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Research Article
*Taq*I Polymorphism in *MYF5* Gene and its Association with Body Weight in Friesian Bull Calves

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Abstract

Objective: The effect of *MYF5* gene polymorphism on body weight was investigated in 100 Friesian bull calves. **Methodology:** Blood samples were collected from each animal for DNA extraction. The PCR-*Taq*I digestion of 1190 bp of a fragment of *MYF5* gene revealed that, two fragments (983 and 207 bp) for genotype BB, three fragments (1190, 983 and 207 bp) for genotype AB and undigested fragment (1190 bp) for genotype AA. The incidence of *MYF5* genotypes and frequencies of alleles were calculated. **Results:** The AA, AB and BB genotype frequencies in the 100 Friesian bull calves were 0.20, 0.46 and 0.34, respectively and the A and B allele frequencies were 0.43 and 0.57. Statistical analysis indicated that there was highly ($p<0.01$) significant association between *MYF5* genotypes and body weight. The AB genotype was higher in body weight than both BB and AA genotypes. However, there was no significant variation between AB and BB genotypes in the body weight. **Conclusion:** This study highlights the effect of *MYF5/Taq*I locus as candidate for body weight in cattle.

Key words: *MYF5* gene, body weight, Friesian cattle, PCR-RFLP

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dual-purpose cattle selection relied on its milk production and growth traits. However, the importance of growth traits increased over the last years due to higher consumer demands as concerns meat quality and market competition. Differences in body weight are probably due to the variation in different genetic and ecological factors which interact and determine factors for manifestation of such quantitative divergences. A candidate gene access may not only furnish a more channeled understanding the phenotypic expression of this variation between individuals on genetic basis but also offers the identification of SNPs in genes that most likely cause mutation in a phenotypic trait relied on physiological and endocrinological revealing specific candidate gene markers associated with target traits[1,2].

Molecular genetic markers have been successfully exploited for animal genetic improvement via identifying and analyzing genes responsible for the principle biosynthetic pathways related to animal growth[3]. The investigations about polymorphisms in these genes permitted the verification of relatedness between genotypes and growth traits in cattle. A study of RFLP involves comparing the DNA fragments number and size when DNA digested with various restriction enzymes. The restriction enzymes cut the DNA molecule at specific recognition sites, originating a set of fragments with different length that could be separated by conventional gel electrophoresis according to their molecular size[4].

Myogenic factor (*MYF5*) gene belongs to the *MYOD* family[5]. One of the characteristics of the onset of skeletal myogenesis is the expression of myogenic factors (*MYF*), particularly *MYF5* and *MYOD*. The plausible role of *MYF5* is the considered to be inherent for innovation, growth, development and sustainment of the phenotype of skeletal muscle. The *MYF5* has been fine mapped on cattle chromosome 5 for QTLS for growth traits[6]. Therefore, it is believed to be candidate gene for growth traits[7,8]. Several studies reported the *MYF5* gene polymorphisms in many cattle breeds and their association with beef production traits particularly growth, carcass and meat quality[9-12]. Nevertheless, the association between *MYF5* gene and body weight in Friesian cattle is scare. Hence, the present study objectives were to reveal the effect *Taq*I polymorphism in *MYF5* gene using PCR-RFLP on body weight in Friesian bull calves.

MATERIALS AND METHODS

Animals and experimental samples: In this study, a total of 100 Friesian bull calves were used. The samples were stored at -20°C until needed for DNA extraction. Based on the farm record, the selected animals were weaned at 90 kg b.wt., have their birth weight ranged from 29-38 kg and their weaning age ranged from 75-100 days. Blood samples were collected into anticoagulant disodium EDTA containing tubes. The genomic DNA was extracted using extraction kit (Jena Bioscience, Germany). This study protocol was approved by the animal welfare and ethics committee, Faculty of Veterinary Medicine, Damnhour University.

***MYF5-Taq*I polymorphism detection:** The PCR was done for amplification of a fragment of *MYF5* gene with expected amplicon size of 1190 bp using available sequence information from bovine *MYF5* published (GenBank accession No. M95684)[13].

Forward : 5'-GATAGCTGGCTGTGAATGAT-3'
Reverse : 5'-CTGGCAACTGGGGAGAGAGAAG-3'

The polymerase chain reaction mixture was done in a 25 µL consisted of: 2 µL DNA, 9.5 µL H_2O (dd H_2O), 12.5 µL PCR master mix (Jena Bioscience, Germany), 0.5 µL of each primer. The final reaction mixture was achieved in a thermal cycler and the PCR temperature schedule program was carried out by 94°C for 4 min as initial denaturation succeeded by 34 cycles of 94°C for 1 min for denaturation, primer hybridization temperature at 58°C for 1 min, primer extension at 72°C for 1°C and the final elongation at 72°C lasts for 10 min.

