

## Marker-Assisted Selection Potential in Kedah-Kelantan Cattle: *DGAT1* Gene Linked to Improvement in Growth Performance Traits

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### ARTICLE HISTORY

Received : 13 May 2025

Accepted : 28 August 2025

Online : 30 June 2026

### KEYWORDS

Kedah-Kelantan cattle, single nucleotide polymorphism (SNP), Marker-assisted selection (MAS), *DGAT1* gene

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### ABSTRACT

Kedah-Kelantan (KK) cattle, an indigenous *Bos indicus* breed from Malaysia, are valued for their high fertility and adaptability to local environments but their slow growth rates hinder their contribution to Malaysia's beef industry. Identifying single nucleotide polymorphisms (SNPs) in the *DGAT1* gene that are associated with growth traits (heart girth, body length) and fertility traits (calving interval, age at first calving) is critical for enabling marker-assisted selection (MAS). SNPs enhance MAS for economically important traits in cattle, but their efficacy requires breed-specific validation. This study aimed to identify SNPs in the *DGAT1* gene associated with growth and fertility traits in KK cattle to enhance MAS. Genotyping of blood samples from 30 mature KK cattle was conducted using polymerase chain reaction (PCR), yielding a 411 bp *DGAT1* fragment in all samples. Phenotypic data comprised heart girth (HG) and body length (BL) for growth, as well as calving interval and age-at-first calving for fertility assessment. Association analyses revealed significant relationships between *DGAT1* SNPs and HG ( $p = 0.036$ ) and BL ( $p = 0.024$ ), while no associations were found for fertility traits. These findings elucidate the genetic architecture of growth in KK cattle, address a knowledge gap in indigenous breed genomics, and support targeted breeding strategies aimed at enhancing Malaysia's beef industry sustainability.

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## 1. INTRODUCTION

The increasing global demand for sustainable beef production has spurred advancements in cattle genetics aimed at improving productivity, carcass quality, and fertility. Molecular markers, particularly single nucleotide polymorphisms (SNPs), have emerged as powerful tools in marker-assisted selection (MAS), enabling the identification of animals with superior traits while overcoming the limitations of conventional breeding methods (Singh et al., 2014; Zalewska et al., 2021). In Malaysia, Kedah-Kelantan (KK) cattle, indigenous KK cattle, which comprise 40% of the national beef population, offer significant potential for genetic enhancement due to their high fertility, heat tolerance, and genetic diversity (Department of Veterinary Services Malaysia, 2023; Islam et al., 2021). Despite these advantages, their small size and slow growth rate hinder their contribution to Malaysia's beef industry, which achieved only 14.7% self-sufficiency in 2022 (Department of Veterinary Services Malaysia, 2023). Addressing these challenges requires innovative breeding strategies to unlock the potential of KK cattle.

While SNP, such as those in genes like *SCD*, *FASN*, *DGAT1* and *PLAG1*, can positively influence carcass and fertility traits in breeds like Japanese Black cattle (Kawaguchi et al., 2020). However, the effects of these genes on non-targeted traits remain underexplored. For instance, genetic selection in Holstein cattle has been linked to reduced fertility, highlighting the need of evaluating the marker effects across traits and populations (Ma et al., 2021). In KK cattle, no studies have investigated SNP-based MAS for growth traits (e.g. body weight, growth rate) or fertility traits (e.g. calving rate), despite their genetic diversity and regional importance. This knowledge gap limits the application of MAS in improving beef production in Malaysia, particularly in tropical environments suited for KK cattle. The decline in beef self-sufficiency underscores the urgency to develop targeted breeding programs that leverage the unique genetic profile of KK cattle to meet national and global demands.

