference with a corresponding P value less than 0.05 was individually plotted to further explore the association between the genotype and the trait. All statistical analyses were completed using Minitab.

3. Results

The 23 samples analysed in this study were all successfully genotyped using a combination of PCR, RFLP analysis, DNA sequencing and real-time PCR. In terms of the RFLP technique, the samples that produced a clean and bright PCR product were subsequently taken forward for the RFLP analysis. The polymorphisms were differentiated using the following criteria; the 411-bp fragment was identified as a KK variant, the smaller fragment at 203-bp and 208-bp was identified as the AA variant, and when both bands were present, a KA variant was identified. The animals that were identified using this method are listed in Table 1.

Real-Time PCR

An example of each genotype was DNA sequenced, and the resulting sequences were used to design real-time PCR assays. The newly designed assays were found to amplify the SNPs efficiently, and the Ct value consistently increased with decreasing DNA template (data not shown). Real-time PCR was used on the samples that did not successfully amplify during the conventional PCR. The two assays were designed to amplify either the AA or the KK variant, with at least a three cycle difference between the final Ct values. For example, if the A allele primers amplified with a Ct value 29, and the K allele primers amplified with a Ct value of 32 or more, then the sample was considered to only contain the A variant, or vice versa. In the case that an animal had both variants, (KA) both assays were simultaneously amplified. All samples were amplified in duplicate to ensure consistency (Appendices 1 and 2 detail example amplification curves, with the results of the real-time PCR noted in Appendix 3). The average Ct values for successful detection of the K allele range from 25.04 ± 0.05 to 30.4 ± 0.73 (Tm 82°C ± 0.34°C), while the average successful Ct values for

the A allele ranged from 21.29 ± 0.05 to 30.3 ± 0.83 9 (Tm 80.8°C ± 0.48°C). If replicates were more than one cycle apart, they were repeated.

The most commonly detected genotype in the herd was the AA variant (n = 10). Eight animals had the KA variant and five animals had the KK variant. Table 1 shows the traits of the animals tested, and their genetic variant of the *DGAT1* gene determined from either the conventional PCR and RFLP approach, or the real-time PCR method.

Table 1: Data of each cow's *DGAT1* genotype, method of genotyping, and associated EBI data. Details of each cow's overall EBI value, and associated sub-index values for each trait analysed in this study were obtained directly from ICBF.

Cow Number	*DGAT1* Variant	Method of Detection	EBI (€)	Milk (€)	Fertility (€)	Calving Difficulty (€)	Beef (€)	Maintenance (€)	Health (€)	Management (€)
1	KK	qPCR	50	-52.8	80.4	16.6	12.5	-9.9	6.3	-3.5
2	AA	RFLP	72	-9.6	82.3	2.2	3	3.3	1.3	-10.3
3	AA	RFLP	71	11.2	67.5	-3.7	19.1	-11.6	-0.9	-10.5
4	AA	RFLP	114	-9.7	91.8	28.6	-1.7	6.6	2	-3.6
5	AA	RFLP	63	21.2	33.8	6.1	15.8	-6.6	-0.8	-6.2
6	KA	qPCR	80	21.2	53.2	1	13	-1.7	-0.8	-6.2
7	KK	RFLP	33	-32.2	38.9	14.6	-8.9	8.3	6.7	5.7
8	KA	RFLP	64	0.1	53.9	12.8	9.8	-3.3	-4.3	-4.5
9	AA	qPCR	41	-11	27.1	29	-2.5	9.9	-6.3	-5.7
10	KK	RFLP	80	21.7	46.8	10	-13	5	4.4	5.5
11	KA	qPCR	104	9.2	69.4	18.2	-7.7	13.2	-1.6	2.8
12	KK	RFLP	71	5	66	5.1	-9.5	14.9	-5.1	-5.1
13	KA	RFLP	45	17.2	16.9	16.9	-9.1	4.1	-2.6	2
14	KK	RFLP	0	17.7	-13.1	4.3	-13	5	-0.3	-0.7
15	AA	qPCR	130	44.5	65	19.4	9	-5.8	4.6	-7
16	AA	RFLP	65	10.7	38.1	3.8	9.9	0	6.7	-4.5
17	AA	RFLP	115	28.7	69	24.2	0.6	0.8	-2.5	-6
18	KA	qPCR	101	20.5	56	23.4	0.3	1.7	2.6	-3.2
19	KA	RFLP	108	46.7	45.8	17.5	2.1	-9.1	1.1	3.8
20	AA	RFLP	9	-5.2	9.1	13.2	17	-15.7	-5.1	-4
21	KA	RFLP	148	21.2	78.6	39.6	-0.6	5	7.4	-3.4
22	AA	RFLP	117	31	42.8	31.8	-1.1	9.1	4.7	-1.6
23	KA	qPCR	60	-10.8	56.4	4.5	2.5	14	2.6	-9