The amplified DNA fragments of *MYF5* gene were digested with fast digest *Taq*I (Thermo Scientific, #FD0674) at 65°C for 5 min. The reaction volume was done in 30 µL consisted of: 10 µL PCR product, 17 µL H_2O (dd H_2O), 2 µL 10x fast digest green buffer, 1 µL restriction enzyme. The obtained cleaved fragments were explored by agarose gel electrophoresis then their patterns were visualized under U.V using gel documentation system.

Statistical analysis

Adjustment or correction of non-genetic factors: Based on farm records, the calf weaning weight was adjusted to 205 days of age by linear interpolation from birth weight, weaning weight and age. Adjustment was carried out using the following equation[14].

$$A = \frac{B\text{-}C}{D} \times 205 + C$$

where, A is for 205 days weight (kg), B is for the weaning weight (kg), C is for the birth weight (kg) and D is for the weaning age (days).

Association analysis: Statistical analysis was performed using Graphpad statistical software program (Graphpad prism for windows version 5.1, Graphpad software, Inc, Sandiego, CA, USA). General Linear Model (GLM) practice of the statistical analysis system package[15] was used for data analysis to determine association of *MYF5* genotypes and body weight.

Gene and genotypic frequencies in *MYF5* locus: Based on the electrophoresis results, gene and genotypic frequencies were calculated by allele simple counting[16]. Chi-square was carried out to test Hardy-Weinberg equilibrium and show genotype distribution in the cattle population.

RESULTS

One pair of specific primers was used to amplify specific DNA fragments 1190 bp of *MYF5* gene (Fig. 1). Restriction analysis of 1190 bp PCR products digested with *Taq*I revealed that, two fragments (983 and 207 bp) for genotype BB, three fragments (1190, 983 and 207 bp) for genotype AB and undigested fragment (1190 bp) for genotype AA (Fig. 2).

Using PCR-RFLP method, the population of 100 Friesian bull calves was genetically described. Where, the incidence and frequency of *MYF5* genotypes and alleles were calculated. In 100 Friesian bull calves, the genotypic frequencies 0.20, 0.46 and 0.34 were for AA, AB and BB

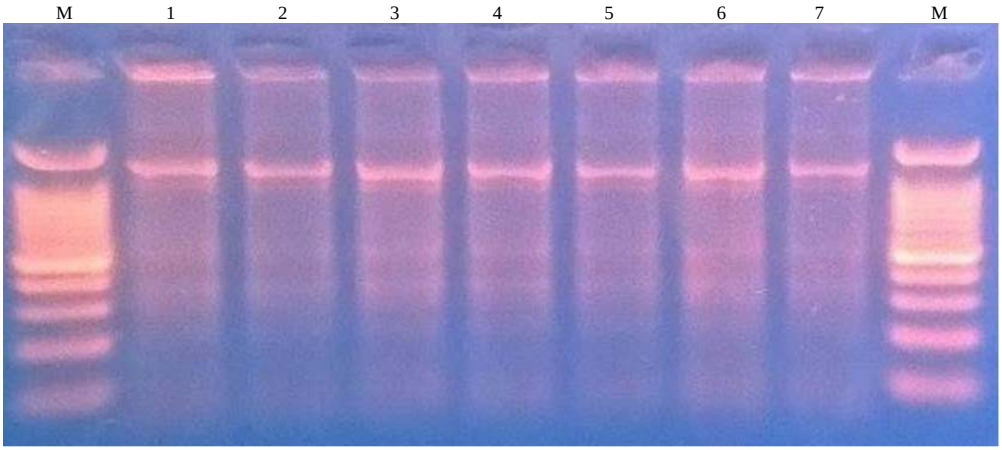

Fig. 1: Representative PCR results of *MYF5* gene, lane M: DNA marker and lanes 1-7: 1190 bp amplified fragment of *MYF5* gene

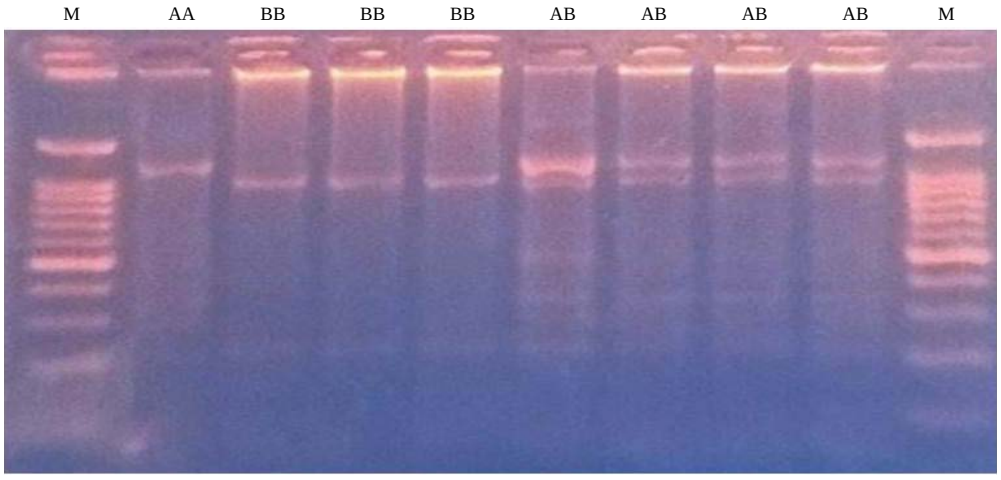

Fig. 2: Representative *Taq*I restriction fragment pattern of *MYF5* gene (1190 bp). BB: Restriction fragment of 983 and 207 bp, AB: Restriction fragment of 1190, 983 and 207 bp, AA: Restriction fragment of 1190 bp and M: DNA ladder