The *DGAT1* gene encodes the microsomal enzyme acyl-CoA:diacylglycerol acyltransferase, which catalyzes the last step of triglyceride synthesis. The gene is located on

chromosome 14 of the cattle genome (Cases et al., 1998). consists of 17 exons and 16 introns. A prominent SNP (K232A) has been identified in the gene. This SNP results in the substitution of genotypes AA to GC, leading to an alteration of amino acid sequence from lysine to alanine in exon 8 of *DGAT1* (Grisart et al., 2002; Winter et al., 2002). *DGAT1* has been found to be associated with meat and milk quality (Anton et al., 2011; Li et al., 2013; Mao et al., 2012; Winter et al., 2002) in both dairy and beef cattle. Significant correlations have been observed between *DGAT1* SNPs and various growth and fertility traits (Hill et al., 2016; Kawaguchi et al., 2020). However, research findings have shown variability regarding which allele is deemed favourable for specific production traits.

This study aims to enhance beef production in Malaysia by exploring the *DGAT1* SNP marker for genetic improvement of KK cattle. The objective is to identify and evaluate the correlations between the *DGAT1* SNP and growth and fertility traits, thereby establishing marker-assisted selection (MAS) as a feasible strategy for breeding high-performing animals. Specifically, this research will identify *DGAT1* genotypes associated with growth and fertility traits in KK cattle and investigate their correlations with phenotypic data. As the first study to examine *DGAT1*-based MAS in KK cattle, this work provides novel insights into their potential as a high-value beef source, supporting sustainable cattle production in tropical regions. Findings will support breeding programs, contributing to Malaysia's beef self-sufficiency goals and advancing global veterinary science.

## 2. MATERIALS AND METHODS

### 2.1. Ethics approval

All procedures performed were reviewed and approved by the Institutional Animal Care and Use Committee of Universiti Malaysia Kelantan (IACUC-UMK). (Approval Code: UMK/FPV/ACEU/PG/004/2022).

### 2.2. Animal selection and sampling

A total of 30 female KK cattle was collected, with 10 mL of blood drawn using an 18G vacutainer needle from the coccygeal vein. Cattle, aged one year and above, were sampled from farms around Kelantan. Selected growth traits and fertility traits parameters were measured or obtained from the farmers, including the age-at-first calving and calving interval.

Selected growth traits parameters, including HG and BL of the cattle were measured. HG was measured by assessing the body circumference immediately behind the front shoulder at the fourth ribs, posterior to the front leg, while BL was determined by measuring the distance from the highest point of the shoulders to the pin bone (Lukuyu et al.,

2016). Both measurements were taken using a standard measuring tape.

### 2.3. Molecular detection of *DGAT1*

#### 2.3.1. DNA extraction

Genomic DNA was extracted from blood samples using gSYNC DNA Extraction Kit (Geneaid, Taiwan), following the manufacturer's protocol.

#### 2.3.2. DNA amplification and electrophoresis

DNA amplifications were performed with PCR using forward primer: GCACCATCCTCTTCCTCAAG and reverse GGAAGCGCTTTC with the following condition: 94 °C for 5 minutes, 30 cycles at 94 °C for 30 seconds, 30 seconds at 58 °C, 40 seconds at 72 °C, 5 minutes at 72 °C with PCR components in listed in Table 1 (Lacorte et al. (2006).

**Table 1:** PCR components and their respective volumes per reaction.

Components	Volume (µL)
GoTaq Green Master Mix	12.5
Forward primer	0.5
Reverse primer	0.5
Extracted DNA	10
Nuclease-free water	1.5

The PCR products were later analyzed by gel electrophoresis in a 1.5% agarose gel stained with Midori Green Advance (Nippon Genetics, Japan). A DNA ladder was loaded as a size reference. The gel was run at 100 V for 40 minutes in 1x TAE buffer. Gel was visualized using Gel Doc™ EZ Imager (Bio Rad, USA) under UV trans-illuminator post electrophoresis. Distinct bands corresponding to the expected size of each PCR product were observed as positive results.