The animals were subsequently grouped into three categories according to their respective *DGAT1* genotype and the associated trait data was statistically compared using the ANOVA function in Minitab. A trait was considered to be significantly associated with a particular genotype, when the P value was 0.05 or less. In this study, only the "Beef" and "Management" traits were found to be significantly associated with the *DGAT1* genotype (Appendix 4). The P value for the beef trait was 0.035, indicating a significant statistical difference between the three genotypes and the beef value. The P value for the management trait was 0.023, indicating that this trait was also significantly influenced by the genotype. To further understand how each of the genotypes influenced the trait values, the data was re-plotted (Figure 1 a & b). The AA genotype was associated with the highest beef values, while the KK animals were associated with the lowest values, and the AA genotype had the lowest management value, while the KK had the highest.

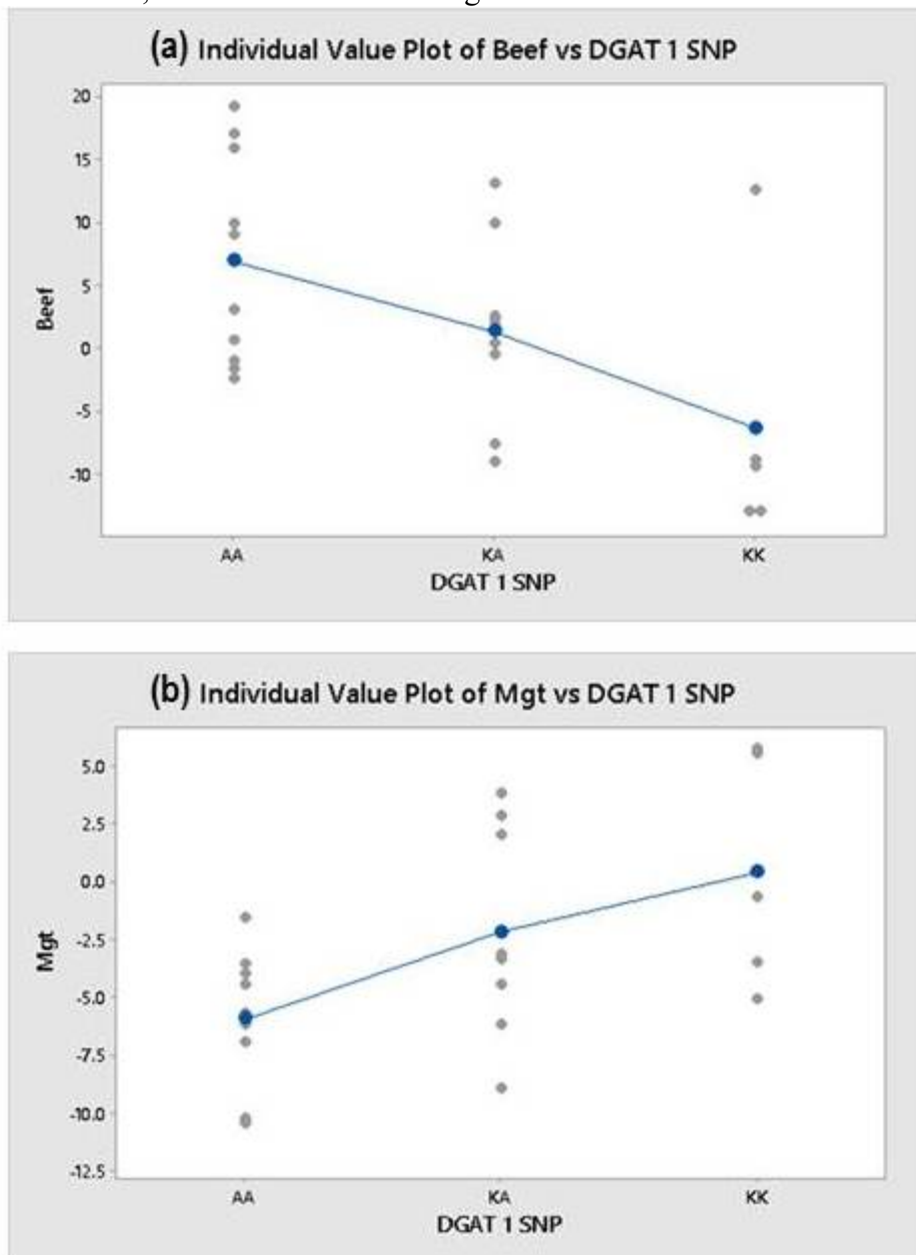

Figure 1: Individual value plot of the beef (a) (p = 0.035) and management (b) (p = 0.023) traits in relation to *DGAT1* genotype. The grey points represent the values associated with each individual animal, and the blue points and plotted blue line shows the mean values.

Science Undergraduate Research Experience Armitage *et al*
Volume (1), Issue (1)

4. Discussion

The *DGAT1* gene is of interest to dairy farmers due to its association with milk quality and quantity. In this study, 23 cows from a dual-purpose dairy farm were genotyped to reveal one of three genetic variants of the *DGAT1* gene. The animals were genotyped using one of two methods using conventional PCR and RFLP analysis or with the use of novel real-time PCR assays. The sample size used in the study was small, and the results should be interpreted with this limitation in mind.