Table 1: Frequency of genotypes and alleles in the *MYF5* locus

	Genotyping frequency				Allele frequency	
Items	Total	AA	AB	BB	Allele A	Allele B
Observed	100	20	46	34	0.43	0.57
Expected		18.49	49.02	32.49		

Chi-square calculated (χ^2) = 0.379-No significant differences, Chi square tabulated (χ^2) at DF = 1 and $p<0.05$ = 3.84

Table 2: Associations of *MYF5* genotypes with corrected body weight (LSM±SE)

LSM±SE of corrected body weight	Genotype
156.18±0.89[b]	AA
175.85±2.56[a]	AB
172.76±1.70[a]	BB

Means of different levels within the same column having different superscripts are significantly different ($p<0.01$)

genotypes, respectively and the allelic frequencies 0.43 and 0.57 were for A and B alleles. The χ^2-test showed that the genotype distributions in the cattle population were in Hardy-Weinberg equilibrium ($p<0.05$) (Table 1).

Statistical analysis indicated that there was highly significant association ($p<0.01$) between *MYF5* genotypes and body weight. The AB genotype was higher in body weight than both BB and AA genotypes. However, there was no significant variation between AB and BB genotypes in the body weight (Table 2).

DISCUSSION

In this study, PCR amplification of a fragment of *MYF5* gene yielded specific PCR product of desirable size (1190 bp). The following DNA restriction fragments were obtained for *MYF5-TaqI* digestion: Digested (983 and 207 bp) fragments for genotype BB, three fragments (1190, 983 and 207 bp) for genotype AB and undigested fragment (1190 bp) for genotype AA. For a population of 100 Friesian bull calves, the genotypic frequencies 0.20, 0.46 and 0.34 were for AA, AB and BB genotypes, respectively and the allelic frequencies 0.43 and 0.57 were for A and B alleles. The χ^2-test showed that the genotype distributions in the cattle population were in Hardy-Weinberg equilibrium ($p<0.05$). Absence of the significant difference between observed and the expected values for genotype counts indicated the population balanced and follow Hardy-Weinberg equilibrium. This balance may originate from the higher number of the heterozygous genotype (AB) than those homozygous genotypes (AA and BB) which keep the balanced allelic frequencies in the population[17]. The association between RFLP-*TaqI* of the *MYF5* gene and body weight was studied. The *MYF5* genotypes were highly significant ($p<0.01$) associated with body weight. The AB genotype was higher in body weight than both BB and AA genotypes. However, there was no significant variation between AB and BB genotypes in the body weight.

Several polymorphisms in *MYF5* gene were indicated in different cattle breeds by several previous studies. However, most of association analyses reported opposing results or failed to demonstrate any relationship to growth traits[9,18-23]. The association of the SNP in *MYF5* with the body weight traits of Nanyang cattle has been investigated[9]. The researchers indicated that there was significant association between the identified SNP in *MYF5* gene and all body weight traits except birth weight. A significant effect of SNP on the average daily gain of cattle was also reported[10].

In another study on RFLP-*TaqI* of the *MYF5* gene and growth traits, association between polymorphism of *MYF5* Gene and body weight in Jiaxian, Nanyang and Qinchuan breeds of cattle was studied[11]. According to results, the DNA restriction fragments were obtained for *MYF5-Taq/*digestion were similar to that denoted in our study. Allele B at *MYF5* locus was dominant in these three populations. The frequency of allele B at *MYF5* locus in the three Chinese breeds was 0.8275/0.7581/0.7523, respectively. No statistically significant variations in body weight traits were observed between the genotypes of the Jiaxian and Nanyang breeds at *MYF5* locus. However, there were statistically significant differences between the genotypes of the Qinchuan breed. The *MYF5* gene polymorphisms and their relatedness with body weight was also reported in Hanwoo (Korean cattle)[24]. Statistical analysis indicated that there was significant association (0.05) between *MYF5* gene polymorphisms and live body weight at 6 months of age (LW6).

CONCLUSION

This study supports the significance effect of *MYF5* gene as plausible candidate for body weight in cattle. Moreover, the effectiveness of RFLP as a molecular genetic marker contains great genetic potential resource to improve such trait results in effective selection.

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