#### 2.3.3. Sequencing

The PCR products were submitted for sequencing, where purification and quantification were carried out prior to sequencing. The PCR products were sent to Apical Scientific Sdn. Bhd. for DNA purification and Sanger sequencing to confirm the correct amplification of genes targeted by the primers used in this study. For the DNA product, 15 µl of amplified PCR product was packed together with forward and reverse primers. Sequencing was carried out using Oxford Nanopore sequencing technology. Following submission, the sequencing results were retrieved and analyzed to verify the target sequence.

The sequence analysis was performed using Bioedit Software Version 7.2. The reverse primer sequence was complemented using the tool provided by Bioinformatics.org ([https://www.bioinformatics.org/sms/rev\\_comp.html](https://www.bioinformatics.org/sms/rev_comp.html)) to reverse the sequence. Both the forward and reverse sequences were aligned using Clustal Omega

(<https://www.ebi.ac.uk/jdispatcher/msa/clustalo>) to improve the accuracy of the DNA sequence. Then, the DNA sequence was also compared with nucleotide sequences in the National Centre of Biotechnological Information (NCBI) database using the Basic Local Alignment Search Tool - Nucleotides (BLASTN) program.

**2.4. Correlation analysis**

Genotypic frequencies were determined through direct counting, identifying homozygous (single peak) and heterozygous (double peak) alleles from chromatograms visualized with Unipro UGENE. Sequence alignment was performed using MEGA11 to locate SNPs. Allelic frequencies expected heterozygosity ( $H_e$ ), effective number of alleles ( $n_e$ ), and polymorphism information content (PIC), chi square test ( $X^2$ ) were calculated using Popgene 1.32, based on established formulas. Association analyses between SNP genotypes and phenotypes used the general linear model (GLM) for normally distributed data and the Kruskal-Wallis test for non-normal data, with Bonferroni correction applied to adjust p-values.

**3. RESULT AND DISCUSSION**

**3.1. Phenotypic parameters**

Phenotypic data concerning growth and fertility traits were successfully recorded during the pre-experimental procedure. The results for each trait are expressed as mean ± standard error (SE), with coefficient of variation (CV%) included to provide a relative measure of variability across the traits, as shown in Table 2.

**Table 2:** Growth and fertility traits expressed as mean ± SE, followed by CV% as a measure of variability with n=30.

	Mean±SE	CV (%)
<b>Growth Traits (cm)</b>		
HG	153.75 ± 2.85	10.09
BL	95.65 ± 1.67	9.01
<b>Fertility Traits (Months)</b>		
Age-at-first calving	32.20 ± 0.68	16.71
Calving interval	13.20 ± 0.29	11.82

HG: Heart girth; BL: Body length

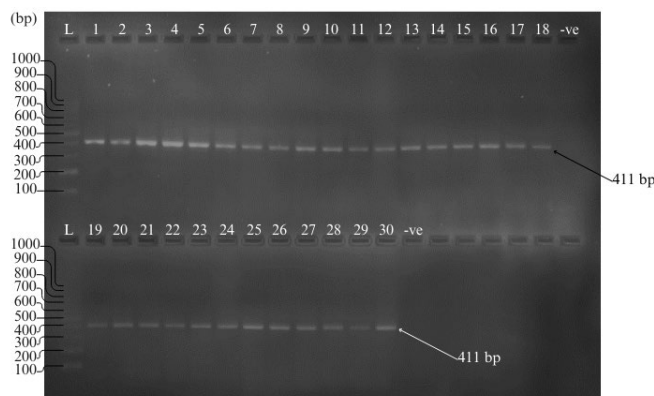
The mean heart girth was recorded at 153.75 cm (± 2.85), which is considerably higher than the baseline measurements for KK cattle (127.4±13.2 cm) established by Islam et al. (2022). In contrast, the mean body length was 95.65 cm (± 1.67), which is comparable to the established baseline (98.3±12.3 cm) according to a study by Islam et al. (2021). The classification of CV% are categorized as low (<10%), medium (10%<CV<20%), high (20%<CV<30%), and

very high (>30%) (Pimentel-Gomes, 2022). A lower CV% indicates uniformity across the data set, while a higher CV% indicates increased variability (Pélabon et al., 2020). The CV% for heart girth and body length were reported at 10.09% and 9.01%, which are considered moderate-to-low.