The breakdown of the genotypes found in this study was as follows: 43% AA, 35% KA and 22% KK. This result was inconsistent with the study carried out by Koopaei and co-authors (2012), who found that KA (57%) was the most prevalent genotype in Iranian Holstein-Friesian herds, followed by the AA (34%) and KK (9%). The findings were, however, consistent with the study carried out by Berry and co-authors (2010) who found the AA (47%) was the most common genotype in Irish Holstein-Friesian herds, followed by the KA (42%) and KK (11%). Berry and co-authors (2010) concluded that the variation of the results across different studies may have arisen due to differences in past breeding goals.

The real-time PCR protocol was developed in this study using known genetic variants which were identified via RFLP and DNA sequencing. Both assays amplified equally for the KA genotype, while samples containing the KK or AA genotype were found to preferentially and reliably amplify with either the K or A primer pairs, respectively. A minimum of three Ct cycles occurred when only one of the alleles was present. In terms of the dilution series, the Ct values were found to consistently increase as the DNA template decreased, with the Ct values increasing approximately by three cycles for each consecutive 10-fold dilution. However, the starting DNA template could not be standardised in this study as the DNA had originated from hair. Further validation using DNA extracted from tissue samples, firstly identified to each genotype, could in the future be used to accurately calculate primer efficiencies using standardised tissue samples.

The advantage of the real-time PCR method developed in this study for genotyping of the samples is that it is quicker and more automated than the RFLP method, making it efficient to test large sample sizes. The real-time PCR is more sensitive than the RFLP method as the fragment of DNA amplified is smaller facilitating lower quality DNA from hair samples to be analysed which may make it easier for farmers to collect and process samples from large herds, while also being less stressful for the cow. The use of SYBR Green chemistry avoids the need to purchase TaqMan probes, typically used for SNP genotyping studies. TaqMan probes typically cost in excess of €300 each, and two would be required to amplify the K and A allele in this case. Such costs would be prohibitive in an undergraduate research project.

The EBI and the sub-EBI traits were compared across the three different genotypes to determine if there were any statistically significant differences between the traits. The P value is an indication of statistical differences between populations, a P value of 1 would indicate that there is no difference whatsoever between the populations and implies that all the data was the same regardless of genotype. If a trait had a P value below 0.05 then it is considered to have significant differences across the genotypes. Studies carried out by Winter and co-authors (2002) and Kaupe and co-authors (2004) found that the *DGAT1* genotypes influenced milk production, but in this study, there was no statistical association found between the genotypes for milk production traits with a P value of 0.178. While a low value does indicate that the genotype of the animal may have some association with milk traits, it was not significant enough to investigate further, in this instance.

The results of this study indicated that there was a correlation between the genotype of the animal and the beef traits of the animals. The beef sub index of the animal is an economic value derived from the animals projected cull cow weight, carcass weight, carcass information and carcass fat. These values are influenced by the performance of the animal's

relatives and the genotype of the animal. The animals with the AA variant of the K232A polymorphism of the *DGAT1* gene were associated with a higher beef value followed by the KA and KK genotypes. The mean Euro value for the AA genotypes was €6.91, KA was €1.29, KK was €-6.38. The standard deviations for all the genotypes were high and a consequence of the wide variation of values within each of the traits, an issue compounded by the low number of samples tested. The positive association of increased beef traits of the AA genotype is inconsistent with the findings of Thaller and co-authors (2003) who found the KK genotype to have a positive effect on intramuscular fat and other beef traits in German Holstein Cows. Our result was also inconsistent with the findings of Berry and co-authors (2010) who found no association between the K232A polymorphism and body fat traits. However, Oikonomou and co-authors (2009) found a significant association between the AA genotype and the body condition score (BCS), a technique used to assess the level of fat, of the cows, while the KK genotype was associated with a lower BCS. The reason Berry and co-authors (2010) found no association with the K232A polymorphism may be caused by the animals that they sampled which were from high yielding Holstein- Friesian dairy cows where beef traits were not a priority in the breeding programme.

The trait that exhibited the greatest difference between the K232A polymorphism genotypes was the management trait. The management trait had a P value of 0.023. The genotype of the animals had more influence on the management trait than the other traits analysed, a novel finding from this current study. The management trait is linked with reduced milking time and better milking temperament, an important consideration for farmers considering expansion of their dairy herd. The AA genotype was associated with a lower management value indicating that they milk faster and are calmer in the milking parlour. Haskell and co-authors (2014) found that there are several QTLs that influence behavioural traits, but the QTLs mentioned in that study did not include the *DGAT1*.

5. Conclusions

This study was carried out to determine the genotype of 23 dairy cows from a mixed dairy/beef farm in in relation to the K232A polymorphism in the *DGAT1* gene. Sixteen samples were genotyped via conventional PCR, RFLP and gel electrophoresis, while the remaining seven samples were genotyped using real-time PCR. In total, 10 animals had the AA genotype, eight animals had the KA genotype, and five animals had the KK genotype.

There was no statistical association found between the milk traits and the three genotypes. There was however, a significant association found between the genotypes and the beef and the management traits. The AA animals had higher beef trait values followed by the KA and KK animals. The AA animals had low management values in comparison to the KA and KK animals. Animals with the A allele of the *DGAT1* gene may be better suited to a dual-purpose dairy/beef enterprise as their offspring are likely to possess good beef and management traits. Further testing using the novel real-time PCR assays and an increased sample size can be used to confirm these findings in the future.

6. Future Work

There was some discrepancy between this study and other studies carried out in relation to the effect that the *DGAT1* gene may have on milk production. The herd that was chosen for this study consisted of animals of various breeds that are not typically bred for high milk yields. Future studies should incorporate cows from different dairy systems e.g. dairy to beef, zero grazing and grass-based systems to better understand how the environment may influence the traits.