For fertility traits, the mean calving age at first calving was 32.20 months (± 0.68), which is closely similar to the baseline (35.50 - 38.20 months) as reported by Islam et al. (2021). The mean calving interval was 13.20 months (± 0.29), consistent with the established range (12.10 to 13.20 months) for KK cattle. The CV% for age at first calving and calving interval were reported within range from 16.71% and 11.82%, respectively, indicating a moderate degree of variation.

**3.2. Genotypic identification**

DNA extraction, amplification, and gel electrophoresis were successfully performed for all 30 samples. Each sample displayed clear and distinct bands corresponding to the expected amplicon sizes (411 bp), with no evidence of non-specific amplification. The results confirmed the successful amplification of target genes, consistent with findings from previous studies (Figure 1).

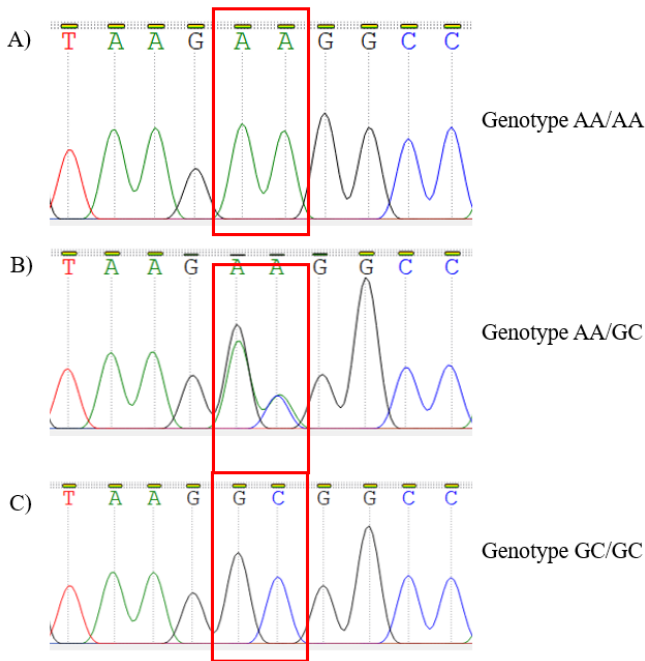


**Figure 1:** Gel electrophoresis image showing amplification of *DGAT1* gene. L refers to 100 bp ladder. 1-30 represent the different samples. -ve indicate negative control. Single clear bands indicate positive results.

The sequencing results confirmed the specific nucleotide variations at the target loci compared to reference sequences, thereby verifying the successful amplification of the intended SNP. The genotype for each sample sequence was determined by visual analysis of the chromatograms.

Figure 2 illustrates the genotypic variation at the SNP site for *DGAT1*, revealing three distinct genotypes; AA/AA, AA/GC, and GC/GC. The homozygous AA/AA was identified by the presence of two single peaks corresponding to the adenine (A) nucleotide. The heterozygous AA/GC genotype was observed by displaying two overlapping peaks for adenine (A) and guanine (G) nucleotides in the first double peak, followed by adenine (A) and cytosine (C) nucleotides in the subsequent double peak, indicating the presence of both

alleles. The homozygous GC/GC genotype was observed by characterizing two distinct single peaks corresponding to the nucleotides guanine (G) and cytosine (C) nucleotides. This result confirms the presence of genetic polymorphism at the targeted site.



**Figure 2:** Chromatogram analysis of DNA sequence assessment for DGAT1 gene. Positions of the SNP is highlighted by a red rectangle with A) homozygous AA/AA genotype, B) heterozygous AA/GC and homozygous GC/GC genotype.