There are some contradictions within the literature in terms of *DGAT1* and its influence on intramuscular fat. This study showed that there was an association between the K232A polymorphism of the *DGAT1* gene and beef traits. A study could be carried out to definitively determine if the gene has an influence on intramuscular fat production in cattle by comparing the animals' genotype and associated post slaughter carcass data. The findings of this study indicated that the *DGAT1* gene may have an influence on the temperament and milking behaviour of dairy cows. Future studies might undertake behavioural and observational studies in combination with *DGAT1* genotyping to further investigate this preliminary finding.

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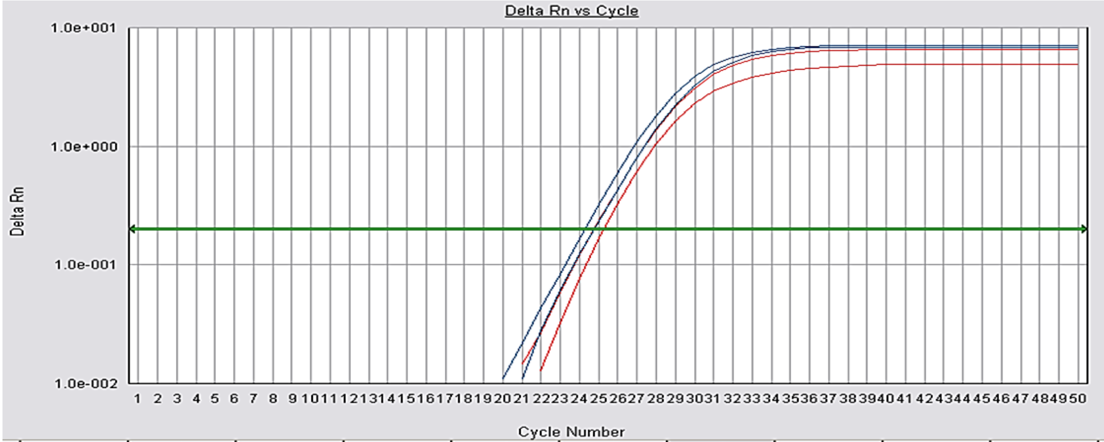
Appendices

Appendix 1: Real-time PCR amplification curves of a sample amplified in duplicate with both the KK and AA primers. In this case, the blue amplification curve representing the K allele PCR product amplified efficiently in duplicate. The A allele products had much higher Ct values and did not amplify consistently. It was concluded that this animal had the KK genotype, a homozygote.



Appendix 2: Real-time PCR of a sample successfully amplified with both the KK and the AA primers amplified simultaneously. It was concluded that the sample contains the KA variant, a heterozygote.

Appendix 3: Average real-Time PCR Ct values for samples amplified for both the K and A alleles. The average (Avg) Ct value, standard deviation (Std Dev) and the Tm or melting temperature for each assay is provided. The final genotype derived from both assays is also included.

Cow Number	Avg Ct (K)	Std Dev	Tm	Avg Ct (A)	Std Dev	Tm	Genotype
9	30.03	0.416	82.2	26.62	0.304	81.5	AA
15	26.55	0.015	81.5	21.29	0.049	81.5	AA
6	29.5	0.732	81.5	29.1	0.132	80.6	KA
11	27.12	0.071	82.4	27.58	0.295	80.6	KA
18	25.04	0.005	82.2	25.23	0.083	80.6	KA
23	30.4	0.731	81.9	30.3	0.846	80.5	KA
1	29.5	0.311	82.05	32.2	0.325	80.6	KK

Appendix 4: The EBI and EBI sub traits corresponding P value, a value below 0.05 indicates significant statistical differences between the three genotypes in relation to the traits

Trait	P Value
EBI	0.131
Milk	0.178
Fertility	0.778
Calving Difficulty	0.583
Beef	**0.035**
Maintenance	0.425
Heath	0.666
Management	**0.023**