The genotypic and allelic frequencies for SNP in DGAT1 were determined based on the observed genotypic counts (Table 3). The genotype distribution of DGAT1 was dominated by genotype AA/AA (53.3%), followed by heterozygous AA/GC (33.3%), and homozygous GC/GC (13.3%). The frequency of the AA allele was notably higher at 70.0% than the GC allele, which was observed at 30.0%.

**Table 3:** Growth and fertility traits expressed as mean ± SE, followed by CV% as a measure of variability with n=30.

SNP	Genotype	Genotypic Frequency	Allele	Allelic Frequency
DGAT1	AA/AA (n=16)	0.533	AA	0.700
	AA/GC (n=10)	0.333	GC	0.300
	GC/GC (n=4)	0.133		

### 3.3. Genetic diversity analysis

Diversity parameters such as,  $H_e$ ,  $n_e$ , and PIC were calculated. Additionally, the chi square test ( $\chi^2$ ) test was performed to assess the Hardy-Weinberg Equilibrium (HWE). The results are summarized in Table 4.

**Table 4:** Diversity parameters of DGAT1 SNP

	$H_e$	$n_e$	PIC	$\chi^2$ (HWE)	$\chi^2$ Test (p-value)
DGAT1	0.427	1.724	0.420	1.517	0.218

The DGAT1 demonstrated moderate genetic diversity with  $H_e = 0.427$ , as values closer to 1 indicate higher genetic variation (Kanaka et al., 2023). The effective number of alleles at  $n_e = 1.724$  likewise reflected moderate genetic diversity, indicating both alleles were maintained within balanced frequencies of the bi-allelic range of 1-2 (Allendorf et al., 2024). The PIC value (PIC = 0.420) indicated moderate marker informativeness, reflecting its capability in identifying polymorphisms among individuals in a population (Kanaka et al., 2023). The genotype distribution conformed to HWE ( $\chi^2 = 1.517$ , p-value = 0.218). Association analyses between DGAT1 SNPs to selected growth and fertility traits are displayed in Table 5.

**Table 5:** Association analysis between DGAT1 to growth and fertility traits. Values are shown as mean ± SE, values with differing superscript letters are significantly different from each other, p-value <0.05 are considered significant (bold)

SNP	Trait	Genotypes			p-value
DGAT1		AA/AA	AA/GC	GC/GC	
Growth traits	HG (cm)	162.50 ± 4.37 <sup>A</sup>	162.56 ± 4.04 <sup>A</sup>	147.81 ± 3.95 <sup>B</sup>	<b>0.036</b>
	BL (cm)	99.00 ± 2.80 <sup>A</sup>	100.26 ± 2.71 <sup>A</sup>	91.94 ± 1.96 <sup>B</sup>	<b>0.024</b>
Fertility traits	Age-at-first calving (months)	22.69 ± 0.68	21.40 ± 1.71	20.75 ± 1.38	0.478
	Calving interval (months)	13.00 ± 0.34	14.20 ± 0.51	12.50 ± 1.04	0.118

The association between SNPs, growth (heart girth and body length) and fertility traits (age-at-first calving and calving interval) revealed significant effects for specific SNPs. DGAT1 showed significant associations with both HG ( $p = 0.036$ ) and BL ( $p = 0.024$ ). The GC/GC genotype showed significantly lower heart girth (147.81 ± 3.95) compared to genotypes AA/GC (162.56 ± 4.04) and AA/AA (162.50 ± 4.37). Similarly, GG/CC genotypes exhibited lower means (91.94 ± 1.96) for body length compared to AA/AA (99.00 ± 2.80) and AG/AC (100.26 ± 2.71) genotypes. In contrast, no significant associations were identified concerning fertility traits.

### 3.4. Discussion

Phenotypic traits, such as HG and BL, as well as age-at-first calving and calving interval, are key indicators of beef cattle production, informing breeding and management decisions (Bila et al., 2024; Kawaguchi et al., 2020). In KK

cattle, body length was consistent with baseline data, but heart girth exceeded it by 30 cm, possibly due to crossbreeding, improved nutrition, or effective management practices (Islam et al., 2022). The low coefficient of variation observed for growth traits suggests a degree of stability advantageous for breeding programs (Tokatlidis et al., 2023). In terms of fertility, a delayed age at first calving, ranging between 35.5 and 38.2 months compared to the optimal 24 months, indicates potential for nutritional enhancements, while a 12-month calving interval signifies commendable reproductive performance (López-Paredes et al., 2018; Cardoso et al., 2014). Moderate CV% in fertility traits reflected environmental influences (Sousa et al., 2015).

The *DGAT1* gene is widely recognized as a key marker for improving cattle production and fertility (Khan et al., 2021). Various genetic selection methodologies, including SNP analysis, genome-wide association studies, and genomic selection, have facilitated the identification of this gene. Its prevalence in cattle populations enables detection using techniques such as PCR and gel electrophoresis followed by sequencing. Alternative identification methods include PCR-RFLP and targeted genotyping technologies, such as iPLEX MassARRAY. These are readily accessible and can be seamlessly integrated into Marker-Assisted Selection (MAS), which is a vital tool for improving cattle genetics. In KK cattle, polymorphism at the *DGAT1* locus was observed, with the homozygous genotype AA/AA (53.3%) and AA allele (70%) had the highest frequency, which coincide with the findings by Avilés et al. (2013). The AA allele have been associated with higher growth traits. The finding explores the genetic landscape of the sampled population, elucidating information on the possible pre-existing selection pressures and breed specificity of *DGAT1*. However, it is important to note that the relatively small population size might limit the representation of these results.

In a broad sense, genetic diversity can be defined as the extent of variability within or between populations (Ennos, 2000; Hughes et al., 2008). Variations in genotypes and alleles present in population reveal differences in physique, psyche or behaviour among individuals (Frankham et al., 2002). Expected  $H_e$  or Nei's genetic diversity is a common parameter used to estimate genetic diversity. It is determined by calculating the probability an individual is heterozygous at a certain locus in the genome (Kanaka et al., 2023). The scale of heterozygosity ranges from zero (indicating a lack of heterozygosity) to one (indicating high heterozygosity), where higher heterozygosity levels reflect significant genetic diversity at the locus within the population. The effective number of alleles ( $n_e$ ) is the number of equally frequent alleles required to achieve a similar expected heterozygosity; therefore,

indicating the expected number of alleles at a locus in a studied population (Allendorf et al., 2024). In populations characterized two alleles contribute equally, the effective number of alleles equals 2. Lower values may indicate that one allele plays a more dominant role in contributing to genetic diversity than the other. PIC acts as a marker quality indicator by gauging the marker's ability to detect polymorphisms among members of a population (Kanaka et al., 2023; Serrote et al., 2020). The values of PIC range from 0 to 1, categorized as follows: values below 0.25 are deemed less informative or uninformative, values between 0.25 and 0.5 are considered somewhat informative, and values exceeding 0.5 are classified as very informative (Botstein et al., 1980). These parameters give an insight into the potential and suitability of selected SNPs as genetic markers.

*DGAT1* exhibited a moderate level of genetic diversity, which is significant since higher genetic diversity contributes to the effectiveness of markers, particularly in reducing the risk of population bottlenecks when utilized in MAS (Abady et al., 2021; Zhong et al., 2016). Selection markers should ideally be chosen when their PIC values surpass 0.5. Nevertheless, it is important to consider that the calculations of the diversity parameters may be affected by the sample size. The HWE states that allelic and genotypic frequencies in a large, randomly-mating population would remain constant throughout generations in the absence of evolutionary forces, such as mutation, migration, selection, and genetic drift. According to the principle, genotypic frequencies (AA, AB, BB) are expected to occur in the relative proportions  $p^2$ ,  $2pq$ , and  $q^2$ , where  $p$  is the allele frequency of A and  $q = 1 - p$  represents the allele frequency of B (Graffelman & Weir, 2016; Mayo, 2008). The estimation of Hardy-Weinberg equation is measured through Chi-square ( $\chi^2$ ) test using in Popgene 1.32 (Yeh et al., 1999). Notably, there are several suppositions that may lead to a deviation from the HWE if compromised. These suppositions include random mating, absence of natural selection, very large population size, absence of migration and mutation, and autosomal loci. Testing for HWE gauges population diversity and identifies any potential genotyping errors. Kanaka et al. (2023) explained that examining genetic markers for HWE may reveal genotyping mistakes that results in heterozygote excess, leading to significant deviations from HWE. Although, disequilibrium may also be caused by selection, inbreeding, migration, and mutation. Meanwhile, SNP with  $\chi^2$  Test P-value  $> 0.05$  classified as disequilibrium. The  $\chi^2$  test revealed *DGAT1* deviated from HWE. This deviation may be attributed to many causes, albeit the small sample size in this study could be the primary reason. Lachance (2016) explained that genetic drift-induced sampling effects can lead to a small

excess of homozygotes or heterozygotes, which are more prominent in very small populations. Another possible cause of deviation is non-random mating, such as selective breeding or inbreeding. However, deviations resulting from non-random mating are typically observed across multiple loci, indicating genome-wide effects.

The *DGAT1* gene is essential for triglyceride synthesis and is associated with meat, milk, growth, and fertility traits. This study involved genotyping K232A (g.10433\_10434 GC>AA) in *DGAT1* of KK cattle. Significant associations were found between SNP K232A and selected growth traits (HG and BL). Cattle with GC/GC genotype exhibited significantly smaller in both heart girth and body length compared to those with AA/AA and AA/GC genotypes. These findings are in accordance with a study by Ribeca et al. (2014), who discovered significant associations between K232A and carcass weight in double muscled Piemontese cattle. The estimated additive effect suggests that the AA allele is associated with increased carcass weight compared to the GC allele. In contrast, Kawaguchi et al. (2020) reported Japanese Black cattle with AA/AA genotype have significantly smaller carcass weight compared to other genotypes. Fortes et al. (2009) highlighted cattle breeds with diverse genetic backgrounds exhibit different responses to the *DGAT1* gene, implying that *DGAT1* polymorphisms are breed-specific.

On the other hand, no significant associations between SNP and selected fertility traits in KK cattle. These findings contradict with study by Ardicli et al. (2019), who reported Holstein cattle with KK genotype have longer calving intervals compared to KA genotypes. Another study by Jecminkova et al. (2016) discovered significant favourable effects of the AA genotype on age at-first-calving and the conception rate after first service in Holstein cattle. There are varying results between the SNP association with fertility traits of the same breed, highlighting the need for extensive research into SNP markers for breeding

#### 4. CONCLUSION

This study explored the associations between the *DGAT1* to growth (HG and BL) and fertility (age-at-first calving and calving interval) traits in KK cattle. It showed significant associations with growth traits, positioning *DGAT1* as a promising marker for selective breeding. Furthermore, the observed polymorphisms within *DGAT1* gene suggest the necessity for further investigation to elucidate its functional implications. To enhance the robustness of the findings, future studies should use larger populations to address the limitations posed by the current small sample size. Overall, this study advocates for the incorporation of SNP-based marker-assisted selection strategies, aimed at improving the

productivity of KK cattle and laying the groundwork for enhanced breeding programs that aspire to elevate beef production in Malaysia.

#### ACKNOWLEDGEMENT

This work was funded by UMK Fundamental 2022 (Reference code: R/FUND/A0600/01870A/001/2022/01100).

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